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Review article

Harnessing polyploidy for vegetable crop improvement: strategies and applications

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Abstract

In the evolution and diversification of plant species, polyploidization is the most common event. Polyploidy, also known as whole-genome duplication (WGD), occurs when a cell or organism has more than two sets of chromosomes per nucleus. Autopolyploidy and allopolyploidy are the two types of polyploidy. The former refers to chromosomal/genome duplication within the same species (AAAA), and the latter refers to genome hybridization followed by chromosome doubling. Scientists are becoming more interested in ploidy because of its benefits in genomic flexibility and long-term functional alterations, as well as its selective ability to respond to environmental changes. The "gigas" effect, is the most visible result. Colchicine, a popular mitotic inhibitor, causes polyploidy in plants by blocking chromosomal segregation during cell division. Ploidy can be detected by using a variety of markers, including morphological, physiological, and molecular ones (counting chromosomes and estimating nuclear genome size using flow cytometry). Watermelon (Pusa Bedana and Arka Madhura), Cassava (Sree Harsha), and Palak (Pusa Jyoti)are among the vegetables that have benefited from polyploidy. As a result, polyploids have a lot of potential for use in breeding programs, particularly in terms of yield and tolerance.

Keywords: Autopolyploids, Allopolyploids, Chromosome doubling, Gigas effect, Whole-genome duplication

The most omnipresent phenomenon for the evolution and diversification of plant species ispolyploidization (Parisod *et al*. 2010; Sattler *et al*. 2016). Polyploidy is the heritable condition of possessing more than two complete sets of chromosomes (Comai 2005). Triploid (3n) and tetraploid (4n) refer to plants containing three and four sets of chromosomes, respectively. Many crop plants have undergone polyploidy, which ranges from 30-70 percent in angiosperms, during the evolutionary process (Chen 2010; Sattler *et al.* 2016). The fertile polyploids not only supplement the species diversity but also an anchorage for polyploidy breeding (Kazi 2015). Some basic terms must be defined before comprehending polyploidy. The letter "x" is used to refer to the basic chromosome number of the ancestor of a polyploid. "2n", refers to the total number of chromosomes. A diploid organism (non-polyploid) is represented as 2n = 2x (Heslop-Harrison, 2013). A somatic cell has 2n chromosomes, whereas gametes only have a haploid (n) set of chromosomes (Otto and Whitton 2000). Whole-genome duplication not only produces variations in the genome (epigenetic changes and modulated gene expression) but also multiplies the copies of existing genes that influence the external characters of the species (Chen *et al*. 2020).

Vegetable crops with increased ploidy levels frequently display higher genetic diversity with anatomical and morphological changes that have resulted in increased size, vigour, biomass, yield and also possess selective abilities to adapt to environmental changes (stress tolerance and disease resistance). Thus, polyploidizationprovides an opportunity for vegetable improvement that put forward many economic and social benefits (Can 2012). The three kinds of polyploidy that have been

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identified are autopolyploidy, allopolyploidy, and segmented allopolyploidy (Stebbins, 1947). In the first one, there is a duplication of a chromosome/genome within the same species (Stebbins 1947). Allopolyploids have two or more different genomes andcan result through the hybridization of two separate species, which is linked to genome doubling (Stebbins 1947; Grant 1975). The third one, *i.e*., segmental allopolyploids, carries more than two incompletely distinct genomes which can lead to the formation of both bivalents and multivalents during chromosome pairing (Stebbins 1947; Levin 2002; Madlung 2013).

Polyploidy gained much importance during the early part of the twentieth century. One of the earliest examples of natural autopolyploidy was the gigas mutant in *Oenothera lamarckiana* (Ramsey and Schemske 1998). In 1916, Wrinkler experimented with Solanum vegetative grafts and chimeras and discovered that some of the plants obtained were polyploid. *Primula kewensis* (sterile interspecific hybrid through chromosome doubling) was the classical example of allopolyploidy (Digby 1912; Dar *et al*. 2017). Tetraploid and octoploid cells in the cortex and pith of Vicia faba have been reported (Ramsey and Schemske 1998; Dar *et al*. 2017). Antimitotic drugs can be used to artificially produce polyploids, with colchicine being one of the most widely utilized (Blakeslee and Avery 1937; Planchais *et al*. 2000). In contrast to their diploid counterparts, induced polyploids exhibit higher flexibility, tolerance to diverse stresses (biotic and abiotic), and a longer reproductive period with increased photosynthetic and genetic action (Gantait and Mukherjee 2021).

Origin

It has been the subject of several reviews in the last century aimed at explaining the causes and effects of polyploidy (Stebbins 1947; Harlan and de Wet 1975; Ramsey and Schemske 1998; Soltis and Soltis 1999 Otto and Whitton 2000).To explain how polyploids originate in nature, various processes have been proposed. Polyploidy in plants is caused by two primary routes: somatic doubling (mitotic nondisjunction) occurs in zygotic, embryonic or sporophytic tissue and the formation of unreduced reproductive cells (non- reduction during meiosis) (Madlung 2013; Sattler *et al*. 2016). Aside from genetic regulation, a variety of environmental factors have contributed to the development of unreduced gametes such as temperature, water-deficient, wounding and nutrient shortage (Ramsey and Schemske 1998). The formation of unreduced gametes can lead to bilateral polyploidization (fusion with another unreduced gamete) or unilateral polyploidization (fusion of an unreduced gamete with a reduced one) (Sattler *et al*. 2016). The two processes that lead to the production of unreduced gametes are cytologically known as first division restitution (FDR) and second division restitution (SDR). During the FDR, there is no chromosome pairing in zygotene or pachytene and/or non-segregation of homologous chromosomes in anaphase I, resulting in two non-sister chromatids with approximately the same heterozygosity level as their parents, whereas in SDR, sister chromatids do not segregate in anaphase I, resulting in the formation of dyads or triads and will have a lower level of heterozygosity as compared to its parents (Bretagnolle and Thompso, 1995; Ramanna and Jacobsen 2003). Other mechanisms that led to the development of polyploid plants are: meiotic or mitotic failures, polyspermy (fertilization of the egg by two male nuclei), and endoreduplication (replication of the DNA but no cytokinesis) (Ramsey and Schemske 1998; Otto and Whitton 2000; Comai 2005; Song *et al*. 2012; Dar *et al*.2017). Chromosome doubling in the zygote or in some apical meristems produces complete polyploids and polyploid chimeras, respectively (Dar *et al*. 2017).

Autopolyploidy

Traditionally, autopolyploids were considered to occur from the doubling of structurally similar, homologous genomes within a single species (AAAA) (Parisod *et al*. 2010). They occur naturally in low frequencies and can be artificially induced by a variety of techniques including: heat and chemical treatments, decapitation, and selection from twin seedlings (Dar *et al*. 2017). Artificially, they are originated using colchicine ($C_{22}H_{25}O_6$), an alkaloid from the autumn crocus (*Colchicum autumnale*) as shown in Table 1, which disrupts the spindle mechanism in mitosis, thereby preventing the migration of duplicate chromosome (Blakeslee and Avery 1937). The meristematic tissue is the most vulnerable to colchicine treatment. Tetraploid crop like potato (Wakchaure and

Table 1. Induction of polyploidy by applying colchicine

Method of application	Optimum treatment	Reference
Colchicine solution is poured over the entire plant.	0.1; 96 hours	Vichiato *et al*. 2014
Seeds that have been immersed in a colchicine solution	0.05%; 24 hours	Balode, 2008
Colchicine is applied to seedlings using cotton plugs.	0.4 %; 3 days	Anurita and GirJesh, 2007
Colchicine was administered to the apical meristems in drops.	0.006% for 3 successive days	Talebi *et al*. 2017

Ganguly 2016) and hexaploid crop like sweet potato are examples of natural autoploids. Meiotic failure occurs in autopolyploidy, leading tothe generation of unreduced gametes, which eventually result in the formation of multivalent (*e.g*., Tetraploids have quadrivalents or bivalents in addition to certain trivalent and univalent) (Dar *et al*. 2017).

The "gigas" effect, which results in individuals having larger leaves, roots, flowers, fruits, and seeds than their diploid counterparts, was the most common consequence of autopolyploid production (Sattler *et al*. 2016; Stebbins 1950). But sometimes, there is reduction in the fertility of autopolyploids as compared to their diploid progenitors (Ramsey and Schemske 2002). During the research on crops, it was observed that genome doubling in autopolyploids contributes prompt acquisition of novel traits (*e.g*., increased cell size and gene expression, changes in physiology and ecological tolerance) (Levin 2002; Ramsey and Schemske 2002; Paterson 2005). Autopolyploidy induction is mainly limited to crops cultivated for their vegetative organs and those with vegetative propagation due to the low rates of viable seed production (Paterson, 2005) with an exception of triploid watermelon as in this case, low number of seeds is a desirable characteristic (Crow 1994). Furthermore, autopolyploids may impact stress tolerance, including nutrient insufficiency, drought, water deficit, temperature, pests, and diseases (Levin 2002). Autopolypoids have higher levels of heterozygosity than diploids due to polysomic inheritance (Moody *et al*. 1993; Osborn *et al*. 2003). In autotetraploid maize (Randolph 1942), potato (Mendoza and Haynes 1974), and alfalfa (Mendoza and Haynes 1974), higher levels of heterozygosity have been associated with increased vigour. Autopolyploidy's two key triumphs in crop development are the formation of triploids and tetraploids.

Autopolyploidy's evolutionary course

Top panel: Genome doubling occurs because of cross-fertilization between individuals, either by the direct fusing of two unreduced gametes (one-step creation) or as a two-step process of cross-fertilization between an unreduced gamete and a triploid intermediate (triploid bridge). In natural populations, spontaneous doubling is uncommon. Central panel: Genome doubling causes genetic and epigenetic alterations, which lead to structural and functional reorganization until full diploidization is achieved in the long run. These genetic mechanisms are associated with the development, establishment, and growth of autopolyploid lineages in wild populations (Parisod *et al*. 2010). The practical applications of autopolyploidy are as under:

Triploid Seedless watermelon

The first triploid seedless watermelon was developed by Dr. Khiara and Nishiyama (Kazi 2015) by treating a normal diploid plant (2x=22) with a tetraploid plant (4x=44) using antimitotic agent *i.e*.,colchicine as shown in Figure1 and thus triploid progeny is produced by using the pollen of the diploid (triploid does not produce viable pollen for pollination and fruit development) to pollinate the stigma of the induced tetraploid (Crow 1994; Pal and Bal 2020). As a result, diploid plants were planted in the same region as triploids to supply the requisite pollen for seedless fruit development (Crow 1994). Seedless watermelons are gradually gaining favour among Indian customers. Through colchiploidy, Kerala Agricultural University (KAU), Trichur, has generated a stable tetraploid line of watermelon called 'KAU-CL-TETRA-1. By crossing this tetraploid line with diploid males, notably CL-4 (red-fleshed) and CL-5 (yellow-

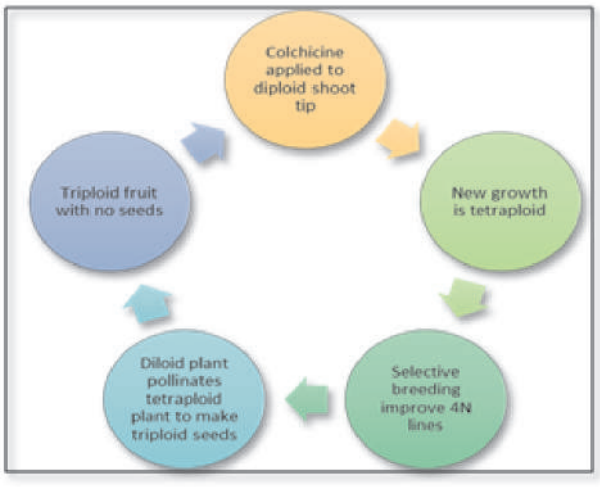

Figure 1: Formation of triploid seedless watermelon

fleshed), two triploid hybrids, Shonima and Swarna, have been created. Both hybrids have been approved for cultivation in Kerala (Thayyil *et al*. 2016).

Triploid sugar beet

Sugar beet comes in diploid (2x = 18), triploid (3x = 27), and tetraploid (4x = 36) forms, and is a major sugar producer in temperate climates (Smulders *et al*. 2010). Male diploid sterile plants are crossed with tetraploid pollinators or by reciprocal crossing, *i.e*., between male tetraploid sterile plants and diploid pollinators, to generate triploid sugar beet (Kinoshita and Takahashi 1969). Sugar beets that are triploid have larger roots and produce more sugar per unit area (Dabholkar 2006).

Triploid asparagus

The most economically important Asparagus species is *Asparagus officinalis* L. (2n = 2x = 20), which is also the only one cultivated as a vegetable crop worldwide. It does, however, have a limited genetic base. As a result, it's critical to introduce agronomically relevant features from wild relatives, thereby expanding the breeding gene pool. In asparagus, a triploid cultivar has been described from crossings between the tetraploid landrace 'Morado de Huetor' and a diploid commercial cultivar (Castro *et al*. 2012).

Tetraploid turnip

Hua-bing *et al*. (2011) investigated a series of physiological characteristics to see if a tetraploid turnip (cv Aijiaohuang, 4n) and its diploid parent (cv. Aijiaohuang, 2n) were tolerant to salinity stress. Tetraploid turnips adapted better to a high salt medium (200 mmol L^{-1}) and had a higher K^+ / Na^+ ratio in the roots, higher glutathione concentration, and antioxidant activity in the leaves, according to the findings.

Allopolyploidy

The prefix 'allo' indicates that the ploidy solely includes non-homologous chromosomes (Wakchaure and Ganguly 2016). Allopolyploids, also known as alloploids, were originated from the hybridization of two or more genomes followed by chromosome doubling or by fusing of unreduced gametes from different species (Ramsey and Schemske 1998; Acquaah 2007; Jones *et al*. 2008; Chen 2010). Rapeseed and mustard are examples of important natural alloploids. (Acquaah 2007; Chen 2010). It is crucial to distinguish the origins of genomes in allopolyploid, hence each genome is assigned a separate letter (*e.g*., Nagaharu explained the origin of the cultivated mustards (Brassica species), in the triangle of U with each species represented by a distinct letter) (Bellostas *et al.* 2007; Nelson *et al*. 2009). Further, there are two subclasses of allopolyploids: true and segmental allopolyploids. If hybridization occurs between distantly related species, then there is the formation of bivalents during meiosis in a disomic inheritance pattern which ultimate giverise to true allopolyploids (Sattler *et al.* 2016). On the contrary, if hybridization occurs between closely related species with partially differentiated genomes, it will result in the formation of segmental allopolyploid (Stebbins 1950). Sometimes allopolyploids are also formed by a two-step process i.e., formation of a triploid bridge (the fusion of reduced 1n gamete with unreduced 2n gamete gives rise to 3n zygote followed by the subsequent fusion of 1n reduced gamete with 3n gamete in the next generation (Dar *et al*. 2017). The increasing number of alleles of a given gene mask detrimental recessive mutations and hence protects against fitness loss (Gu *et al*. 2003). The second benefit is polyploids' ability to outperform their diploid counterparts due to heterosis (Birchler *et al*. 2010). The neo-functionalization or sub-functionalization that leads to ecological niche expansion is the third and most crucial advantage (Adams and Wendel, 2005; Moore and Purugganan,

2005; Lynch 2007). Okra (2n=130) is a natural amphidiploid (Joshi and Hardas1956) that results from chromosome doubling in a hybrid between *Abelmoschus tuberculatus* as one parent and *Abelmoschus ficulneus* as the other probable parent (Lata *et al*. 2021). The important applications of allopolyploidy are enumerated as under:

1. Contribution of ploidy in the evolution of new species:

Amphidiploid *Brassica* species

Brassica contains 330 genera and 3800 species, making it the most important genus (Bailey *et al*. 2006; Huang *et al*. 2016). The six key cultivated species are *Brassica rapa, Brassica juncea, Brassica nigra, Brassica carinata, Brassica oleracea,* and *Brassica napus*. Based on artificial inter-specific hybridization experiments, a well-known model, U's triangle, was proposed to demonstrate the genetic links among these six species (Figure 2; Nagaharu U's triangle, 1935). *B. rapa* (AA, 2n = 2x = 20), *B. nigra* (BB, 2n = 2x = 16), and *B. oleracea* (CC, 2n = 2x = 18) are the three fundamental diploid species, and three allotetraploid species, *B. juncea* (AABB, 2n = 4x = 36), *B.carinata* (BBCC, 2n = 4x = 34), and *B.napus* (AACC) are formed through natural hybridization and chromosome doubling (Chalhoubetal 2014; Yang *et al*. 2016).

Hakuran

An artificially manufactured interspecific hybrid and a promising breeding bridge plant created by crossing Chinese cabbage with cabbage and combining the heading qualities of both parents. Bacterial soft rot resistance and low-temperature sensitivity were introduced from common cabbage and Chinese cabbage, respectively. Hakuran is self-incompatible, although it is cross-compatible with other Brassica species (Nishi 1981).

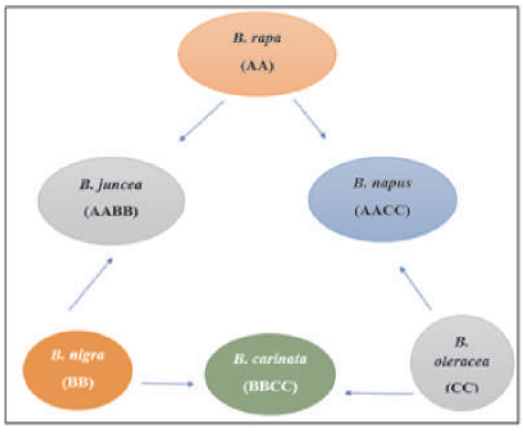

Figure 2: Brassica U's triangle

Cucumis hytivus

Cucumis hytivus (Cucumis hystrix × *Cucumis sativus)*, a novel synthetic Cucumis (Cucurbitaceae) species resulting from interspecific hybridization via embryo culture and chromosome doubling. It has many advantages, including resistance to root-knot nematodes, sticky stem blight, downy mildew, and low temperature and irradiance tolerance (Chen and Kirkbride 2000).

Raphanobrassica

Karpechenko (1927), developed Raphanobrassica viable hybrid (artificial alloploid) by crossing radish (*Raphanus sativus*, n=9) and cabbage (*Brassica oleracea,* n=9).The goal was to create a plant with radish roots and cabbage leaves. By accident, a fertile amphidiploid (4n=36) with cabbage roots and radish leaves was created by spontaneous chromosomal doubling (Bharadwaj 2015).

Polyploid onion

McCallum (1988), used colchicine to treat F_1 of *Allium cepa* × *Allium fistulosum* and reciprocal crosses, and found a C_2 population with good seedling vigour and cold hardiness at Beltsville during the winter, compared to normal diploids.

2. Bridge crossing

Another breeding approach that takes advantage of polyploids is bridge crossing. When ploidy levels induce sexual incompatibilities between two species, intermediary crosses can be used to produce fertile bridge hybrids, followed by chromosomal doubling. For onion breeding, *Allium fistulosum* has several beneficial agronomical features. Direct sexual hybridization of *A. fistulosum* for onion breeding is problematic. As a result, we investigated whether using *A. roylei* as a bridging species in a bridge cross could be a realistic alternative (Khrustaleva and Kik 1998).

3. Polyploids are employed for mutation breeding

Polyploids are utilized for mutation breeding because, despite their ability to withstand harmful cistron alterations after mutation, they require a higher mutation frequency due to their huge genomes, which result from the duplicated condition of their bountiful chromosomes (Gaul 1958). The high mutation

frequencies found in polyploids could be used to try to generate mutations in diploid cultivars that don't produce enough genetic variation when subjected to an agent treatment. This method has been used to generate mutants ofthe hot water plant species(nut orchids). It is the first to develop autotetraploid after being treated with colchicine and exposed to X-rays. During this research, autotetraploid were discovered to have a 20-40 times higher mutation frequency than diploid varieties with big genomes (Broertjes 1976).

4. Genetics effects

4a. Cell and body size changes

Polyploid cell sizes typically rise in tandem with genome doubling and increases in genetic resources. Plants may use a variety of ways to deal with the increased cell size that comes with polyploidy. Polyploid plants have the same number of cells as diploid plants, which allows them to grow larger organs and bodies (Mable 2004; Gregory and Mable 2005).

4b. Genomic Shift

The novel polyploid's primary distinguishing characteristics are genomic instability and fast recombination, in an endeavourto establish the peaceful coexistence of several genomes within one nucleus. Within five generations of artificial synthetic polyploid Brassica hybrids, for example, substantial genomic rearrangements and fragment loss were detected (Song 1995; Comai 2000; Chen *et al*. 2007).

4c. Gene expression changes in polyploids

Polyploids undergo alterations in gene expression, including gene silencing, up-regulation or down-regulation of expression, non-functionalization, sub-functionalization, and neofunctionalization, in addition to chromosomal structural changes. Both genetic and epigenetic processes are essential (Chen 2007; Song 2012).

4d. Diploidization

The allopolyploid generally undergoes diploidization to maintain stability following genome merging and doubling, to eliminate a wide spectrum of incompatibilities. Because homoeologous chromosomes may pair during meiosis, preventing the generation of functional gametes, allopolyploids frequently exhibit bivalent rather than multivalent chromosome pairing, indicating a diploid-like meiotic behaviour (Song 2012).

Disadvantages

Polyploidy alters the structure of the genome and the arrangement of cells. It imposes several significant constraints on cell cycle events (mitosis, meiosis), cell physiology (metabolism, growth), gene expression regulation, and genome stability.

Ploidy level measurement

Traditionally, meristematic tissue chromosomes (i.e., root tips during the metaphase phase of cell division) are counted to determine ploidy levels. There are numerous indirect approaches for determining ploidy: the number of chloroplasts in the stomatal guard cell can be counted. The length of the stomata can be measured (the longer the stomata, the higher the ploidy). Pollen grain diameter can also be used to determine ploidy levels. Flow cytometry has recently been the most widely used and accurate method. It determines the amount of nuclear DNA in plants (Pal and Bal 2020).

Table 2. The varieties of vegetable crops developed using polyploidy

Crop	Variety	Feature
Watermelon	Arka Madhura	TSS 13-14 percent, higher shelf life, and transit quality, appropriate for year-round cultivation under protected conditions, yields 50-60 t/hectare
Watermelon	Pusa Bedana	Aborted embryos and fake, rudimentary, barely discernible seeds characterize this seedless triploid hybrid.
Cassava	Sree Harsha	Plants are triploid and non-branching, yielding 35-40 t/ha and containing 39.05 percent starch.
Palak	Pusa Jyoti	Tetraploid with large, thick, soft, succulent dark green leaves, fast rejuvenation, and yields of 50 tonnes per hectare.

Conclusion

Polyploidy is a valuable tool to employ in breeding programs. Polyploidy causes plant gigantism, which is especially helpful for vegetative crops like potatoes, sweet potatoes, taro, and green vegetables. Polyploidy can be used to create new plant types with improved disease resistance, adaption, yield, and quality. With distantly related species, polyploidy helps to overcome fertilization obstacles. Triploidy is used to achieve seedlessness. Polyploidy is a widespread phenomenon in vegetable crops that has a significant impact on plant evolution.

Future prospects

Initially, polyploidy drew interest because of its distinct cytogenetics and reproductive isolation, but it was quickly discovered that polyploids also exhibited unusual phenotypic features and hybrid vigour, both of which are beneficial to agriculture. Polyploidy species that arise from heterozygous diploid progenitors may be a significant source of genetic variation. It is possible to generate new crops, transmit interspecific genes, and track the origins of crops. Despite the risk of displaying unwanted traits and the potential for a variety of obstacles, polyploidy breeding will unveil many of the plant's mysteries. In the realm of vegetable breeding, unveiling the evolution of crop plants and exploiting their variety is currently an intriguing research topic.

Conflict of interest: The authors declare no conflict of interest in relation to this research article.

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Status Paper

Beekeeping in Himachal Pradesh: way to an economic entrepreneurship

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Abstract

Beekeeping is considered as an ancillary activity and an alternate to farmer for increased farm income. It also has been regarded as a sole profession in stationary and migratory mode. Annual profit estimate from migratory unit of 100 colonies is about six lakh and seventy-five thousand per year. In contrary to generalized statement that beekeeping is a low input and high output profession, an effort to practically understand the economics, limiting factors and thereafter alternatives to make unit economically viable has been made. Among the limiting factors; various diseases and enemies, low honey price, high input cost and less profit. To sustain apiaries in state, a requirement of about 20.8 kg of dry sugar/honey bee colony/year has been worked out. To overcome these impediments, proper infrastructure, channelized methodology and enhanced awareness is needed. Skill based trainings would be instrumental in growth of beekeeping sector in the state and large-scale involvement of beekeeping enthusiasts.

Key words: Honey, hive products, beekeeper, honey bees, Himachal Pradesh, beekeeping

Scope of beekeeping in Himachal Pradesh

Himachal Pradesh is nestled in the lap of the western Himalayan Mountain range with a vast area under forest, horticulture, and agricultural crops. The state has a total geographical area of 55,673 km^2, forest cover of 15,433.52 km^2 (ISFR 2019). Of its total 10.3 lakh hectares of gross cropped area, 2.3 lakh hectares is under horticultural crops and an annual fruit production of 565.3 thousand metric tons. Most of these crops are insect-pollinated and require cross pollination. Beekeeping is an excellent pollination support to the fruit growers of the state besides providing outcomes to the beekeepers. Annual honey production in the state is about 5.85 thousand metric tons (NBB 2023). The earliest record of beekeeping in state was reported in 1882-1884 when Sir Louis Dane, kept honey bees in modern hives. The modern beekeeping in H. P. was introduced in 1934 in Kullu valley and in 1936 in Kangra valley (Sharma *et al*. 2022). Himachal is blessed with different agro-climatic conditions and crop diversity. Such diversity of geographical pockets allows varied flora and fauna to flourish and offer great potential for commercial beekeeping and vast scope to expand beekeeping as an entrepreneurial activity.

The state is a hub of beekeepers and known for its first-time successful establishment of *Apis mellifera* in India. Before 1971 there were only 1250 honey bee colonies in modern bee hives in Himachal Pradesh. The number of honey bee colonies maintained by beekeepers is on the rise and now has increased to 20.05 lakhs colonies in 2022 (NBB 2023). Diverse flora in different geographical pockets has tremendous potential for entrepreneurial and stationary beekeeping. Traditionally, the native bee, *Apis cerana* was utilized for beekeeping. *A. cerana* considered as natural heritage of mountain communities was reared in log, wall, pitchers, and box hives (Verma and Partap 1993). In Chamba district on an average 2.45 hives per house with hive occupancy rate of 53.94% has been

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documented that realizes the richness of this bee culture (Verma and Attri 2008). In another district Sirmaur where average annual production of honey remains between 1.72 to 3.38 kg per wall hive and on an average 2.92 colonies in wall hives per house with occupancy rate of 71.94% (Kumar and Thakur 2014). But recently *A. mellifera* beekeeping due to its suitability as a managed bee for honey production and efficient pollinator in crops is being preferred by beekeepers. It is therefore an increase of 15 per cent in honey production every year in the state has been documented (NBB 2023). Beekeeping constitutes a wholesome venture, as other than honey, bees also provide bees wax, bee pollen, propolis, bee venom and royal jelly.

For promotion of organic farming, the State Department of Agriculture intends to increase the area under organic agriculture and organic certification (approximately 2000 ha area) in all districts. State administration has also approved the organic policy for the state so the major emphasis be laid on the promotion of Organic Farming in the coming years. Multifloral and unifloral honeys produced in the state from different organic medicinal flora and from various geographical regions has a huge demand in the country. The most preferred honeys in market are; multi-floral Himalayan honey, litchi honey, wild thyme honey, white honey, and *Acacia* honey. Besides honey production, in recent years much attention is given to commercial utilization of honey bees as pollinators. Beekeeping for diversified hive products and maintaining colonies for efficient pollination in temperate horticultural crops can be regarded as an entrepreneurial activity for unemployed youth. Pollinators affect 35 per cent of the world's production (Roubik 1995; Delaplane and Mayer 2000) and honey bees are among the most exploited pollinators worldwide. Therefore, beekeeping industry is an excellent pollination support to the fruit growers of the state besides providing reaping outcomes to the beekeepers.

Progressive beekeepers intend to organic certification and proper processing for their honey and other hive products to do well in the domestic market and subsequently enter the export market. But few or none of farm certification and lack of processing infrastructures are the reasons for halt in the revenue and trade value of hive products. Honey marketing is major constraint that discourages the beekeepers as they are not aware of honey standards. About 65-70 per cent of the beekeepers have highlighted the problem of honey marketing and low price of their bee products (Kumar and Singh 2002; Bansal *et al*. 2013). The raw bulk honey produced by the beekeepers fails to meet the national and international standards. Non-fixation of minimum support prices for honey and unorganized market are the other bothersome limitations in the marketing of bee products. Also, there is no specific market for the sale of honey, and the beekeepers are selling their products mostly locally or in nearby areas without any brand name and are getting very less price Gatoria *et al*. (2003).

Honeybee predators, wasp species are the major limiting factor in the state desolate colonies up to 20 to 25 percent annually (Gulati and Kaushik, 2004). Wasp intercept honey bee at hive entrances and fly back to his nests carrying bee to feed their larvae Adlakha *et al*. (1975). Five wasp species namely; *Vespa mandarinia, V. auraria, V. orientalis, V. basalis* and *V. tropica* predate honey bees in rainy season. Maximum attack by *Vespa auraria, V. basalis* and V. mandarinia occurs during July to November in the mid hills zone of Himachal Pradesh when temperature and relative humidity remains high Rana *et al*. (2000). The beekeepers often tend to escape dwindling of their colonies due to wasp attack and make their way to plains.

Various insect pollinators coexist with honey bees include: solitary bees, bumble bees, and dipteran flies. Bumble bee, *Bombus haemorrhoidalis* has been commercially reared under laboratory conditions and used for pollination of some polyhouse crops that are otherwise not pollinated effectively by honey bees Dayal and Rana (2004). While foraging, on varied flora, these efficient creatures are also keeping alive many entomophilous and wild crops and helping in maintenance of ecological balance.

Economic model presented in the paper is

exclusively considered for the normal climatic conditions and with the normal yield and product values (Table 1). The economics may vary with the congenial climate prevailing during honey flow season, good price in the market and absence of natural calamities. This economic model would be helpful for beginners and the old beekeepers to redefine the management practices to maintain strong colonies for higher honey production and further colony multiplication.

Table 1. Economic modeling of Beekeeping to Profitability

		Number of colonies			
Capital costs (₹)		**10**	**50**	**100**	**500**
Bee Hives (Teak, Mango, Acacia) @ (₹) 2100/-*		21000	105000	210000	1050000
Bees @ (₹) 1750/- (Five frames)		17500	87500	175000	875000
Iron Hive stand @ (₹) 250/-		2500	12500	25000	125000
Essential equipment**					
Extractor capacity (8 kg) =₹ 8000/-, hive tool HT=₹ 200/-, bee veil BV=₹ 150, smoker ₹ =600/-, CFS =₹ 26/-piece, uncapping knife= ₹ =100, Tent=₹ ,10000 /- extraction net =₹ 6000/-, stainless steel honey filter=₹ 6000/-, stainless steel honey drums=₹ 2000/-, steel tub=₹ 200/-, glass bottles=₹ 32/- (1kg) and ₹ 17/- (500 g), labels and stickers=₹ 10/- a set, etc.					
		33250	104000	164300	753000
	Total	74250	309000	574300	2803000
Recurring Costs					
Depreciation (hives & other equipment excluding bees @10%		5675	22150	39930	192800
Labour (Full time @ ₹ 10000/- p.m.)		Self	1 Full Time 120000	1 Full time 120000	4 Full time 480000
Migration charges (to & fro to Rajasthan/ Haryana)		Stationary	30000	60000	180000
Other (sugar, feeders, gunny bags, medicines, etc.)	Sugar	20.80 kg/ col/yr	11.05 kg / col/yr	11.05 kg/ col/yr	11.05 kg/ col/yr
Sugar	@₹ 45	9360	24863	49725	248625
Feeder	@₹ 50	500	2500	5000	25000
Sulphur (@₹ 18.30/colony/year)	@₹ 48.3	483	2415	4830	24150
Gunny bags for winter packing	@₹ 12.5	125	625	1250	6250
Pollen traps	@₹ 200	1000	2000	6000	20000
Venom collector	@₹ 9800	-	9800	19600	39200
Propolis screens	@₹ 175	-	1750	3500	35000
Pollen substitute (min. 0.4 kg/ colony/year)	@₹ 50/kg	200	1000	2000	10000
Total expenditure		17343	217103	311835	1261025
Income from sale of colonies (end of first year)		3000	30000	75000	375000
Income from wax		-	1600	3200	8000
Honey production (kg)***		100 kg	2000 kg	4000 kg	20000 kg
Income from sale of honey (₹)**** 200/kg		100x 200=20000	4 lakh	8 lakh	40 lakh
Pollen production (kg)***		3 kg	15 kg	30 kg	150 kg
Income from sale of pollen(₹)**** 800/kg		2400	12000	24000	120000
Bee venom production (mg)***		350 mg	1750 mg	3500 mg	17500 mg
Income from sale of bee venom (₹)**** 10,000/g		3,500	17,500	35,000	1,75,000
Propolis production (kg)***		1kg	5kg	10 kg	50kg
Income from sale of propolis(₹)**** 1000/kg		1000	5000	10,000	50,000
Profit (₹)		12,557	2.49 lakh	6.35 lakh	34.6 lakh

* Cost of hive made of kail wood/superior timber wood in Himachal Pradesh

** 500 colony apiary require 5 times and equipment cost is also variable for small apiary

*** Honey yield 10 kg for stationary and 40 kg for migratory beekeeping (minimum/year)

**** Farmgate marketing of honey doubles the profit

Beekeeping as an option for Covid-19 migrants of Himachal Pradesh

Amid the crisis of COVID-19 pandemic, small farmers and migrants have been left stranded and rendered jobless. As per Himachal Pradesh Government, 94,819 migrants had gone out from Himachal to various states of the country by June 2020 and over 1.5 lakh have returned back to state (SDMA, 2020). To such homeless migrants and job seeking youth, beekeeping can be a safer job option in the lockdown. The migrant workers can be provided with bee boxes and the tool kit so that they become able to make a better livelihood through setting up small apiaries. In this way, the youth would no longer need to leave their home state in search of jobs.

Sustaining colonies in long dearth periods

Honey bee colonies need extra care during long winters and monsoon seasons. A study at Bee Research Station, Nagrota Bagwan was conducted to work out the prevailed floral dearth periods in mid hill zone of Himachal Pradesh and found that there was a dry period of 32 weeks. To supplement for honey or nectar, an artificial sugar syrup as feed an average strength single colony of honey bees require 0.650 kg of dry sugar in a week. A total of 20.8 kg of sugar is required during one year for one colony. We can shift our colonies in nearby area with *Acacia* plantation in July to August to have 5-week natural feed for colonies and extractable honey as well. Commercial *A. mellifera* beekeepers often migrate their colonies to plains on mustard and this makes them to avoid dwindling of colonies and artificial feed for 10 standard weeks.

Indian Beekeeping scenario

At present, there are about 1.934 million honeybee colonies of native *A. cerana* and exotic *A. mellifera* maintained in traditional and modern beehives in India. Honey production has increased from 76150 metric tons in the year 2013-2014 to about 133,000 metric tons in the year 2021-2022 (NBB 2022). India is one of the major exporters of raw honey to USA, Saudi Arabia, UAE, Nepal, Bangladesh etc. During 2015-16, an export of 38.2 thousand metric ton of honey valued[1] 706 crores while it was increased to 79,929.17 MT worth Rs. 23.3 billion in 2022 and is expected to grow at a CAGR of 8.4% to Rs 38.8 billion by 2028 (APEDA 2023). In honey production India ranks 2nd in world while China has its supremacy globally in honey production. As per an estimate, India has a potential to rear 200 million bee colonies that can provide self-employment to over 12 million rural and tribal families (Bhatnagar *et al*., 2020). It would also create opportunities to boost the output of bee related products and overall enhancement in agricultural and horticultural productivity.

Utilization of honey bees as pollinators looks to be imperative world over to increase crop productivity and restoring biodiversity. As per an estimate the value of honey bees as pollinator is 18-20 times more than their value as producers of honey and other hive products. In India crops benefitted by insect pollination are grown in an area of about 50 million hectares (Gupta and Gupta 1997). A few commercial beekeepers of Himachal are renting their bee apiaries @ of Rs. 1900/- per colony during 2022 for pollination in mustard crops in Gujarat.

Wasp and hornets are widely distributed and cause 30% losses globally, Asian hornet, *V. velutina auraria* has recently established in Europe and has alone been reported to cause losses up to 30 per cent Monceau *et al*. (2014). In India, wasps are considered most serious predators of honey bees which pose considerable threat to both the domesticated species *viz., A. cerana* and *A. mellifera* colonies (Abrol 1994).

Methodology: The survey was conducted in different districts of Himachal Pradesh namely, Kangra, Mandi, Kullu, Una, Chamba and information was collected from 10 beekeepers from each district. The data maintained at Beekeeping Research Station have contributed in developing this report for the benefit of entrepreneurs in Himachal Pradesh. Survey data from different locations in HP have been compiled and correlated. Status of beekeeping with established economics was analyzed to realize required changes. Various practical level factors have been considered for development of beekeeping enterprise in the state.

Limiting factors in expansion of beekeeping

Many restraints impair the development of beekeeping in the state. Bee mortality due to excess use

of pesticides in orchards and blooming crops cause losses to honeybees Kumar and Kundal (2016). Majority of beekeepers are not aware of scientific management during dearth periods and apiaries succumb to attack by various pests and predators. Pests like wax moths, wasps, mites, and diseases pose a significant threat to beekeeping (Sharma and Kumar 2011; Sharma *et al*. 2013; Thakur *et al*. 2021). In honey production, natural factors like bad weather are a major constraint, consistent inclement weather prevailing during build up and honey flow periods lead to failure of crop and extractable honey Singh *et al*. (2002). Migration of honey bee colonies is carried out by most of the beekeepers of the state during lean periods when bee flora is in scarce (Sharma *et al*. 2011). Migration of bee colonies to rich bee flora areas require a lot of manpower, high transport cost, mortality of bees during transportation and interference of highway police are other problems of beekeepers. Also, every year increasing cost of honey bee colonies, high cost of equipment particularly the hives is another limiting factor in its expansion.

Steps to minimize limiting factors

The impediments in the way of developing this activity as an entrepreneurial industry need conscious efforts at community level. The interest of young entrepreneurs through awareness programs needs to be developed by providing them with initial support and estimates of economical budgeting. Efforts should be made to promote and encourage small farmers for beekeeping, so that they may earn extra income along with farming. Establishing big regional nucleus apiaries in the potential areas for quality queen rearing of honey bees and establishment of honey processing and grading facilities is the need of the hour. Plantation of bee friendly flora at appropriate places and engaging women self help groups in their maintenance can be a viable option. These women groups can also be trained as local artisans for stitching bee veils and manufacturing other bee keeping equipment. Supply of bee colonies on rental basis from the registered beekeepers to the orchardists for pollination during flowering season may be ensured by the line department. Arrangements for marketing of honey produced by private bee keepers following good management practices with minimum support price should be the priority of the concerned department. Value of additional yield from pollination services by honey bees alone has been estimated about 15-20 times more than the value of all hive products put together. Beekeeping has been included as an activity for promoting cross pollination under National Horticulture Mission since May 2005 with a view that honey bee pollination increase yield up to 20 per cent and under this mission a provision is must for additional support to beekeeper against the additional revenue generated through crop yield.

Conclusion

Honey having several medicinal properties has rising demand in the nutraceutical, pharmaceutical and cosmetic industries. Honey processing is an important income avenue and it has emerged out as a multidisciplinary approach where small and marginal farmers can also opt its desired components to start their direct income. Valiant hive products other than honey, need careful modeling for their production so that they too can be incorporated in more profitable integrated beekeeping systems also bee flora rich sites must be harnessed for stationary apiaries (20-25 colonies) to get local honey and for pollination in integrated cropping system. In the present scenario, beekeeping would provide food & energy supplements as balanced nutrition and employment to youth affected due to COVID-19 by establishing apiaries. Therefore, there is an urgent need to frame suitable strategies for this emerging enterprise as a source of permanent income.

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Heterosis for fodder and seed yield traits in common oat (*Avena sativa* L.)

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Abstract

The manifestation of heterosis has brought an economic revolution to the agricultural production and seed sector in the last few decades. The present research was examined to estimate the comparative performance of newly developed F_1 crosses of oat with commercially used modern oat cultivars in North-western Himalayan regions. Forty hybrids were developed using 10 female and 4 male parental lines of oat during *Rabi*, 2021-22. These crosses were evaluated in Randomized Complete Block design with three replications along with 3 checks during *Rabi* 2022-23 at CSK Himachal Pradesh Agricultural University, Palampur. Thirteen seed and fodder yield traits were analyzed for heterosis. The oat hybrids showed heterotic superiority up to -9.50% for earliness, 36.79% higher number of leaves, 67.51% higher number of tillers and 44.38% increment in plant height over the best commercial check. For green fodder yield and dry matter yield, heterosis was observed up to 117.78% and 141.94%, respectively. The magnitude of heterosis for seed yield per plant over the best check HJ-8 ranged from -26.68% to 44.49%. Conclusively, based upon earliness, high seed yield, plant height and fodder yield per plant, five cross combinations viz., PO-1×JHO-851, OS-6×HFO-114, PO-1×UPO-212, JHO-813×JHO-851 and OS-377×JHO-851 were adjudged to be the best.

Keywords: Oat, Heterosis, Green fodder yield, Seed yield

Oat (*Avena sativa* L.) is one of the most important nutricereal fodder crop grown during *Rabi* season in many parts of the country including North Western, Central, and extending up to the parts of Eastern India (Kumar *et al*. 2022; Sood *et al*. 2022). In India, oat is cultivated in Himalayan states like Punjab, Haryana, Uttar Pradesh, Madhya Pradesh, Kashmir, Himachal Pradesh, some parts of Maharashtra and Uttarakhand (Kumar *et al*. 2022). Cultivation of oat offers numerous benefits, including nutritional value, versatility, adaptability to different growing conditions, and contributions to soil health and sustainability (Sanadya *et al*. 2023). These factors contribute to oats' status as an important cereal crop worldwide. Oats are a highly nutritious grain and are commonly incorporated into the human diet in various forms due to their versatility and health benefits. For human food oat groat is desired, which is high in protein, β-glucan and low in oil, whereas high oil and low β-glucan with the high protein is desired for livestock feed to maximize the energy (Peterson *et al*., 2005 and Sood *et al*., 2022). Ongoing research and innovations in oat breeding and agronomy could contribute to improved productivity and resilience in the face of environmental challenges (Sanadya *et al*., 2024).

Heterosis, commonly known as hybrid vigor, plays a crucial role in crop production. Exploitation of heterosis has been recognized as an essential tool in providing the breeders means for improving yield and related attributes of different crops (Sood *et al*. 2022). Oats are an essential cereal crop globally, valued for their nutritional content and versatility. Heterosis plays a vital role in oat crop improvement by enhancing yield potential, genetic diversity, disease resistance, grain quality, and overall stability. Incorporating hybridization techniques into oat breeding programs contributes to sustainable agricultural practices and ensures the continued success of oat cultivation in meeting global food and nutrition demands.

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Identification of parents and elite cross-combinations with high yield and other related traits is the major objective(s) of any breeding programme (Lata *et al*. 2023). Although heterosis being ubiquitous, it does not mean it occurs in every cross between two parents and also not necessarily always from good parents (Liu *et al*. 2021). Therefore, information on the extent of heterosis, is rather essential to identify potential cross combinations which can be further exploited through inter varietal hybridization programme (Kumar *et al*. 2022 and Rana *et al*. 2021). In oat, heterosis has been reported by several researchers *viz.,* Vishwakarma *et al*. 2010; Kapoor and Bajaj, 2013; Mishra *et al*. 2014 and Dumlupinar *et al*., 2015. Top of FormAnother important aspect from the practical point of view, which needs consideration, is the identification of potential cross combinations with respect to seed yield and its related traits and superior recombinants in the segregating generations based on their heterotic performance and combining ability (Sharma *et al*. 2007). Recently, a considerable attention has been paid to increase the yield potential by the possible use of heterosis in autogamous crops. Taking the importance of heterosis in consideration, the present research was undertaken to determine the heterotic potential of newly developed oat cross combinations.

Materials and Methods

The experiment comprised 40 F_1 hybrids developed by crossing 10 female lines with 4 male lines during *Rabi* 2021-22. The detail of parents and checks used in the present study is given in Table 1. These crosses along with three commercial checks *viz*., HJ-8, Kent and PLP-24 were evaluated in Randomized Complete Block design with three replications during *Rabi*, 2022-23.

Each entry was raised in two rows of 1.7 m length with 30 cm row to row and 10 cm plant to plant distance. One row was utilized for recording fodder yield while another one was used for seed yield. The crop was raised following the recommended package of practices under irrigated conditions. The location of experiment is situated in the mid-hill zone of Himachal Pradesh (Zone-II) and represents humid sub-temperate climate with high average annual rainfall (2500 mm per annum). Data were recorded for thirteen agro-morphological and fodder yield traits *viz.,* days to 50% flowering, number of leaves per plant, number of tillers per plant, flag leaf area (cm^2), leaf:stem ratio, plant height (cm), green fodder yield per plant (g), dry matter yield per plant (g), days to 75% maturity, biological yield per plant (g), seed yield per plant (g), harvest index (%) and 100-seed weight (g). Observations for days to 50% flowering and days to 75% maturity were recorded on plot basis while for remaining traits five randomly tagged plants were selected and averaged them for analysis and interpretation.

Data was analyzed to estimate of heterosis over best commercial check (selected based on mean performance) and tested the significance of heterosis for all traits studied. The 't' calculated values for heterosis over standard check (SC) were compared

Table 1. Oat genotypes used in the study

S. No.	Female Genotypes	Source	S. No.	Male Genotypes	Source
1.	OS-6	CCS HAU, Hisar	**1.**	PLP-1	CSKHPKV, Palampur
2.	RO-19	MPKV, Rahuri	**2.**	UPO-212	GBPUAT, Pantnagar
3.	PO-1	NBPGR, New Delhi	**3.**	HFO-114	CCS HAU, Hisar
4.	EC-608834	NBPGR, New Delhi	**4.**	JHO-851	IGFRI, Jhansi
5.	JPO-29	JNKVV, Jabalpur			
6.	JHO-813	IGFRI, Jhansi	**Checks**		
7.	RO-11-1	MPKV, Rahuri	**1.**	HJ-8	CCS HAU, Hisar
8.	OS-377	CCS HAU, Hisar	**2.**	Kent	PAU, Ludhiana
9.	OS-403	CCS HAU, Hisar	**3.**	PLP-24	CSKHPKV, Palampur
10.	TRS-106	NBPGR, New Delhi			

with 't' tabulated values at error degree of freedom at 5% of level of significance. The 't' calculated ≥ 't' tabulated values were marked significant and an asterisk (*) was put on per cent values. The standard heterosis was estimated as per the procedure suggested by Liang *et. al*. (1971).

Results and Discussion

The analysis of variance for the experimental design revealed that mean sum of squares due to genotypes were significant for all the thirteen agro-morphological traits. This indicated the presence of sufficient genetic variability among the studied genotypes (Table 2).

The estimation of heterosis would be useful to judge the best hybrid combinations for exploitation of superior hybrids (Al-Juhaishi *et al*. 2020; Garkoti and Pandey 2022). The heterosis responses in oat are not well defined. The difficulty of crossbreeding for producing large numbers of F_1 seeds prompted most researchers to compare the performance of the F_1 hybrid and their parents in space-grown populations (Murphy 1966). In the present study, three check varieties *viz.,* HJ-8, Kent and PLP-24 were used to calculate standard heterosis (economic heterosis) of forty newly developed oat crosses for seed and fodder yield traits. The values of heterosis (%) for all the studied traits are given in Table 3 and Figure 1.

The magnitude of heterosis for days to 50% flowering over the best check (PLP-24) ranged from -9.50% to 10.00%. The significant negative heterotic

Table 2. Analysis of variance for various agro-morphological traits

	Source of variance		Replication	Genotypes	Error
S. No.	Traits	d.f.	2	56	112
1.	Days to 50% flowering		9.8	111.56*	13.71
2.	Number of leaves per plant		31.03	1428.54*	26.27
3.	Number of tillers per plant		2.39	36.01*	0.65
4.	Flag leaf area		15.28	578.90*	8.56
5.	Leaf:stem ratio		0.0001	0.013*	0.001
6.	Plant height		1.6	1936.92*	10.38
7.	Green fodder yield per plant		12.87	11159.41*	136.87
8.	Dry matter yield per plant		1.58	759.03*	12.08
9.	Days to 75% maturity		93.81	118.65*	19.67
10.	Biological yield per plant		27.27	1878.26*	66.89
11.	Seed yield per plant		2.69	129.34*	7.1
12.	Harvest index		8.17	62.27*	12.61
13.	100 seed weight		0.001	1.76*	0.01

*** Significant at P d≤ 0.05**

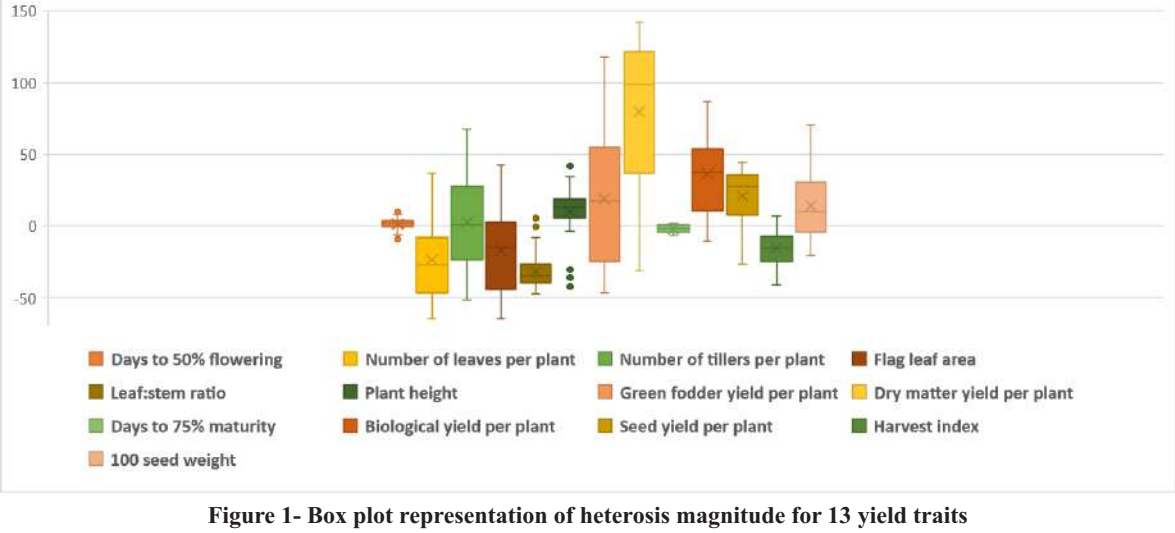

Figure 1- Box plot representation of heterosis magnitude for 13 yield traits

Table 3. Estimates of standard heterosis (%) over best check for various agro-morphological traits

Traits	DTF (PLP-24)	NOL (Kent)	NOT (Kent)	FLA (PLP-24)	LSR (Kent)	PH (PLP-24)	GFY (Kent)	DMY (Kent)	DTM (HJ-8)	BYP (HJ-8)	SYP (HJ-8)	HI (Kent)	SW (HJ-8)
OS-6×PLP-1	0.75	-65.10*	-50.89*	-3.42	-27.27*	-0.89	-16.48*	99.08*	-3.68	6.16	17.35*	3.98	41.74*
OS-6×UPO-212	-9.50*	23.88*	59.05*	9.52	-41.67*	18.89*	64.81*	126.24*	-3.86	1.85	6.40	-0.75	15.79*
OS-6×HFO-114	1.50	8.63	51.05*	-12.52*	-14.39*	16.79*	106.52*	139.07*	-5.33*	5.9	16.95*	4.96	40.26*
OS-6×JHO-851	8.25*	36.79*	67.51*	0.50	-31.06*	12.46*	71.38*	130.09*	-2.57	35.62*	28.25*	-11.17	2.59
RO-19×PLP-1	2.50	-28.73*	-1.82	-9.66	-47.73*	18.69*	-40.78*	3.14	-4.78*	5.47	-26.68*	-34.70*	4.99*
RO-19×UPO-212	3.75	-9.20	-6.03	-37.92*	-25.00*	17.78*	37.59*	116.30*	-4.41*	-4.83	-14.40	-15.40*	-4.25
RO-19×HFO-114	6.25*	27.98*	52.56*	-43.30*	-43.18*	25.68*	76.48*	141.94*	0.00	2.88	-14.40	-21.70*	-4.71*
RO-19×JHO-851	7.50*	-7.57	18.22*	-12.71*	-39.39*	8.66*	58.91*	127.35*	-0.55	46.95*	38.23*	-11.46	-20.50*
PO-1×PLP-1	1.75	-11.85*	18.25*	27.56*	-35.61*	44.38*	68.92*	134.92*	-5.51*	74.66*	33.74*	-27.91*	9.23*
PO-1×UPO-212	4.00	-20.62*	-12.16*	-8.13	-40.15*	26.90*	-26.76*	73.98*	-6.25*	51.77*	41.15*	-12.79	1.20
PO-1×HFO-114	0.25	-60.52*	-45.44*	-36.04*	-26.52*	5.62*	-35.31*	33.81*	-6.07*	45.75*	21.51*	-21.58*	-10.34*
PO-1×JHO-851	2.25	27.20*	39.41*	42.57*	-47.73*	32.22*	88.50*	134.10*	-4.04	86.70*	44.49*	-27.00*	18.28*
EC608834×PLP-1	1.00	14.04*	48.53*	-38.95*	-32.58*	20.06*	48.04*	107.41*	-2.94	60.80*	40.04*	-17.33*	3.79
EC608834×UPO-212	-6.75*	-16.78*	25.04*	-54.07*	-45.45*	13.22*	32.88*	119.26*	-1.47	55.64*	37.30*	-16.64*	-20.22*
EC608834×HFO-114	0.25	-35.53*	-27.25*	-47.84*	-34.09*	14.44*	28.09*	99.93*	1.65	27.33*	-0.59	-26.34*	4.71*
EC608834×JHO-851	-1.00	-26.32*	-18.16*	-20.07*	-35.61*	15.07*	37.17*	97.65*	1.29	38.18*	18.36*	-19.25*	-15.79*
JPO-29×PLP-1	-6.75*	-26.32*	4.61	-50.88*	-21.21*	34.50*	-33.36*	-6.31	-2.21	33.78*	-5.08	-32.24*	-5.17*
JPO-29×UPO-212	-4.50*	-35.09*	12.12*	-56.54*	-37.12*	41.72*	-20.60*	35.85*	-0.92	62.61*	28.65*	-24.74*	23.18*
JPO-29×HFO-114	10.00*	4.61	31.86*	-16.30*	-35.61*	-3.50	5.59	51.51*	1.10	-3.31	9.90	6.65	-11.54*
JPO-29×JHO-851	-3.75	-18.42*	36.44*	-49.75*	-38.64*	-3.64	-2.14	112.38*	-0.18	54.34*	10.98	-32.41*	-7.02*
JHO-813×PLP-1	1.50	-42.54*	-30.25*	-16.69*	-10.61*	9.80*	-38.93*	88.04*	0.55	34.75*	34.78*	-5.99	5.91*
JHO-813×UPO-212	2.50	-22.37*	4.61	20.31*	-40.15*	10.18*	48.93*	110.36*	-0.18	31.35*	38.58*	-0.26	60.30*
JHO-813×HFO-114	3.25	-23.68*	11.37	-44.66*	-33.33*	-1.98	38.93*	102.47*	1.29	80.68*	31.71*	-31.23*	70.27*
JHO-813×JHO-851	-3.50	-20.06*	2.33	-2.59	-41.67*	14.49*	38.60*	95.29*	-4.41*	36.33*	29.71*	-10.71	16.53*

RO-11-1×PLP-1	1.00	-58.78*	-51.53*	-42.13*	-36.36*	8.29*	8.00	75.20*	0.18	-5.37	-1.22	-1.81	6.93*
RO-11-1×UPO-212	2.50	-39.47*	-27.25*	-60.95*	-46.21*	11.89*	14.1	113.59*	1.29	30.72*	-17.75*	-40.93*	-7.48*
RO-11-1×HFO-114	3.75	-58.22*	-27.25*	-36.64*	-34.85*	21.58*	-44.24*	-11.26	0.55	58.03*	31.29*	-20.89*	45.89*
RO-11-1×JHO-851	6.00*	-47.37*	-9.03	-38.46*	-25.76*	13.22*	20.54*	90.06*	0.00	68.63*	39.81*	-21.68*	-1.94
OS-377×PLP-1	-6.25*	-59.64*	-24.25*	-65.22*	-28.03*	5.24*	-46.57*	-31.27*	-6.43*	38.73*	35.50*	-8.01	-6.56*
OS-377×UPO-212	-8.25*	-35.52*	4.61	-48.56*	-40.15*	13.22*	-45.28*	-16.81	-3.49	43.53*	26.55*	-17.13*	10.53*
OS-377×HFO-114	2.50	-39.47*	-9.06	-55.64*	-41.67*	3.57	-13.14	39.01*	-4.41*	23.24*	10.94	-15.25*	30.75*
OS-377×JHO-851	4.25	30.26*	54.59*	32.07*	-34.09*	18.35*	117.78*	136.37*	-7.17*	69.73*	26.62*	-29.86*	10.06*
OS-403×PLP-1	2.75	-44.73*	-27.28*	2.75	-30.30*	16.43*	56.20*	116.60*	-6.07*	42.68*	14.15	-24.69*	12.47*
OS-403×UPO-212	2.5	15.79*	28.22*	-4.18	-39.39*	14.44*	8.02	90.93*	-5.15*	4.57	0.77	-9.49	11.17*
OS-403×HFO-114	2.75	-43.42*	-15.85*	37.68*	-40.91*	12.72*	74.68*	124.49*	0.55	33.28*	31.32*	-7.70	42.66*
OS-403×JHO-851	0.25	-30.26*	9.12	27.44*	-32.58*	11.33*	51.53*	122.66*	1.29	30.28*	34.74*	-2.97	45.71*
TRS-106×PLP-1	-0.75	-47.37*	-18.13*	22.52*	-8.33	-36.45*	14.49	97.58*	-4.04	-10.37	2.12	6.89	30.01*
TRS-106×UPO-212	0.75	-54.60*	-13.64*	-13.24*	-15.15*	-42.25*	-19.94*	28.95*	-0.18	52.38*	38.20*	-14.71	30.47*
TRS-106×HFO-114	1.75	-47.36*	-21.16*	23.64*	5.30	-30.47*	-43.06*	-26.90*	1.1	50.57*	42.03*	-11.6	44.04*
TRS-106×JHO-851	6.00*	-60.52*	-45.44*	-3.3	-0.76	-39.82*	-43.09*	-28.63*	0.55	48.63*	33.81*	-15.37*	36.01*
SE ±	2.08	3.02	0.45	1.74	0.01	1.89	7.16	2.14	2.62	5.01	1.63	2.01	0.06

DTF: Days to 50% flowering, NOL: Number of leaves per plant, NOT: Number of tillers per plant, FLA: Flag leaf area, LSR: Leaf:stem ratio, PH: Plant height, GFY: Green fodder yield per plant, DMY: Dry matter yield per plant, DTM: Days to 75% maturity, BYP: Biological yield per plant, SYP: Seed yield per plant, HI: Harvest index, SW: 100 seed weight

* Significant at P d" 0.05; check variety in parenthesis represent the best check for the particular trait.

values are desirable for this trait showcasing the genotypes' ability for early flowering. Six cross combinations *viz.,* OS-6×UPO-212 (-9.50%), OS-377×UPO-212 (-8.25%), EC608834×UPO-212 (-6.75%), JPO- 29×PLP-1 (-6.75%), OS-377×PLP-1 (-6.25%) and JPO-29×UPO-212 (-4.50%) exhibited significant and desirable negative heterosis. The range of heterosis for number of leaves per plant over check variety Kent was -65.10% to 36.79%. Seven cross combinations *viz.,* OS-6×JHO-851 (36.79%), OS-377×JHO-851 (30.26%), RO-19×HFO-114 (27.98%), PO-1×JHO-851 (27.20%), OS-6×UPO-212 (23.88%), OS-403×UPO-212 (15.79%) and EC608834×PLP-1 (14.04%) exhibited significant and desirable positive heterosis value over Kent.

For number of tillers per plant it was ranged from -51.53% to 67.51% over the best check i.e. Kent. Fourteen cross combinations *viz.,* OS-6×JHO-851(67.51%), OS-6×UPO-212 (59.05%), OS-377×JHO-851 (54.59%), RO-19×HFO-114 (52.56%), OS-6×HFO-114 (51.05%), EC608834×PLP-1 (48.53%), PO-1×JHO-851 (39.41%), JPO-29×JHO-851 (36.44%), JPO-29×HFO-114 (31.86%), OS-403×UPO-212 (28.22%), EC608834×UPO-212 (25.04%), PO-1×PLP-1 (18.25%), RO-19×JHO-851 (18.22%) and JPO-29×UPO-212 (12.12%) showed significant and desirable positive heterosis for this character. The range of heterosis for flag leaf area over the best check i.e. PLP-24 was -65.22% to 42.57%. Highest value of heterosis for this trait was recorded for cross PO-1×JHO-851 (42.57%), followed by OS-403×HFO-114 (37.68%), OS-377×JHO-851 (32.07%) and PO-1×PLP-1(27.56%).

The range of heterosis for leaf:stem ratio over the best check i.e. Kent was recorded from -47.73% to 5.30%. None of the cross combination exhibited significant and desirable positive heterosis value over Kent. For plant height, it was ranged from -42.25% to 44.38%. Thirty-one cross combinations exhibited significant and desirable positive heterosis value over PLP-24. The cross PO-1×PLP-1 (44.38%) showed highest heterosis for plant height, followed by JPO-29×UPO-212 (41.72%), JPO-29×PLP-1 (34.50%) and PO-1×JHO-851 (32.22%).

Green fodder yield per plant is an important character to increase the fodder productivity. The extent of heterosis for green fodder yield per plant over the best check Kent ranged from -46.57% to 117.78%. Twenty cross combinations found significantly higher heterosis value for this trait. The cross OS-377×JHO-851 (117.78%) exhibited highest value of heterosis, followed by OS-6×HFO-114 (106.52%), PO-1×JHO-851 (88.50%) and RO-19×HFO-114 (76.48%). The heterosis for dry matter yield per plant over the best check i.e. Kent ranged from -31.27% to 141.94% (RO-19×HFO-114). Thirty-three cross combinations exhibited significant and desirable positive heterosis value over best check Kent. The study also compared heterosis values with previous research findings by Kapoor and Singh (2017) and Chauhan *et al*. (2018) which reported positive heterosis for green fodder yield and dry matter yield, corroborating the current findings.

For days to 75% maturity, it was ranged from -7.17% to 1.65% over HJ-8. Twelve combinations *viz*., OS-377×JHO-851 (-7.17%), OS-377×PLP-1 (-6.43%), PO-1×UPO-212 (-6.25%), PO-1×HFO-114 (-6.07%), OS-403×PLP-1 (-6.07%), PO-1×PLP-1 (-5.51%), OS-6×HFO-114 (-5.33%), OS-403×UPO-212 (-5.15%), RO-19×PLP-1 (-4.78%), RO-19×UPO-212 (-4.41%), JHO-813×JHO-851 (-4.41%) and OS-377×HFO-114 (-4.41%) exhibited significant and desirable negative heterosis value over HJ-8. The value of heterosis for biological yield per plant over the best check i.e. HJ-8 ranged from -10.37% to 86.70%. Thirty cross combinations showed significant higher value for this character. The cross PO-1×JHO-851 (86.70%) exhibited highest heterosis, followed by JHO-813×HFO-114 (80.68%), PO-1×PLP-1 (74.66%) and OS-377×JHO-851 (69.73%).

The extent of heterosis for seed yield per plant over the best check i.e. HJ-8 ranged from -26.68% to 44.49%. Twenty-six cross combinations exhibited significant and desirable positive heterosis and maximum value was recorded for cross PO-1×JHO-851 (44.49%), followed by TRS-106×HFO-114 (42.03%), PO-1×UPO-212 (41.15%), EC608834×PLP-1 (40.04%) and RO-11-1×JHO-851 (39.81%). Vishwakarma et al. (2010) and Rana et al. (2021) also reported almost similar range of standard heterosis for this trait.

None of the cross combination exhibited significant and desirable positive heterosis value over Kent for harvest index. The range of heterosis for

harvest index was ranged from -40.93% to 6.89%. For 100 seed weight, twenty-four cross combinations exhibited significantly higher magnitude of heterosis over best check HJ-8. It was ranged from -20.50% to 70.27% for this character. The cross JHO-813×HFO-114 (70.27%) showed highest value, followed by JHO-813×UPO-212 (60.30%), RO-11-1×HFO-114 (45.89%) and OS-403×JHO-851 (45.71%). Thukral and Verma (2003) while studying heterosis in five crosses of oat observed that the range of heterosis varied substantially among various crosses and characters. Overall, based on the superiority over the best check varieties, five cross combinations *viz.,* PO-1×JHO-851, OS-6×HFO-114, PO-1×UPO-212, JHO-813×JHO-851 and OS-377×JHO-851 were desirable for earliness, seed yield, fodder yield, and plant height, suggesting their potential utility in future oat improvement programs. These findings contribute to the understanding of hybrid vigor in oat breeding programs and offer potential avenues for improving productivity and performance in oat cultivars.

Conclusion

The exploitation of heterosis is extremely effective method for the genetic improvement of different traits. Any of a multitude of genetic phenomena known to influence qualitative or quantitative characters is expected to influence heterosis but over the years that dispersion of completely or incompletely dominant genes and over-dominance along with some contributions of non-allelic interactions have been the main causes of heterosis. In the present study, the magnitude of heterosis in studied traits was recorded up to 141.94%, indicated the importance of heterosis in future breeding programs. Many genotypes showed the trait specific enhancement for heterosis value providing the scope to study the genetic inheritance pattern of individual trait in oat. Based upon earliness, high seed yield, plant height and fodder yield per plant, five cross combinations *viz*., PO-1×JHO-851, OS-6×HFO-114, PO-1×UPO-212, JHO-813×JHO-851 and OS-377×JHO-851 were found promising in the present investigation can be evaluated in multi-location trials and further exploited through heterosis breeding. The identified cross combinations also offer promising avenues for oat improvement and warrant further investigation and utilization in breeding programs aiming to enhance oat productivity and resilience.

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Comparative efficiency of randomized complete block design versus alpha lattice design in wheat breeding experiments

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Abstract

Evaluating large number genotypes in complete block design is impractical due to the need for all treatment combinations in each block, which increases experiment size and reduces accuracy. To minimize errors and enhance the precision and effectiveness of breeding programs, a high-quality experimental design is essential. To address this limitation, the current experiment was conducted to evaluate the suitability of alpha lattice design (ALD) compared to randomized complete block design (RCBD) in plant breeding trials. In this study, three hundred six wheat genotypes were sown in ALD of 17×18, consisting of 17 blocks with 18 genotypes each, replicated in triplicates under both irrigated and rainfed conditions. The study estimated two traits: the number of grains per spike and grain yield per plant, using data from both environments. These traits were then analyzed using both RCBD and ALD to evaluate the efficiency of ALD in comparison to RCBD. The relative efficiency based on grain yield per plant under both trials indicates that ALD was more efficient than RCBD. A relative efficiency close to one for the number of grains per spike could be attributed to the large number of entries evaluated in the RCBD. The anomalies regarding low EMS in the RCBD trial instead of the ALD could potentially be explained by this factor. Therefore, in field experiments with many entries, ALD should be preferred over RCBD.

Keywords- Wheat, Alpha lattice design, RCBD, Breeding programs, Relative efficiency

Bread wheat (*Triticum aestivum* L., 2n=6x=42) is the major cereal crop across the globe. It caters as a staple food for a large population worldwide, particularly in West Asia, North Africa and Europe. According to the FAO (2022), India ranks second in terms of global wheat production, accounting for 13.3% after China at 17%. Wheat plays a pivotal role in global food security. The grains are mainly composed of carbohydrates (~70-80%) and proteins (~8-22%) (Slafer *et al.* 2021). Wheat can grow in diverse climate, water and soil regimes. The growing global population, shrinking arable land, and shifts in climate and precipitation patterns make addressing drought stress more urgent (Valizadeh *et al.* 2014; Trenberth 2011). Wheat production in semiarid and arid regions faces constraints from drought stress, making the selection of high yielding drought-tolerant cultivars crucial for rainfed conditions.

Breeders often conduct field trials to select the best entries, choosing the optimal design and analysis method based on the population's genetic architecture. In the last few decades, the development and adoption of new experimental designs have grown significantly due to expanding applications, statistical complexity and appeal they offer. It is evident that newly originated agricultural field experimental designs were inspired by applications across a wide range of experimental studies (Hinkelmann and Kempthorne 2005). One of the key principles of experimental design is the minimizing experimental error. In field experimentation, the randomized complete block design (RCBD) is commonly used because it applies randomization, replication, and local control, dividing experimental units into homogenous blocks and ensuring each block contains a complete replication (Gupta *et al*. 2016). One disadvantage of RCBD is that

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it is only suitable for 25-30 genotypes per block due to heterogeneity within larger blocks. However, most plant breeding trials involve many entries, making incomplete blocking designs, such as an alpha lattice design (ALD), a more effective choice (Williams *et al*. 2002; Patterson and Williams 1976). Lattice designs, including balanced and partially balanced incomplete block designs, were developed for large-scale agricultural trials to compare large number genotypes with greater accuracy (Yates 1936), balanced designs often require many replications. The results suggest replacing RCBD with ALD when there are more than ten treatments, as ALD provides better control of experimental variability and improves precision by reducing mean square error, coefficient of variation, and standard error of difference.

Globally, breeding and field trials now commonly employ alpha designs due to their flexibility and capacity to accommodate any number of varieties. They are recommended for trials with large numbers of genotypes on variable soil, especially when variability between lines is very less. ALD are popular in wheat breeding for their adaptability with entry numbers, block size, and error control (Kumar *et al*. 2020). They can also handle situations where the entry count isn't an exact multiple of block size by omitting treatments. Multi-environment replicated trials are essential in crop improvement programs for evaluating numerous entries and identifying the best performers. Therefore, this study aimed to compare the efficiency of alpha lattice design (ALD) with randomized complete block design (RCBD) in evaluating grain yield and related components in wheat genotypes.

Materials and methods

Experimental design

The experimental material comprised of 306 diverse wheat genotypes including doubled haploids, landraces from North-western Himalayas, exotic lines and popular Indian cultivars. The experiment was conducted at the Experimental Farm of the Department of Genetics and Plant Breeding, CSK HPKV, Palampur, during the *rabi* season of 2021-22 in irrigated vs. rainfed trials with three replications. Employing the Alpha lattice design (Patterson and Williams 1976), randomization of 306 cultivars was conducted using PB tools software. The experimental material was assessed for various morpho-physiological traits within an ALD framework of 17×18, comprising 17 blocks with 18 genotypes each, replicated in triplicates for each trial. In RCBD, each treatment appears once in every block, making the number of treatments equal to the block size. Additionally, as each block serves as a complete replication, the number of blocks equals the number of replicated treatments.

Each entry was planted in a 1meter-long row, with two rows per plot, with the intra and inter-row spacing of 10 cm and 20 cm respectively. Data was recorded for two agro-morphological traits: Grains per spike (GPS) and Grain yield per plant (g) (GY). The data collected were analyzed using the "agricolae" package of R statistical software (R Core Team 2020) in both RCBD and ALD. The linear mathematical model of ALD is:

$$y_{iju} = \mu + \tau_i + \beta_i + e_{iju}$$

(y_{iju} is the response of variable; μ is the general mean effect; τ_i is the effect of the i^{th} treatment; β_j is the effect of the j^{th} block; e_{iju} are uncorrelated random error components with response)

Estimation of efficiency of ALD versus RCBD

The comparative effectiveness of the ALD in contrast to the RCBD was assessed using the error mean square (EMS) and coefficient of variation (CV) from each analysis, as per the following equations:

Relative Efficiency (RE) = (EMS for RCBD) / (EMS for ALD)

Relative Efficiency (RE) = (CV for RCBD) / (CV for ALD)

If the value RE > 1, it indicates that the ALD is more efficient than the RCBD (Masood *et al*. 2008), if RE ≈ 1, it suggests that both designs yield similar results, while RE< 1 indicates that the RCBD is a more efficient.

Results and Discussion

Analysis of variance for RCBD

The ANOVA (RCBD) for individual and the pooled analysis of the studied traits are presented in Tables 1 and 2, respectively. The mean square due to replications showed significant differences for GPS and GY in both environments and pooled across environments.

Homogeneity of variances for both traits was indicated by Bartlett's test, thus enabling a pooled

ANOVA to be performed. The mean square due to environment in the pooled data revealed highly significant differences for both traits. The highly significant genotypic differences among GPS and GY in both environments and the pooled environment. Consequently, the germplasm can help identify genotypes with high potential for GPS and GY.

The large number of entries evaluated in the RCBD, can lead to higher variability within blocks and anomalies in data analysis regarding low EMS.

Analysis of variance (alpha lattice design)

The ANOVA (alpha lattice design) for individual and the pooled analysis of the studied traits are presented in Tables 1 and 2, respectively. The mean square of the blocks (replication) did not show significant differences for both GPS and GY across all individual and pooled environments. The mean square due to replications showed significant differences for GPS and GY in both the environments. Similar with the RCBD, highly significant genotypic differences were found for both GPS and GY in both environments and across pooled environment, suggesting that the germplasm pool in this study provides a rich source of genetic diversity. This can be useful for identifying genotypes with high GPS and GY potential.

The mean square of the environment in the pooled data exhibited highly significant differences for both traits. In the pooled data, mean square due to environment revealed significant differences for both the traits. A significant replication and environment interaction was observed for the trait GPS. While, Genotype × Environment interaction was highly significant for GY but not significant for GPS. This suggests that there was wide range of genetic

Table 1. Analysis of variance (RCBD and Alpha lattice design) for studied traits in wheat in irrigated vs. rainfed trials

Sources of variation	df	Irrigated		Rainfed	
		Grains per spike	Grain yield per plant	Grains per spike	Grain yield per plant
Randomized Complete Block Design					
Replication	2	1395.36*	15.14*	475.22*	10.56*
Genotypes	305	72.93*	9.96*	77.73*	4.91*
Error	610	29.3	1.13	19.34	0.40
Alpha Lattice Design					
Replication	2	1395.36*	15.14*	475.22*	10.56*
Blocks (Replication)	48	21.28	1.28	20.04	0.30
Genotypes	305	72.78*	9.38*	74.89*	4.64*
Error	562	29.6	1.07	19.28	0.40

Table 2. Pooled Analysis of variance (RCBD and Alpha lattice design) for studied traits in wheat in irrigated vs. rainfed trials

Sources of variation	df	Grains per spike	Grain yield per plant
Pooled RCBD			
Replication (environment)	2	1733.90*	33.00*
Genotypes	305	103.90*	11.04*
Environment	1	2,313.57*	169.21*
Pooled Error	610	24.54	0.75
Pooled ALD			
Replication (environment)	2	1686.40*	25.18*
Block	16	23.80	0.86
Genotype	305	135.60*	12.88*
Environment	1	9106.70*	1415.50*
Replication × Environment	2	184.20*	0.52
Genotype × Environment	305	15.10	1.57*
Residuals	1124	24.60	0.73

variations among studied genotypes and environments for GY, with varying responses to different environments. These findings indicate that the studied wheat germplasm responded to G × E interaction across different environments.

Relative efficiency of ALD versus RCBD

Relative efficiency of ALD versus RCBD (Table 3) was recorded RE ≈ 1 for GPS suggests that both designs yield similar results, while higher values for error mean square (EMS) for GY in irrigated environment (1.06) and pooled environment (1.03), indicating that the ALD is more efficient than the RCBD. Whereas for relative efficiency based on coefficient of variation (CV) was observed RE ≈ 1 for GPS and higher for GY in irrigated environment (1.06) and pooled environment (1.01) which indicate that analysis in ALD resulted in reducing the experimental error and thus enhancing the capability of the researcher to detect significant differences among the 306 wheat genotypes. The lower CV values under rainfed trial over irrigated for both the studied traits, suggest that rainfed conditions exhibit reduced variability, which may be attributed by the broader range of grain yields observed under irrigated environments, indicating the full expression of genetic potential of the genotypes. A relative efficiency close to one for the GPS can be associated with the large number of genotypes evaluated in the RCBD. The unexpected occurrence of low EMS in the RCBD trial, compared to the ALD, may potentially be accounted for by this factor.

The results are consistent with the findings of Abd El-Mohsen and Abo-Hegazy (2013); Idrees and Khan (2009); and Masood *et al*. (2006). In a study by Priyanka *et al*. (2023), sixty-six bread wheat varieties were evaluated in alpha-RBD with three replications, finding significant differences for all studied traits over environments. Sood *et al*. (2021) and Thakur & Sharma (2023) also evaluated black gram and cauliflower breeding material, respectively for yield and related traits in RCBD. Sanadya *et al*. (2022) evaluated 98 (12×8) genotypes in ALD over two consecutive years (2019-2021) for green forage and seed yield per plant, found ALD more effective than RCBD and pooled data varied significantly due to genotype-environment interaction. The relative efficiency of ALD studied by Kumar *et al*. (2020) indicated that these designs were more effective than the RCB design.

The study revealed that using alternative experimental designs leads to significant improvements when working with a large number of genotypes displaying notable variability. Statistical analysis of grain yield per plant data showed that the RCBD was less effective than the alpha lattice design ALD, resulting in lower experimental accuracy. Moreover, the experimental designs utilized fewer plots compared to the traditional design of randomizing entire blocks. The relative efficiency based on EMS and CV in the pooled data increased precision for grain yield per plant, leading to better experiment management. These results indicate that ALD is more suitable for wheat experimental trials than the conventional RCBD, making it a more effective approach for agricultural research.

Conclusion

An irrigated vs. rainfed trial was carried out to compare the efficiency of ALD over RCBD in enhancing wheat breeding experiments by reducing experimental error. This was accomplished by

Table 3. Relative efficiency (RE) of Alpha lattice design over RCBD for studied traits in wheat in irrigated vs rainfed trials

Traits	Irrigated			Rainfed			Pooled		
	RCBD (EMS)	ALD (EMS)	RE	RCBD (EMS)	ALD (EMS)	RE	RCBD (EMS)	ALD (EMS)	RE
Grains per spike (EMS)	29.3	29.6	0.99	19.38	19.28	1.01	24.54	24.6	1.00
Grains per spike (CV)	8.78	8.89	0.99	7.69	7.68	1.00	8.34	8.35	1.00
Grain yield per plant (EMS)	1.13	1.07	1.06	0.4	0.4	1.00	0.75	0.73	1.03
Grain yield per plant (CV)	19.72	18.54	1.06	16.45	16.61	0.99	18.46	18.21	1.01

minimizing soil heterogeneity through smaller block sizes and adjusting the mean performance of each treatment within blocks. Additionally, notable genotypic differences were observed among genotypes, indicating that the studied germplasm may offer substantial genetic diversity for future breeding programs. In multi-environment crop field experiments for assessing economic traits involving many entries, ALD should be preferred over RCBD.

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Genetic diversity of tartary buckwheat (*Fagopyrum tataricum* Gaertn.) genotypes based on cluster and principal component analyses in Organic Agriculture

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Abstract

A total of 24 accessions of tartary buckwheat with some local germplasm were analyzed by cluster and principal component using 17 quantitative characters to group them on the similarity at phenotypic level, and to identify the most relevant characters in explaining the variation. The genotypic variance of all the traits was lower than the phenotypic variance. Highest GCV and PCV were recorded for seed yield per plant, seed per plant and protein content. The non-hierarchical Euclidean cluster analysis using Mahalanobis statistic, grouped the genotypes into five distinct clusters among which two clusters were polygenotypic and three clusters were monogenotypic. Cluster I was the largest group comprising of 15 genotypes characterized with highest primary branch per plant, inflorescence length, leaf per plant, seed per plant, seed yield per plant, magnesium content, iron content and zinc content. Most of the high yielding germplasm along with Himpriya were grouped under this cluster. Cluster II composed of the genotypes having high amount of protein, calcium and phosphorus that can be used as a source for introgression of genes responsible for high nutrition. The results of PCA confirmed the findings of cluster analysis. Six principal components having eigen value greater than one, accounted for nearly 78.58% of the total variation. According to eigen vector analysis, the observed variation for first, second, third, fourth, fifth and sixth principal component were about 26%, 15%, 12%, 10%, 8% and 6%, respectively. In the first principal component, number of seed per plant and seed yield per plant were the most contributing traits whereas days to maturity, calcium and iron were the principal traits of the second principal component.

Key words: *Fagopyrum*, variation, multivariateanalysis, micronutrients

Buckwheat (*Fagopyrum* spp.) contains more than 18 species and among them, two species *i.e.*, Common buckwheat (*Fagopyrum esculentum* Moench, 2n = 2x = 16) and Tartary buckwheat (*Fagopyrum tataricum* Gaertn., 2n = 2x = 16) are being utilized for food and feed (Campbell 2003). Grain yield of tartary buckwheat is high and stable due to its self-compatibility, low seed abortion and tolerance to stresses such as frost (Campbell 1995). In addition to higher grain yield of tartary buckwheat, it is nutritionally and medicinally considered superior to common buckwheat and other cereals. Due to its short growing period and cold tolerance, it is suitable to cultivate in high hills, where summer season is short. However, most of the landraces of tartary buckwheat have a tightly adhering husk, low grain yield and bitter components. In mountain areas of Himachal, farmers are maintaining different landraces of tartary buckwheat (also called mountain buckwheat), some of which are unique e.g., *Bhate Phaper* (rice Tartary buckwheat) which has a non-adhering hull (Bhardwaj and Kaur 2020).

Despite its importance, area under its cultivation has decreased over years, primarily due to its

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replacement with more remunerative cash crops likeapple, green pea, hops and potato apart from non-availability of improved varieties having high yield potential, quality flour and resistance to biotic and abiotic stresses (Joshi and Rana, 1997).Therefore, breeding work is necessary to increase their productivity. To initiate the breeding work, it is important to group the large number of diverse accessions based on the multiple characters. Characterization, systematic documentation and conservation of germplasm are important for its utilization in crop improvement programmes (Sekhon *et al*. 2019; Singh *et al*. 2024).

Therefore, under the present investigation, local germplasm of Tartary buckwheat (*F. tataricum*) from higher northern hilly region of the Himalayas along with a few important exotic and other indigenous accessions were characterized for 17 important agro-morphological and biochemical traits to assess the extent of genetic diversity. The information generated can be useful for identifying groups of accessions that have desirable characters for crossing, planning efficient germplasm collecting expeditions, revealing the patterns of variation in germplasm collections and investigating some aspects of crop evolution (Sharma *et al*. 2020).

Among the different methods of multivariate analysis, cluster analysis and principal component analysis (PCA) are commonly used. Cluster analysis used to group accessions according to similarity in certain characteristics. PCA is a technique for analyzing relationships among several quantitative variables measured on a number of accessions. It provides information about the relative importance of each variable in characterizing the genotypes and to identify the most relevant characters in explaining the variation (Sharma *et al*. 2022).

Materials and methods

Plant Materials

A total of 24 accessions of tartary buckwheat were characterized and evaluated in 2023 (Table 1). These accessions were selected from the Reginal Station, ICAR-National Bureau of Plant Genetic Resources, Shimla.

Experimental Design

The field experiment was conducted in Organic Agriculture and Natural Farming Farm, Holta, CSKHPKV, Palampur in the *Rabi* season of 2023. Farm is located at an elevation of 1,290.8 metres above mean sea level, with latitude of 32° 6' N and longitude of 76° 3' E.

The experimental material was sown in Randomized Complete Block Design (RCBD) with two checks namely Shimla B1 and Himpriya. The plot design was two rows, 1 m long with a 25 cm row to row

Table 1. List of 24 buckwheat genotypes

Genotypes	Code	Source	Genotypes	Code	Source
IC 26755	3	ICAR-NBPGR, Shimla	IC 37288	15	ICAR-NBPGR, Shimla
Sangla B 444	4	CSKHPKV, Palampur	IC 341667	16	ICAR-NBPGR, Shimla
Sangla B 214	5	CSKHPKV, Palampur	IC 345059	17	ICAR-NBPGR, Shimla
Sangla B 129	6	CSKHPKV, Palampur	IC 323723	18	ICAR-NBPGR, Shimla
Sangla B 5	7	CSKHPKV, Palampur	IC 341674	19	ICAR-NBPGR, Shimla
IC 46160	8	ICAR-NBPGR, Shimla	IC 341683	20	ICAR-NBPGR, Shimla
Himgiri 109728	9	ICAR-NBPGR, Shimla	IC 371665	21	ICAR-NBPGR, Shimla
IC 318859	10	ICAR-NBPGR, Shimla	IC 42430	22	ICAR-NBPGR, Shimla
IC 109729	11	ICAR-NBPGR, Shimla	EC 286377	23	ICAR-NBPGR, Shimla
IC 47929	12	ICAR-NBPGR, Shimla	Chitkul	24	Local landrace
IC 341589	13	ICAR-NBPGR, Shimla	Shimla B1 (C)	1	ICAR-NBPGR, Shimla
IC 356112	14	ICAR-NBPGR, Shimla	Himpriya (C)	2	ICAR-NBPGR, Shimla

Where, ICAR-NBPGR - Indian Council of Agricultural Research, National Bureau of Plant Genetic Resources
CSKHPKV - Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur

distance and 75 cm block to block. Block was little raised for proper drainage because, stagnate water could damage buckwheat completely. Under organic input conditions soil was treated with *jeevamrit* (10%), *beejamrit* was used for seed treatment, vermicompost was added @ 5 t/hafollowed by sprays of organic liquid manure (vermiwash @ 10%) at 15 days interval.

Data Collection and Statistical Analysis

The observations for 17 agro-morphological and biochemical traits *viz*., Days to 50% flowering (DTF), Days to 80% maturity (DTM), Inflorescence length (IL), Inflorescence per plant (IPP), Leaves per plant (LPP), Primary branches per plant (PB), Plant height (PH), Seed index (SI), Seed per plant (SPP), Straw yield per plant (SYPP), Seed yield per plant (YPP), Protein, Calcium content (Ca), Phosphorus content (P), Magnesium content (Mg), Iron content (Fe) and Zinc content (Zn) were made on individual plants as well as on a plot basis depending on the nature of the characters based on Descriptors for buckwheat (IPGRI 1994). For measurement on an individual plant basis, ten individual plants per plot or plant's parts were considered and the averaged data was used for analysis. Thoroughly washed plant samples were dried in oven at 70° C for 48 hr, ground in a stainless-steel Wiley mill, and digested in a di-acid mixture of HNO_3 and $HClO_4$ (Jackson 1973). Micronutrient was determined in aqueous extracts of the digested plant material by atomic absorption spectrophotometer (AAS). Protein fractionation was done by dissolving different fractions of proteins in double distilled water, 1M NaCl, 1% NaOH and 90% ethanol. The total crude protein estimation was done using the standard micro-Kjeldahl method and the amount of total nitrogen in the raw materials were multiplied with the traditional conversion factor of 6.25.

Based on this averaged data, descriptive statistics were estimated, and cluster and PC analyses were applied. The genetic divergence among genotypes was computed following Mahalanobis D^2 technique (1936) and the genotypes were grouped into different clusters following Tocher's method as described by Rao (1952) using Windostat 8.0 software package developed by Indostat Services, Hyderabad, India. The grouping pattern of the 24 buckwheat genotypes was computed by principal component analysis (PCA) in PAST (Paleontological Statistics) software v.4.03 (Hammer *et al*. 2001) using Eigen procedure based on correlation coefficient between two genotypes and the principal components with eigenvalues >1.0 were selected.

Results and discussion

Determination of Genetic Variations

The experimental results revealed a wide range of variation among 24 buckwheat genotypes for both morphological and biochemical traits (Table 2). The genotypic variance (σ^2G) of all the traits was lower than the phenotypic variance (σ^2P). Genotypic variance ranged from 0.08 (Zn) to 640.02 (P). GCV values ranged from 4.71% for days to 80% maturity to 31.05% for seed yield per plant. Similarly, the PCV values ranged from 4.95% for days to 80% maturity to 31.12% for yield per plant. PCV values were higher than the GCV values for all the traits which indicates the environmental role in trait expression (Soharu *et al*. 2022). Deshmukh *et al*. (1986) suggested that PCV and GCV values greater than 20% are regarded as high, values between 10% to 20% as medium, whereas values less than 10% are considered to be low. Accordingly, highest GCV was recorded for seed yield per plant (31.12%), seed per plant (20.74%) and protein (20.12%) whereas medium GCV was recorded for inflorescence length (20.34%), inflorescence per plant (11.94%), leaf per plant (19.16%), primary branches per plant (14.68%), plant height (10.02) and seed index (14.04%) and lowest GCV was observed for days to 50% flowering (9.53%), days to 80% maturity (4.95%), straw yield per plant (8.40%), calcium content (9.40%), phosphorus content (7.15%), magnesium content (7.85%), iron content (8.40%) and zinc content (9.02%). Similarly, the highest PCV was also recorded for seed yield per plant (31.05%), seed per plant (20.70%) and protein (20.06%). Medium PCV was recorded for inflorescence length (18.61%), inflorescence per plant

Table 2. Assessment of genetic variability of eleven morphological and six biochemical traits of buckwheat

Traits	Mean	MSG[1]	MSE[2]	Genotypic variance	Phenotypic variance	GCV[3] (%)	PCV[4] (%)	Heritability (%)	Genetic Advance	GAM[5]
DTF	52.10	73.93*	2.03	23.96	24.64	9.40	9.53	97.25	9.94	19.09
DTM	89.20	58.34*	5.42	17.65	19.45	4.71	4.95	90.70	8.24	9.24
IL	3.21	1.28*	0.21	0.36	0.43	18.61	20.34	83.69	1.13	35.06
IPP	19.14	15.66*	0.23	5.14	5.22	11.85	11.94	98.53	4.64	24.23
LPP	27.09	80.86*	0.36	26.83	26.95	19.12	19.16	99.50	10.65	39.30
PB	2.94	0.56*	0.01	0.18	0.19	14.50	14.68	97.52	0.87	29.50
PH	80.71	196.30*	4.42	63.96	65.43	9.91	10.02	97.75	16.29	20.19
SI	2.30	0.31*	0.01	0.10	0.10	13.97	14.04	99.04	0.66	28.64
SPP	120.26	1867.04*	8.37	619.56	622.35	20.70	20.74	99.55	51.16	42.54
SYPP	7.30	1.13*	0.04	0.36	0.38	8.24	8.40	96.20	1.22	16.65
YPP	2.68	2.09*	0.01	0.69	0.70	31.05	31.12	99.57	1.71	63.82
Protein	13.04	20.66*	0.12	6.84	6.89	20.06	20.12	99.41	5.38	41.20
Ca	58.27	90.10*	2.40	29.24	30.03	9.28	9.40	97.35	10.99	18.86
P	361.78	2005.04*	85.00	640.02	668.35	6.99	7.15	95.76	51.00	14.10
Mg	207.63	796.64*	29.77	255.62	265.55	7.70	7.85	96.26	32.32	15.56
Fe	3.94	0.33*	0.02	0.11	0.11	8.24	8.40	96.36	0.66	16.66
Zn	3.08	0.23*	0.01	0.08	0.08	8.93	9.02	98.06	0.56	18.22

DTF= Days to 50% flowering, DTM= Days to 80% maturity, IL= Inflorescence length, IPP= Inflorescence per plant, LPP= Leaves per plant, PB= Primary branches per plant, PH= Plant height, SI= Seed index, SPP= Seed per plant, SYPP= Straw yield per plant, YPP= Seed yield per plant, Ca= Calcium, P= Phosphorus, Mg= Magnesium, Fe= Iron, Zn= Zinc

[1]MSG- Mean square of genotypes; [2]MSE- Mean square of error; [3]GCV- Genotypic coefficient of variation; [4]PCV- Phenotypiccoefficient of variation; [5]GAM- Genetic advance as percent of mean

(11.85%), leaf per plant (19.12%), primary branches per plant (14.50%) and seed index (13.97%) while low PCV was recorded for days to 50% flowering (9.40%), days to 80% maturity (4.71%), plant height (9.91), straw yield per plant (8.24%), calcium content (9.28%), phosphorus content (6.99%), magnesium content (7.70%), iron content (8.24%) and zinc content (8.93%). Dutta *et al*. (2008) also observed high PCV and high GCV for the number of secondary branches, number of leaves and seed yield per plant in buckwheat. Hiremath *et al*. (2017) observed high genotypic and phenotypic coefficients of variation for seed yield. Similar results were obtained by Bisht *et al*. (2018) hence the selection for these traits could be effective.

The difference between PCV and GCV was less (<1) in all the traits except for inflorescence length which indicates higher contribution of genotypic effect towards phenotypic expression of such traits. For inflorescence length, the gap was high indicating that environmental factors are playing an important role in addition to the genotype for expression of this trait. The genotypic coefficient of variance offers insight into the genetic variability of quantitative traits. However, it does not directly indicate the proportion of variation that is heritable.

To gain a comprehensive understanding of the potential advancement through selection, it's crucial to complement the genotypic coefficient of variance with heritability estimates. The genetic coefficient of variance, combined with these heritability values, provides the most accurate depiction of the anticipated progress achievable through selection. Heritability values play a pivotal role in forecasting the expected gains attainable via selection processes (Chauhan and Sharma 2021). Estimation of heritability in broad sense ranged from 99.57% for seed yield per plant to 83.69% for inflorescence length. All traits showed very high heritability with values greater than 80%. In similar case, Rana *et al*. (2023) found high heritability for days

to 50% flowering while moderate and low heritability for rest of the traits in wheat.

According to Fehr (1987), the heritability of a trait is influenced by factors such as the studied population, environmental conditions and the methodology employed. However, heritability alone does not reveal the extent of genetic improvement achievable through individual genotype selection. A more comprehensive understanding is obtained by considering both heritability and genetic advance. Genetic advance (GA) in selection context refers to the enhancement of traits in genotypic value within a new population compared to the base population after one selection cycle at a specified selection intensity (Hamdi *et al*. 2003). This combined knowledge of heritability and genetic advance provides a more practical insight into the potential improvements achievable through selection processes.

Genetic advance as percent of the mean (GAM), in the present study, ranged from 9.24% to 63.82% for days to 80% maturity and seed yield per plant, respectively. Johnson *et al*. (1955) categorized genetic advance as percent of mean as low (<10%), moderate (10-20%) and high (>20%). Using this classification as the basis, inflorescence length, inflorescence per plant, leaf per plant, primary branches per plant, plant height, seed index, seed per plant, seed yield per plant and protein had the highest genetic advance as percent of mean followed by moderate in days to 50% flowering, straw yield per plant, calcium content, phosphorus content, magnesium content, iron content and zinc content whereas low for days to 80% maturity. High heritability along with high genetic advance as percent mean is more helpful in predicting gain under selection than heritability alone. Accordingly, high heritability along with high genetic advance was observed in inflorescence length, inflorescence per plant, leaf per plant, primary branches per plant, plant height, seed index, seed per plant, seed yield per plant and protein indicating the presence of additive gene action for the expression of these traits and selection in next population based on these traits would be ideal. Traits with high values of heritability coupled with moderate genetic advance as percent of mean *viz*., days to 80% maturity, straw yield per plant, calcium content, phosphorus content, magnesium content, iron content and zinc contents uggesting that selection for improvement of these characters may be rewarding. It also indicates the greater role of non-additive gene action in their inheritance.

Cluster analysis

The non-hierarchical Euclidean cluster analysis using Mahalanobis statistic, grouped the genotypes into five distinct clusters among which two clusters were polygenotypic and three clusters were monogenotypic (Fig 1). Cluster I was the largest group comprising of 15 genotypes *viz*., Himpriya, IC 323723, IC 341674, Sangla B 5, IC 345059, Sangla B 444, IC 341589, IC 109729, IC 318859, IC 371665, IC 42430, Himgiri 109728, IC 37288, IC 341667 and IC 47929. This cluster was characterized with highest primary branch per plant, inflorescence length, leaf per plant, seed per plant, seed yield per plant, magnesium content, iron content and zinc content.

Most of the high yielding germplasm along with widely grown variety Himpriya were grouped under this cluster. Hence this group may be used for selection of genotypes with high grain and foliage yield. Cluster II had six genotypes viz., Sangla B 214, EC 286377, IC 356112, IC 46160, IC 341683 and Chitkul while Cluster III, IV and V comprised of single genotype each *viz*., Sangla B 129, IC 26755 and Shimla B1, respectively indicating towards the diverse origin of these genotypes. Cluster II is composed of genotypes having high amount of protein, calcium content and phosphorus content that can be used as a source for introgression of genes responsible for high nutrition. The intra-cluster distance varied from 32.82 (cluster II) to 31.00 (cluster I), whereas it was zero for the mono-genotypic clusters (Table 3). The inter-cluster distance ranged from 35.11 (clusters IV and V) to 92.24 (clusters II and V). Rana and Sharma (2000) also found that inter-cluster distances were greater than intra-cluster distances in forty-three buckwheat genotypes; similar results were also obtained by Deng *et al*. (2011) in tartary buckwheat and by Rana *et al*. (2023) in cauliflower.

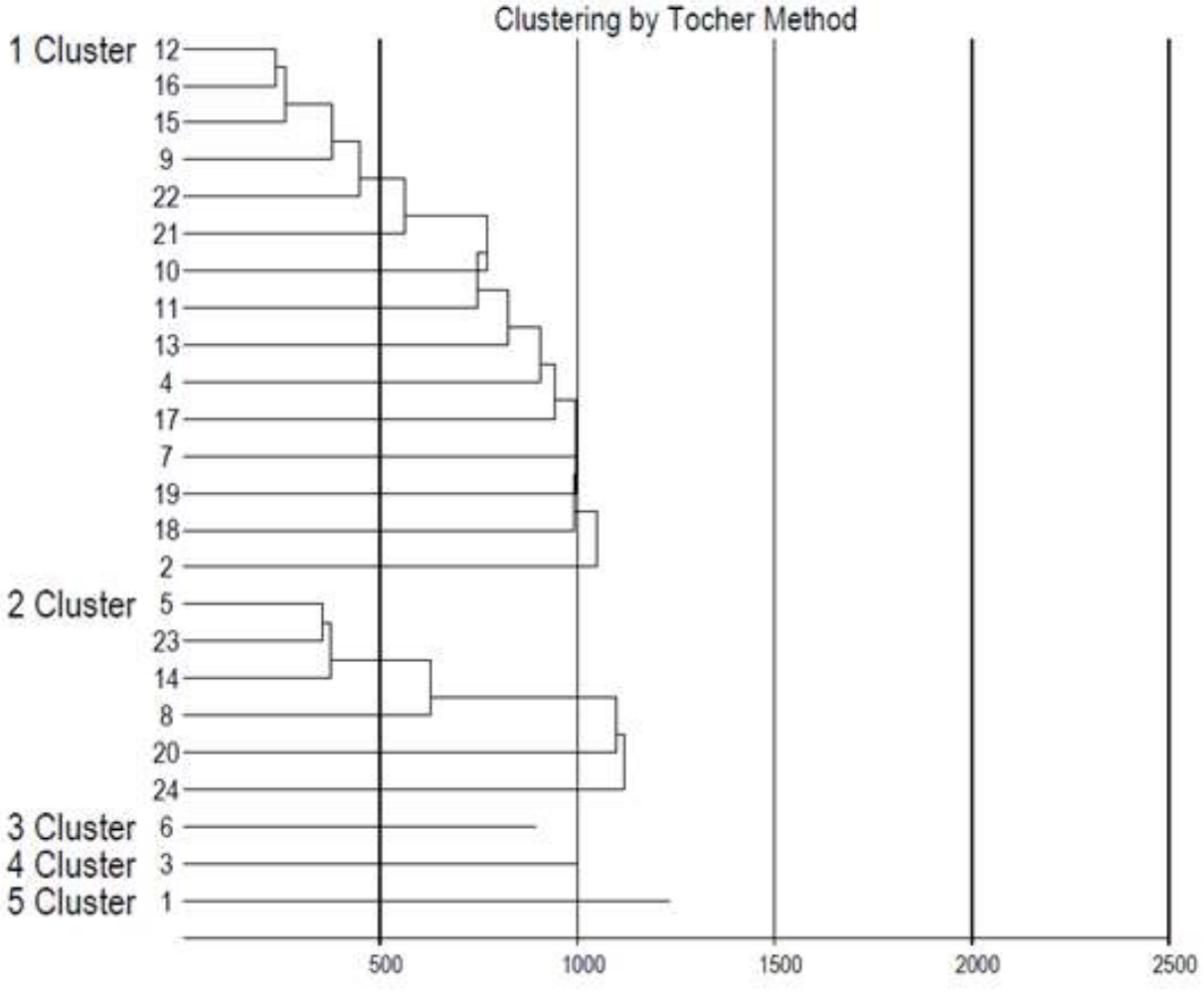

Fig. 1 Dendrogram of 24 buckwheat genotypes constructed using Tocher's method

1= Shimla BI 2= Himpriya, 3= IC 26755, 4= Sangla B 444, 5= Sangla B 214, 6= Sangla B 129, 7= Sangla B 5, 8= IC 46160, 9= Himgiri 109728, 10=IC 318859, 11=IC 109729, 12=IC 47929, 13=IC 341589, 14=IC 356112, 15=IC 37288, 16=IC 341667, 17=IC 345059, 18=IC 323723, 19=IC 341674, 20=IC 341683, 21=IC 371665, 22=IC42430, 23=EC 286377, 24=Chitkul

Table 3. Average intra and inter-cluster distances of D^2 among clusters

Cluster	I	II	III	IV	V
I	31.00	39.50	38.30	49.28	69.63
II		32.82	41.09	71.36	92.24
III			0.00	61.82	79.38
IV				0.00	35.11
V					0.00

Principal Component Analysis (PCA)

PCA reduces the dimensionality of data by converting the original variables into a smaller set of variables while retaining the essential information from the initial variables.The results of PCA confirmed the findings of cluster analysis. Six principal components, PC1 to PC6, which were extracted from the original data and having eigenvalue greater than one, accounted for nearly 78.58% of the total variation.

According to eigen vector analysis, the observed

variation for first, second, third, fourth, fifth and sixth principal component were about 26%, 15.44%, 11.95%, 10.44%, 8.35% and 6.40%, respectively (Table 4).

In the first principal component, number of seed per plant (0.40) and seed yield per plant (0.40) were the most contributing traits whereas days to 80% maturity (0.42), calcium (0.41) and iron (0.38) were the principal traits of the second principal component. Genotypes no. 1, 2, 3, 10, and 24 in the PCA biplot stood out from the other genotypes, which may be the result of their unique ancestry and extensive variance in morphological traits (Fig 2). Rana and Sharma (2000) revealed direct positive effects of 100-seed

Table 4. Eigenvectors for the first six components of 17 morphological and biochemical traits in buckwheat genotypes

	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	4.42	2.63	2.03	1.77	1.42	1.09
% variance	26.00	15.44	11.95	10.44	8.35	6.40
Cumulative	26.00	41.44	53.39	63.83	72.18	78.58
DTF	-0.05	0.37	0.02	0.44	-0.08	-0.18
DTM	-0.17	0.42	-0.08	0.29	-0.12	-0.31
LPP	0.23	-0.04	-0.14	0.30	0.55	-0.04
PH	0.25	0.27	-0.01	-0.01	0.48	0.09
PB	-0.05	0.18	-0.17	0.03	0.08	0.77
IL	0.34	-0.08	0.09	-0.07	-0.06	0.10
IPP	0.34	-0.03	0.18	0.03	-0.21	-0.09
SI	-0.16	0.10	0.30	0.44	-0.11	0.34
SPP	0.40	-0.06	0.22	-0.08	-0.17	0.06
SYPP	0.20	0.09	0.33	-0.05	0.41	-0.17
YPP	0.40	0.03	0.19	0.07	-0.22	0.18
Ca	-0.13	0.41	0.17	-0.33	0.03	0.10
Fe	0.27	0.38	-0.01	0.07	-0.30	0.04
Mg	0.21	0.25	-0.34	-0.31	-0.14	-0.19
P	-0.07	0.25	0.51	-0.14	0.13	-0.09
Zn	0.27	0.18	-0.46	0.09	0.03	0.03
Protein	-0.17	0.28	-0.04	-0.43	0.04	0.09

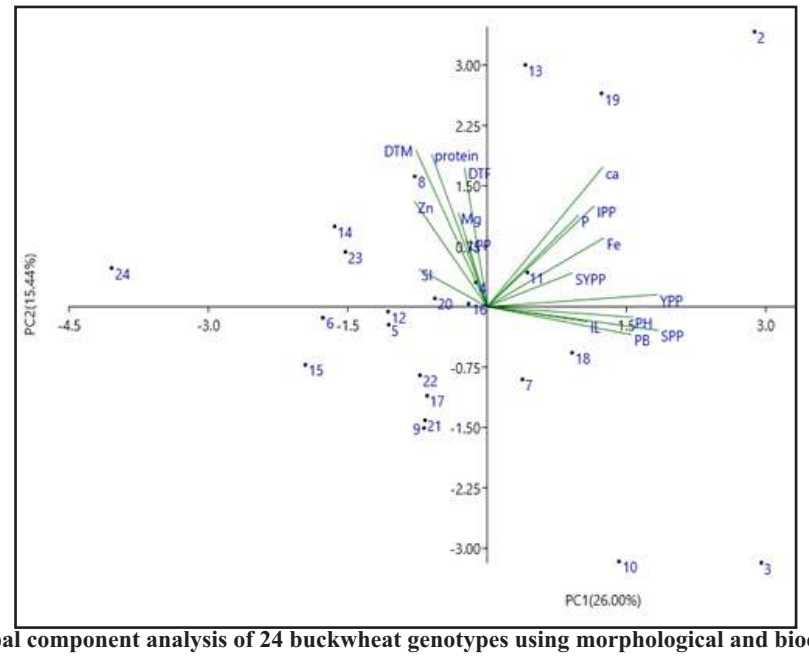

Fig 2. Principal component analysis of 24 buckwheat genotypes using morphological and biochemical traits

weight on seed yield. Joshi (2005), Dutta *et al*. (2008) and Bisht *et al*. (2018) also observed similar results.

Conclusion

In the resent study, PCV values were higher than the GCV values for all the traits which indicates the environmental role in trait expression. Highest GCV and PCV were recorded for seed yield per plant, seed per plant and protein which shows that these traits can be improved through these genotypes. Cluster analysis using Mahalanob is statistic, grouped the genotypes based on Jaccard's coefficient, into five distinct clusters shows that there are 3 groups intercrossed for heterosis breeding. First cluster was characterized with highest primary branch per plant, inflorescence length, leaf per plant, seed per plant, seed yield per plant, magnesium content, iron content and zinc contents how this cluster carries genotypes that can be utilized for these traits. The results of PCA confirmed the findings of cluster analysis. Six principal components showed eigen value more than one where first principal component showed maximum number of seed per plant and seed yield per plant genotypes in this group which could be exploited for their direct release as a variety(s) after testing under wide range of environments. Moreover, these genotypes can also be used as parents in hybridization programs to develop high yielding buckwheat varieties.

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Conflicts of Interest: The authors declare that there is no conflict of interest.

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Genetic analysis of variation in rice (*Oryza sativa* L.) for yield and yield components under organic *vis-a-vis* chemical input conditions

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Abstract

The present study was undertaken during *Kharif* 2020 under conventional inorganic (E_1) and low input organic (E_2) conditions at RWRC, Malan with an objective to evaluate 40 diverse rice germplasm lines in RBD with three replications for grain yield and other agro-morphological traits for genetic variation studies and to identify reliable selection criteria for low input conditions. The mean and range for all the traits except days to flowering and days to maturity were found to be lower under low input organic system as compared to high input chemical system. The overall mean of the genotypes for grain yield was 9.58 g/plant in organic input system as against the mean value of 12.98 g/plant in chemical input system. The top performing genotypes in organic input system were HPR 2795, Desidhan, Jattu, Chohartu, Deval and Sukara Red while in chemical input system top yielders were HPR 2795, HPR 2911, IC 191886, Varun Dhan, Bhrigu Dhan, HPR 2720 and Sukara. Based upon the correlation and path studies days to flowering, days to maturity, total tillers per plant and effective tillers per plant were considered as target traits to improve rice grain yield under organic input condition, while plant height, total tillers per plant and 1000-seed weight were found important traits for selection under chemical input conditions. The traits exhibiting positive association with yield under chemical input conditions were found to be non-significant under organic conditions and this change in correlation patterns under the two different conditions was due to the influence of genetic interactions. Hence, the present study showed that exposure to organic inputs conditions may induce positive or negative correlation among traits due to the expression of new gene advocating thereby that a separate breeding program is required for breeding varieties for organic agriculture.

Key words: Organic Agriculture, genetic analysis, chemical input system, correlations

Green revolution in India was mainly realized with the introduction of high yielding semi dwarf varieties of wheat and rice with the use of high responsive fertilizers. With the introduction of semi dwarf varieties during mid 1960's, India has made a spectacular progress in the production during last few decades enabling the country to become self-sufficient in food grains. The yield of the crops using semi dwarf varieties integrated with high inputs has reached a plateau, but valuable soil nutrients are destroyed due to excessive and imbalanced use of fertilizers and pesticides. This has caused greater depletion of micronutrient reserves in soil and thereby accentuated wide spread deficiencies of micronutrients (Alloway, 2008).

A self-sustaining system of agriculture like organic farming may offer solution to these problems in different agricultural ecosystems (Bhardwaj *et al*. 2020). Organic farming is basically a sustainable low-input eco-friendly practice. It is based on minimal use of off-farm inputs and on management practices that restore, maintain, and enhance 'ecological harmony'. The role of organic farming in Indian rural economy can be leveraged to mitigate the ever-increasing problem of food security. Hossard *et al*. (2016) concluded that low-input system could be less damaging for the environment than the conventional systems in reducing yield losses but this system is

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associated with many challenges and one of the main challenges is developing varieties with the capacity to achieve high yields in reduced chemical input systems /organic input conditions. As the popularity of organic farming is increasing, plant breeding concerns are however a bottleneck in the further development of organic agriculture. Currently, organic farming largely depends upon varieties supplied by conventional plant breeding, even though organic farming conditions demand varieties with the different characteristic than the conventional varieties. In the developing countries like India, where organic movement is at the initial stage of development, varieties that are specifically bred for organic and low- input systems are almost nil, whereas in developed countries it is estimated that more than 95% of organic agriculture is based on crop varieties that were bred for the conventional high-input sector with selection in conventional breeding programme. It has been observed that such varieties lack important which are traits required under organic and low-input production condition. This is primarily due to selection in conventional breeding programmes being carried out in the background of high inorganic fertilizer and crop protection inputs. Hence, in the present study, an effort has been made to find out important traits of rice contributing to yield under organic *vis-a- vis* in-organic input conditions for selection under organic input conditions.

Materials and methods

The present study was conducted during *Kharif* 2020 under low input organic conditions and high input chemical conditions at RWRC, Malan. The experimental material comprised of 40 diverse rice germplasm lines including local land races and released cultivars of Northern Hill Zone. These genotypes varying in their adaptability and yield potential were evaluated under conventional inorganic (E_1) and low input organic (E_2) conditions. The two sets of material were raised in Randomized Block Design with three replications. One set of the experimental material was raised in organic block of the farm while another set was raised in inorganic block on the same date of sowing. Each plot consisted of four rows, each 3.0 m long with 20 cm spacing between rows. Recommended package of practices for organic and conventional agriculture were followed for raising the crop in both the conditions. The data was recorded on yield and other contributing traits and was subjected to correlation analysis following Burton and De Vane (1953).

Results and discussion

A suitable variety is of utmost importance to harness the yield potential in any crop and for the best utilization of the resources (Sharma *et al*. 2014). Significant variation among the genotypes was observed for all the traits studied in the present investigation. The mean values for all the traits under study were lower in organic input system as compared to the high input chemical input system (Table 1 & 2). Grain yield/plant ranged from 9.70g- 20.53g with a mean of 12.98 g/plant in inorganic system while the range was from 6.08g-16.91g with a mean of 9.58g in organic system. 1000-grain weight ranged from 12.78g-30.73g with a mean of 19.71g in organic input system as compared to the inorganic system where range was 17.28g-35.23g with a mean of 24.36g. Similar trend was observed for plant height, spikelets /plant, total tillers/plant and effective tillers /plant with low mean and range values in organic input system as compared to chemical input system. The top performing genotypes in organic input system were HPR2795, Desidhan, Jattu, Chohartu, Deval and Sukara Red while in chemical input system top yielders were HPR 2795, HPR 2911, IC 191886, Varun Dhan, Bhrigu Dhan, HPR 2720 and Sukara. HPR 2901, IC 191886, Hatiali, Byla and Varun Dhan were poor yielders in organic input system while Phulpatas, Hatiali, IC-3131186, Naggar Dhanand Roda Dhan were the poor yielders in chemical input system. IC 191886 and Varun Dhan which were among the top yielders in chemical input system exhibited poor yield in organic input system (Table 1&2). Noori *et al* (2023) also observed differential response of different varieties of chilli and garden pea under conventional, organic and natural farming system.

Correlation studies (Table 3) revealed that under organic conditions grain yield per plant was significantly positively correlated with days to flowering, days to maturity, total tillers per plant and effective tillers per plant. On the other hand, under chemical input conditions, grain yield per plant was significantly positively correlated with plant height, total tillers / plant, effective tillers/plant, and 1000-grain weight. Days to flowering and maturity exhibited

Table 1: Mean performance of rice genotypes under organic input conditions

S.No	Genotypes	Days to 50% flowering	Days to 75% maturity	Plant height(cm)	Spikelets/ panicle	Total Tillers / Plant	Effective Tillers / Plant	1000-grain weight (g)	Grain Yield/ Plant(g)
1	Kaluna	78.00	108.33	87.84	50.85	6.00	5.21	16.1	8.85
2	Ramjuwain	81.00	108.00	119.74	52.92	7.00	6.00	17.74	7.81
3	Chohartu	81.33	110.00	146.20	37.95	8.00	7.00	23.47	13.68
4	Sukara	86.00	114.00	143.17	37.85	7.20	6.00	13.82	11.65
5	Karad	93.00	118.33	111.90	42.02	6.21	4.13	14.62	8.48
6	Kalaina	91.00	116.00	123.17	31.32	6.20	5.53	17.85	8.85
7	Phulpatas	76.00	105.00	102.7	47.45	5.41	4.20	15.64	9.28
8	Acchoo	90.33	117.00	144.77	39.39	5.20	4.13	16.74	9.61
9	Begmi	91.00	118.33	125.94	39.69	7.00	5.40	12.79	7.31
10	Bathidhan	79.00	108.00	112.00	40.15	7.43	6.73	23.6	10.41
11	Byla	85.00	105.33	136.64	32.29	6.20	5.70	19.7	6.71
12	Desidhan	88.00	116.00	138.57	27.35	7.20	6.00	18.24	15.35
13	Kalijhini-2	76.00	101.33	135.60	43.92	6.33	5.10	12.96	7.88
14	Sukara Red	91.33	120.67	130.04	57.29	6.23	5.00	22.02	13.21
15	Hatiali	89.00	109.33	123.64	32.65	5.37	4.60	17.23	6.15
16	HPR-2800	82.00	106.67	132.90	58.09	6.20	5.37	19.88	9.71
17	Naggar dhan	88.00	120.33	117.90	40.59	5.10	4.37	22.91	6.88
18	Roda dhan	73.00	102.00	123.17	47.35	7.43	6.13	24.85	6.95
19	Nailina	77.33	106.33	124.20	57.89	8.21	5.00	19.52	7.65
20	Deval	73.00	102.00	114.60	50.62	4.33	4.00	17.74	13.31
21	Bhrigu dhan	82.00	108.33	149.44	34.05	5.32	5.00	18.24	6.91
22	Kalijhini	79.33	106.67	125.87	35.15	6.21	5.33	13.95	12.61
23	Matali	86.00	110.33	92.50	42.89	5.77	5.00	18.04	8.71
24	Doda dhan	74.33	101.00	141.00	62.85	6.43	6.13	29.19	10.75
25	HPR 2902	86.00	108.33	156.27	49.92	4.83	4.13	19.88	7.58
26	Gosha	83.00	112.00	126.20	35.09	8.23	7.90	19.53	8.01
27	SailaDhan	77.67	102.00	135.80	50.12	7.10	6.83	23.51	11.05
28	Kalijhini	75.00	104.33	119.60	64.49	5.21	4.17	19.56	7.53
29	IC-191886	75.33	103.67	89.77	70.22	5.21	3.47	21.64	6.31
30	Jattu	74.00	101.00	87.74	72.82	6.23	5.00	21.4	14.49
31	Sattu Dhan	75.00	104.00	141.17	67.99	8.97	7.00	25.95	8.91
32	Kalijhini-1	77.33	105.33	124.74	51.35	8.21	6.97	12.78	9.36
33	IC-3131180	88.67	110.67	81.74	53.75	9.03	7.67	18.81	7.11
34	HPR-2901	102.00	130.33	90.40	44.75	7.23	6.23	20.01	6.08
35	HPR-2907	114.33	142.00	120.40	40.55	8.43	7.00	20.6	9.61
36	HPR-2911	108.33	133.00	79.20	34.02	7.21	6.70	24.7	8.88
37	HPR-2912	76.00	101.67	122.60	46.89	8.23	6.20	20.99	8.11
38	Varun Dhan	75.00	104.00	146.34	51.62	7.40	6.90	30.73	6.78
39	HPR-2720	96.00	126.67	127.27	55.82	10.27	8.20	19.82	10.81
40	HPR-2795	88.33	107.33	146.30	64.62	8.40	7.20	21.62	16.91
	Mean	84.07	110.89	122.48	47.41	6.80	5.72	19.71	9.58
	Max	114.33	142.00	156.27	72.82	10.27	8.20	30.73	16.91
	Min	73.00	101.00	79.20	27.35	4.33	3.47	12.78	6.08
	C.D	0.76	1.31	3.24	6.51	1.02	1.10	2.12	2.03
	C.V	1.20	0.72	4.51	7.52	5.41	9.20	6.50	4.78

Table 2: Mean performance of rice genotypes under chemical input conditions

S.No	Genotypes	Days to 50% flowering	Days to75% maturity	Plant Height(cm)	Spikelets/ panicle	Total Tillers/ Plant	Effective Tillers / Plant	1000-grain weight(g)	Grain Yield/ Plant(g)
1	Kaluna	82.00	110.67	98.07	64.13	10.37	10.17	20.60	13.47
2	Ramjuwain	79.33	109.67	129.97	66.20	6.30	6.13	22.24	11.43
3	Chohartu	77.67	108.00	156.43	41.23	3.67	3.60	27.97	12.30
4	Sukara	91.33	115.33	153.40	41.13	8.13	7.77	18.32	15.27
5	Karad	91.67	116.00	122.13	55.30	5.77	5.40	19.12	12.10
6	Kalaina	89.00	114.00	133.40	34.60	7.70	6.83	22.35	12.47
7	Phulpatas	76.33	106.67	112.93	50.73	6.73	6.53	20.14	9.70
8	Acchoo	91.33	115.00	155.00	52.67	4.57	4.13	21.24	13.23
9	Begmi	90.00	120.33	136.17	42.97	6.20	5.40	17.29	10.93
10	Bathidhan	75.33	105.00	122.23	53.43	4.33	3.73	28.10	14.03
11	Byla	77.00	107.33	146.87	35.57	5.50	4.70	24.20	10.33
12	Desidhan	89.00	121.33	148.80	40.63	8.90	7.90	25.74	8.97
13	Kalijhini-2	74.33	105.33	145.83	57.20	8.50	8.10	17.46	11.50
14	Sukara Red	93.00	123.67	140.27	70.57	7.83	6.70	26.52	10.83
15	Hatiali	88.00	111.33	133.87	45.93	8.37	7.60	23.73	9.77
16	HPR-2800	82.33	110.67	143.13	71.37	7.10	6.27	24.38	13.33
17	Naggar dhan	89.00	120.33	128.13	53.87	6.70	6.37	27.41	10.50
18	Roda dhan	71.67	102.00	133.40	60.63	9.10	8.57	29.35	10.57
19	Nailina	76.33	106.33	134.43	71.17	10.43	6.63	24.02	11.27
20	Deval	71.67	101.67	124.83	63.90	6.20	6.00	27.24	13.89
21	Bhrigu dhan	80.33	110.67	159.67	47.33	7.37	6.63	24.74	15.53
22	Kalijhini	77.33	108.67	136.10	48.43	7.87	7.33	18.45	13.23
23	Matali	88.00	112.67	124.63	56.17	6.23	6.13	22.54	12.33
24	Doda dhan	72.33	102.00	151.23	76.13	8.77	8.13	33.69	14.37
25	HPR 2902	84.00	111.33	166.50	63.20	5.43	5.13	24.38	11.20
26	Gosha	84.00	117.00	136.43	48.37	9.83	8.90	24.03	11.63
27	SailaDhan	75.67	101.67	146.03	63.40	8.17	7.83	28.01	14.67
28	Kalijhini	73.33	105.67	129.83	77.77	8.10	7.17	24.06	11.15
29	IC-191886	75.67	104.00	100.00	83.50	6.13	5.47	26.14	14.93
30	Jattu	72.33	102.00	97.97	86.10	8.23	5.43	25.90	13.11
31	Sattu Dhan	76.33	105.33	151.40	81.27	8.23	7.50	30.45	12.53
32	Kalijhini-1	76.67	106.67	134.97	64.63	7.97	4.97	17.28	12.98
33	IC-3131180	89.00	111.67	91.97	67.03	8.03	7.67	23.31	10.73
34	HPR-2901	101.33	132.33	100.63	58.03	8.10	7.73	24.51	12.90
35	HPR-2907	116.33	146.00	130.63	43.83	12.43	12.07	25.10	13.23
36	HPR-2911	106.33	136.00	89.43	37.30	9.07	8.70	23.20	19.50
37	HPR-2912	75.33	102.33	132.83	60.17	7.70	6.67	25.49	11.73
38	Varun Dhan	73.33	108.33	156.57	54.90	8.40	7.23	35.23	16.40
39	HPR-2720	94.00	124.33	137.50	59.10	8.27	8.20	24.32	15.43
40	HPR-2795	89.00	108.00	156.53	77.90	10.40	8.57	26.12	20.53
	Mean	84.69	112.18	133.25	58.19	7.68	6.90	24.36	12.98
	Max	116.33	146.00	166.50	86.10	12.43	12.07	35.23	20.53
	Min	73.33	101.67	89.43	34.60	3.67	3.60	17.28	9.70
	C.D	0.89	1.76	5.54	8.25	1.20	2.24	2.61	0.62
	C.V	2.10	0.88	3.12	8.30	6.53	8.65	6.30	7.23

Table 3. Estimates of correlation coefficients at phenotypic level for different traits under organic and chemical input conditions

Traits		Days to 75% maturity	Plant height	Spiklets/ plant	Total tillers/ plant	Effective tillers/ plant	1000-grain weight	Grain yield/ plant
Days to 50% flowering	E1	0.504*	0.067	-0.232	-0.153	-0.462**	-0.101	-0.491*
	E2	0.597*	0.281*	-0.296*	0.499**	0.118	0.116	0.282*
Days to 75% maturity	E1		-0.001	0.009	0.116	-0.034	0.156	-0.170
	E2		0.412*	-0.121	0.492*	0.310*	0.107	0.341*
Plant height	E1			0.121	0.301*	0.021	0.142	0.245*
	E2			-0.122	0.070	-0.001	0.347*	-0.061
Spikelets/panicle	E1				0.101	0.104	-0.005	0.072
	E2				0.021	-0.021	-0.256*	0.172
Total tillers/plant	E1					-0.001	0.342*	0.433*
	E2					0.362*	-0.009	0.615*
Effective tillers per plant	E1						0.036	0.250*
	E2						-0.265*	0.470*
1000- grain weight	E1							0.331*
	E2							-0.290*

E1=Inorganic E2= Organic; * Significant at 5% level

significant positive correlation with plant height, total tillers per plant andgrain yield under organic input conditions which was absent in chemical input conditions. Plant height exhibited significant positive correlation with total tillers per plant under chemical input conditions and with 1000-grain weight under organic input conditions. Total tillers /plant had significant positive correlation with effective tillers under organic conditions and with 1000-grain weight under chemical input conditions. Similar results of correlation analysis were also observed by Ratna *et al*. (2015), Tiwari *et al*. (2019) and Sadhana *et al*. (2022).

Table 4: Estimates of direct and indirect effects at phenotypic level for different traits under organic and chemical input conditions

Traits		Days to 50% flowering	Days to 75% maturity	Plant height	Spiklets/ plant	Total tillers/ plant	Effective tillers/ plant	1000-grain weight	Grain yield/ plant
Days to flowering	E1	-0.48	0.01	0.01	0.01	-0.04	0.02	-0.02	-0.491*
	E2	0.14	-0.02	-0.03	-0.04	0.24	0.01	-0.02	0.282*
Days to maturity	E1	-0.26	0.03	0.00	0.00	0.04	0.00	0.02	-0.170
	E2	0.13	-0.04	-0.03	-0.02	0.27	0.05	-0.02	0.341*
Plant height	E1	-0.03	0.00	0.18	-0.01	0.08	0.00	0.02	0.245*
	E2	0.05	-0.02	-0.05	-0.01	0.04	0.00	-0.07	-0.061
Spikelets/panicle	E1	0.06	0.00	0.01	-0.03	0.03	0.00	0.00	0.072
	E2	-0.05	0.01	0.01	0.14	0.01	0.00	0.05	0.172
Total tillers/plant	E1	0.10	0.00	0.03	-0.01	0.26	0.00	0.05	0.433*
	E2	0.09	-0.03	0.00	0.00	0.49	0.06	0.00	0.615*
Effective tillers per plant	E1	0.26	0.00	0.00	-0.01	0.00	-0.01	0.01	0.250*
	E2	0.04	-0.01	0.00	0.00	0.18	0.21	0.05	0.470*
1000- grain weight	E1	0.06	0.00	0.02	0.00	0.10	0.00	0.15	0.331*
	E2	0.02	-0.01	-0.02	-0.03	-0.01	-0.04	-0.20	-0.290*

E1=Inorganic E2= Organic; * Significant at 5% level

Correlation analysis has revealed some interesting facts about changes in input conditions. Exposure to organic inputs conditions may induce positive or negative correlations among traits. Due to the expression of new genes, the variances and covariances among traits are changed and correlation values show a congenial effect of organic input conditions that will help in selection among different traits under organic input conditions, making direct and indirect contributions of component characters towards grain yield. The negative correlation of grain yield with days to flowering and maturity under high input chemical system changed to significant positive under low input organic conditions while significant positive correlation of grain yield with 1000-grain weight and plant height under chemical input conditions got transformed to significant negative under organic conditions. There are evidences that change in conditions can influence genetic interactions among traits as well as genetic variance in traits themselves. Bhardwaj *et al*. (2012) Bhardwaj *et al*. 2014 and Kaur and Bhardwaj (2019) made comparative studies on correlation of yield and yield components under organic vis-a-vis non-organic input conditions in wheat and gram and found that correlation patterns under the two different conditions indicated the influence of genetic interactions. Sharma *et al.* (2022) construed that morphological characters are largely influenced by the environmental factors.

Association of various plant characters with the traits of major interest and economic importance like grain yield is the consequence of their direct and indirect effects. It becomes, therefore essential to partition such association into direct and indirect effects of component characters through path coefficient analysis. The path coefficient analysis required to determining the degree of relationship between yield and its component effects, as well as for examining specific factors that contribute to a given correlation (Sekhon *et al*. 2019; Lata and Sharma 2023). In the present study, to obtain a relevant and clear understanding of the association among various traits, the estimates of the direct and indirect effects at phenotypic and genotypic level were worked out under organic and inorganic environments. The positive correlation of grain yield with days to flowering, total tillers/plant and effective tillers/plant under organic conditions and plant height, tillers/plant and 1000-grain weight under chemical input conditions was due to high direct effects. On the other hand, the positive correlation of days to maturity with grain yield was due to indirect effects via days to flowering and total tillers / plant. Path analysis showed a high magnitude of direct effects for days to flowering, effective tillers / plant and total tillers /plant under organic conditions and for plant height, total tillers / plant and 1000- grain weight only under chemical input conditions.

Conclusion

The results indicated that different selection parameters operate for grain yield improvement under both the conditions. Thus, the traits *viz*; days to flowering, days to maturity, total tillers per plant and effective tillers per plant are to be considered as selection criteria for high yield for low input organic conditions while plant height, total tillers / plant, effective tillers/plant and 1000-grain weight are important selection criteria for high input chemical conditions. Similar studies were also conducted by Sood and Sood (2001) for the effects of cropping systems on some genetic parameters in soybeans, and observed different criteria in each cropping system. Bhardwaj *et al*. (2012) made comparative studies on correlation of yield and yield components under organic *vis-a-vis* non-organic input conditions in wheat and lentil and found that correlation patterns under the two different conditions indicated the influence of genetic interactions. Exposure to organic inputs conditions may induce positive or negative correlation among traits due to the expression of new gene (Manal 2009), advocating thereby that a separate breeding program is required for both the conditions.

Conflict of interest: The authors declare no competing interest.

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Sulphur distribution in acidic soil profiles of Himachal Pradesh

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Abstract

Acidic soils of Northwestern Himalayas are showing deficiency of sulphur and this is emerging as major limitation to grow quality crops. The vertical distribution of sulphur fractions down the soil profile was studied in the major cropping system; maize-wheat, rice–wheat, maize-potato and vegetable based cropping system. Five soil profiles were selected randomly in the acidic region of Himachal Pradesh, the pH of particular soil profile and mobilization of sulphur down the soil profiles in different layers was studied by analyzing different fractions of sulphur *viz*., sulphate-S, water soluble-S, heat soluble S, organic-S, and total-S. All soil profiles have sufficient to deficient level of available sulphur at surface level (8.3-27.6 mg kg^{-1}). All the sulphur fractions decreased with increase in the depth and soil profiles under vegetable cropping system have comparatively higher levels of all forms of sulphur. Among different forms of sulphur organic sulphur is predominant.

Keywords: soil profile, sulphur, acidic soils, sulphur fractions

Essentiality of sulphur (S) for plants growth and development is widely known and established. In modern agriculture, S is considered as fourth major plant nutrient after nitrogen, phosphorus and potassium and it is crucial for animals and humans as well. Sulphur is best known for its role in the synthesis of proteins, oils, vitamins and flavored compounds in plants. It is a constituent of three amino acids, *viz*., methionine (21% S), cysteine (26% S), and cystine (27% S), which are the building blocks of proteins. About 90% of plant sulphur is present in these amino acids (Tandon and Messick 2002). S deficiency has restricted the sustainable growth and development of various field crops. Use of concentrated fertilizers having no or less S, decreased emission of sulphur dioxide (Lehmann *et al*. 2008), intensive cropping have aggravated the S deficiency in soil around the world (Scherer 2009).

Sulphur application had a significant impact on the yield-related characteristics of crops (Udaykumar & Jemila 2016 and Singh *et al*. 2022). Aside from nutrient sources, the soil is the primary source of sulphur (Scherer 2009). A significant factor in determining the amount of sulphur absorbed by crops is the status of other major nutrients, particularly nitrogen and phosphorus and other physicochemical properties of soil (Singh *et al*. 2022; Paul and Mukhopadhyay 2015; Hembram *et al.* 2012). Therefore, even under excellent management practices and regardless of all other nutrient applications, the absolute yield potential ofa crop cannot be obtained in soils that are lacking in S content (Singh *et al*. 2022). The importance of S in long term fertilization has been also established by Chauhan *et al*. 2018 and Suri *et al*. 2022 in Palampur conditions of Himachal Pradesh.

On an average, 41 percent of Indian soils have reported S deficiency (Sharma *et al*. 2014). Reports indicated that, S deficiency was widespread in red-lateritic, coarse-textured alluvial, leached acidic hill soils and black clayey soils (Shukla *et al*. 2020). It is more pronounced in Alfisols than Vertisols (Singh *et al*. 2022). Though the efficiency of sulphur is only 8–10% (Tiwari and Gupta 2006), the severity of this deficiency varies according to these regions' physicochemical characteristics of soil as well as the climatic conditions (Das *et al*. 2021).

As 90% of the total S is present in organic form, it is preferable to study the various forms of S rather than

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the available ones to determine a soil's capacity to supply S (Basumatari and Das 2012). The availability of sulphur is influenced by a number of soil conditions, and as a result, the status of various forms of sulphur in soils varies greatly with soil type (Trivedi *et al*. 2000). Both inorganic and organic forms of sulphur are found in soil. Sulphur exists in soil in different forms, *viz*, water soluble S, sulphate S, organic S, adsorbed S, heat soluble S and total S. Due to different losses, mainly through leaching, sulphate sulphur only makes up a small portion of total sulphur (1.25 to 17.7%), especially in soils with a coarse texture (Singh *et al*. 1993). The sulphur-supplying capacity of a soil is determined by the types of sulphur and how they interact with soil characteristics to affect the release and its dynamics (Mohammed Nisab *et al*. 2023). Different forms of sulphur and their relationship with some important soil characteristics decide the sulphur-supplying power of soil by influencing its release and dynamics (Gourav *et al*. 2018). However, knowledge of different forms of sulphur in soil along with their distribution in the zone of penetration is of much relevance in assessing the long-term availability of nutrients. The information of vertical distribution of S forms in acid *Alfisol* is scanty. In view of this the present study was undertaken to study the distribution of S forms in the soil profile under different cropping system.

Materials and Methods

Five soil profiles were selected randomly in the acidic region of Himachal Pradesh in the major cropping system; maize-wheat, rice–wheat, maize-potato and vegetable-based cropping system. Soil profiles were also classified taxonomically by following taxonomy map of Himachal Pradesh prepared by National Bureau of Soil Survey and Land use Planning (Regional centre, Delhi) in cooperation with Department of agriculture Himachal Pradesh, Shimla.

The pH of particular soil profile and mobilization of sulphur down the soil profiles in different layers was studied by analyzing different fractions of sulphur *viz*., sulphate-S, water soluble-S, heat soluble S, organic-S, and total-S by following standard procedures as follows:-

a) Soil pH: It was determined in the ratio (1: 2.5, soil: water) by following standard procedure given by Jackson (1973).

b) Total sulphur: It was estimated turbidimetrically using $BaCl_2$, after digesting the soil with HNO_3 and $HClO_4$, di-acid mixture in a ratio of 4:1(Chapman and Pratt 1961).

c) Water soluble sulphur: It was estimated turbidimetrically using de-ionized water as extracting solution (Chesnin and Yien 1950).

d) **Heat soluble sulphur:** Soil samples were hydrolyzed with the addition of distilled water and then evaporated to dryness on a gently boiling water bath. Thereafter, soil was dried in an oven at 102^0 C for 1 hour and then extracted with 0.15 per cent $CaCl_2$. The sulphur in the solution was determined turbidimetrically (Williams and Steinbergs 1959).

e) Sulphate sulphur: The soil was extracted with 0.15 per cent $CaCl_2$, using a soil: extractant ratio of 1:5. The sulphate sulphur in soil extract was determined colorimetrically by developing $BaSO_4$ turbidity in the presence of sodium acetate-acetic acid buffer (Chesnin and Yien 1950).

f) Organic sulphur: Organic sulphur content in soils was estimated as described by Bardsley and Lancaster (1965).

Selected soil profiles have been represented geospatially in Figure 1.

Results and Discussion

Location I (Palampur)

Typically, Palampur soil is deep, well drained, fine loamy soils with loamy surface and slight erosion. Taxonomically it can be named as *Typic Hapludalfs* (Anonymous 1996). This soil profile was under maize-wheat land use. The values of different sulphur fractions (available S, water soluble S, heat soluble S, organic S and total S) have been presented in table 1. Available S ranged from 14.5 mg kg^{-1}in 0-0.15 m soil layer to 6.9 mg kg^{-1} in 0.90-1.20 m soil depth. Water soluble S varied between 7.2 mg kg^{-1} in surface layer (0-0.15 m) and 1.8 mg kg^{-1} in 0.90-1.20 m soil layer. Heat Soluble S which include organic plus sulphate S ranged from 80.2 mg kg^{-1} in surface soil (0-0.15 m) to 22.4 mg kg^{-1} in 0.90-1.20 m soil depth. Similarly organic S and total S ranged from 163.4 mg kg^{-1} to 68.8 mg kg^{-1} and from 210.2 mg kg^{-1} to 98.6 mg kg^{-1}, in

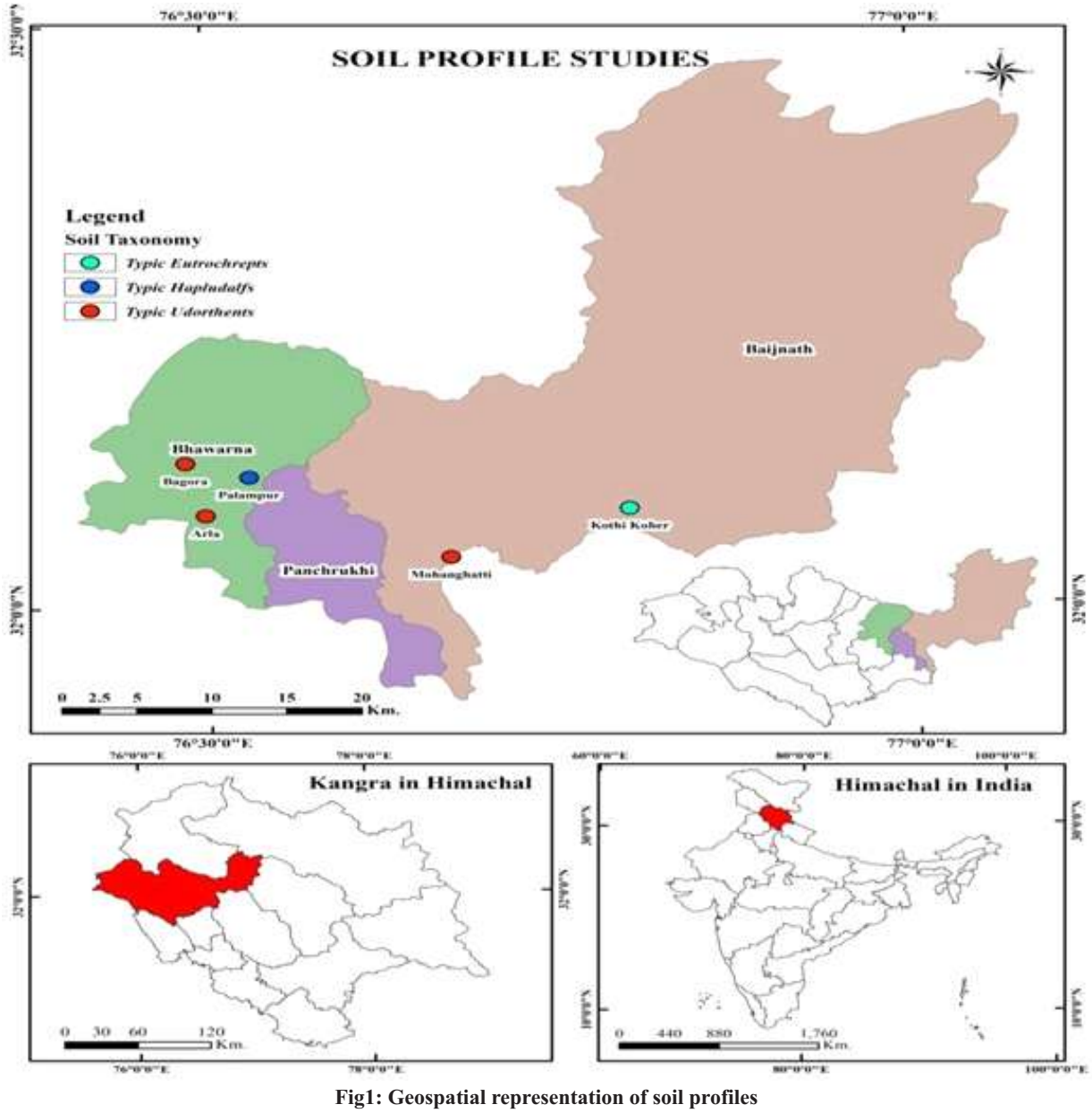

Fig1: Geospatial representation of soil profiles

Table 1.Vertical distribution of S fractions (mg kg^{-1}) in soil profile at Palampur (pH: 5.80)

Depth (m)	Available sulphur	Water Soluble Sulphur (WSS)	Heat Soluble Sulphur (HSS)	Organic Sulphur	Total Sulphur
0-0.15	14.5	7.2	80.2	163.4	210.2
0.15-0.30	12.7	5.7	60.4	138.3	179.2
0.30-0.60	10	3.1	45.8	110.2	150.8
0.60-0.90	9.2	2.5	30.6	98.2	135.4
0.90-1.20	6.9	1.8	22.4	68.8	98.6
Mean±SD	10.66±2.98	4.06±2.29	47.88±23.20	115.78±36.48	154.84±42.47
Range	6.9-14.5	1.8-7.2	22.4-80.2	68.8-163.4	98.6-210.2

Optimum limit of available sulphur in surface soil is >10 mg kg^{-1}

surface layer (0-0.15 m) and 0.90-1.20 m soil layer, respectively.The mean values of available S, water soluble S, heat soluble S, organic sulphur and total sulphur in soil profile were 10.66±2.98, 4.06±2.29, 47.88±23.20, 115.78±36.48 and 154.84±42.47, mg kg^{-1} respectively.

Location II (Bagoda)

Typically, Bagoda soil is medium deep, well drained, coarse loamy soils with loamy surface and moderate erosion. This soil is a member of the family of *Typic Udorthents* (Anonymous 1996). This profile was situated in the area, where maize- potato was grown widely. A perusal of the data given in table 2 revealed that the mean values of available S, water soluble S, heat soluble S, organic sulphur and total sulphur in soil profile were 6.76±3.65, 3.52±2.36, 43.58±22.90, 102.28±38.98 and 135.14±45.95, mg kg^{-1} respectively. Available S ranged from 12.2 mg kg^{-1} in 0-0.15 m soil layer to 3.2 mg kg^{-1} in 0.90-1.20 m soil depth. Water soluble S varied between 6.7 mg kg^{-1} in surface layer (0-0.15 m) and 1.3 mg kg^{-1} in 0.90-1.20 m soil layer. Heat Soluble S which includes organic plus sulphate S ranged from 77.9 mg kg^{-1} in surface soil (0-0.15 m) to 20.3 mg kg^{-1} in 0.90-1.20 m soil depth. Similarly organic S and total S ranged from 163.4 mg kg^{-1} to 64.5 mg kg^{-1} and from 207.2 mg kg^{-1} to 90.6 mg kg^{-1}, in surface layer (0-0.15 m) and 0.90-1.20 m soil layer, respectively.

Location III (Kothi Kohar)

Soils of Kothi Kohar are medium deep to deep, well drained, fine loamy soils with loamy surface and moderate erosion. Typically, Kothi Kohar soil is a member of the family of *Dystric Eutrochrepts* (Anonymous 1996). Soil profile was selected in the area where major land use is vegetable production. The status of different sulphur fractions (available S, water soluble S, heat soluble S, organic S and total S) in soil profile of Kothi Kohar have been embodied in table 3. The range of available S was from 27.6 mg kg^{-1} in 0-0.15 m soil layer to 12.8 mg kg^{-1} in 0.90-1.20 m soil

Table 2.Vertical distribution of S fractions (mg kg^{-1}) in soil profile at Bagoda (pH: 4.9)

Depth (m)	Available sulphur	Water Soluble Sulphur	Heat Soluble Sulphur	Organic Sulphur	Total Sulphur
0-0.15	12.2	6.7	77.9	163.4	207.2
0.15-0.30	8.6	5.3	54.2	115.7	150.8
0.30-0.60	5.5	2.5	36.7	89.4	120.3
0.60-0.90	4.3	1.8	28.8	78.4	106.8
0.90-1.20	3.2	1.3	20.3	64.5	90.6
Mean±SD	6.76±3.65	3.52±2.36	43.58±22.90	102.28±38.98	135.14±45.95
Range	3.2-12.2	1.3-6.7	20.3-77.9	64.5-163.4	90.6-207.2

Optimum limit of available sulphur in surface soil is >10 mg kg^{-1}

Table 3. Vertical distribution of S fractions (mg kg^{-1}) in soil profile at Kothi Kohar (pH: 4.6)

Depth (m)	Available sulphur	Water Soluble Sulphur	Heat Soluble Sulphur	Organic Sulphur	Total Sulphur
0-0.15	27.6	12.2	127.8	362.4	444.5
0.15-0.30	23.5	9.2	93.6	296.5	370.2
0.30-0.60	21.8	4.8	60.2	248.2	314.2
0.60-0.90	16.7	2.8	37.6	204.8	260.3
0.90-1.20	12.8	1.2	24.6	182.4	232.7
Mean±SD	20.48±5.80	6.04±4.56	68.76±42.12	258.86±72.50	324.38±85.36
Range	12.8-27.6	1.2-12.2	24.6-127.8	182.4-362.4	232.7-444.5

Optimum limit of available sulphur in surface soil is > 10 mg kg^{-1}

depth. Water soluble S and heat soluble S varied between 12.2 mg kg^{-1} in surface layer (0-0.15 m) to 1.2 mg kg^{-1} in 0.90-1.20 m soil layer and from 127.82 mg kg^{-1} in surface soil (0-0.15 m) to 24.6 mg kg^{-1} in 0.90-1.20 m soil layer, respectively. Similarly organic S and total S ranged from 362.4 mg kg^{-1} and 444.5 mg kg^{-1} in surface layer (0-0.15 m) to 182.4 mg kg^{-1} and 232.7 mg kg^{-1} in 0.90-1.20 m soil layer, respectively. The mean values of available S, water soluble S, heat soluble S, organic sulphur and total sulphur in soil profile were 20.48±5.80, 6.04±4.56, 68.76±42.12, 258.86±72.50 and 324.38±85.36, mg kg^{-1} respectively.

Location IV (Mohanghati)

Soils of Mohanghati are shallow, well drained, thermic, loamy soils on very steep slopes with loamy surface and very severe erosion. Typically, Mohanghati soil is a member of the family of *Lithic Udorthents* (Anonymous 1996). Soil profile was situated in the rice-wheat growing belt. A perusal of the data about different S fractions given in table 4 depicted that available S ranged from 8.3 mg kg^{-1} in 0-0.15 m soil layer to 3.2 mg kg^{-1} in 0.90-1.20 m soil depth. Water soluble S and heat soluble S varied between 3.4 mg kg^{-1} in surface layer (0-0.15 m) to 0.7 mg kg^{-1} in 0.90-1.20 m soil layer and from 63.7 mg kg^{-1} in surface soil (0-0.15 m) to 28.6 mg kg^{-1} in 0.90-1.20 m soil layer, respectively. Similarly, organic S and total S ranged from 126.7 mg kg^{-1} and 157.2 mg kg^{-1} in surface layer (0-0.15 m) to 53.2 mg kg^{-1} and 68.2 mg kg^{-1} in 0.90-1.20 m soil layer, respectively. The mean values of available S, water soluble S, heat soluble S, organic sulphur and total sulphur in soil profile were 5.34±1.86, 1.82±1.04, 42.46±14.11, 84.22±28.10 and 106.14±34.11 mg kg^{-1}, respectively.

Location V (Arla)

Soils of Arla are deep, somewhat excessively drained, thermic, coarse-loamy soils on gentle slopes with loamy surface and moderate erosion. Typically, Arla soil is a member of the family of *Typic Udorthents* (Anonymous 1996). Soil profile was located where rice-wheat was grown throughout the year. The value of different sulphur fractions (available S, water soluble S, heat soluble S, organic S and total S) have been presented in table 5. The mean values of available S, water soluble S, heat soluble S, organic sulphur and total sulphur in soil profile were 10.42±3.71, 2.7±1.63, 65.56±22.14, 146.32±50.00 and 190.58±61.85, mg kg^{-1} respectively. Available S ranged from 15.3 mg kg^{-1} in 0-0.15 m soil layer to 5.0 mg kg^{-1} in 0.90-1.20 m soil depth. Water soluble S varied between 4.8 mg kg^{-1} in

Table 4. Vertical distribution of S fractions (mg kg^{-1}) in soil profile at Mohanghati (pH: 6.31)

Depth (m)	Available sulphur	Water Soluble Sulphur	Heat Soluble Sulphur	Organic Sulphur	Total Sulphur
0-0.15	8.3	3.4	63.7	126.7	157.2
0.15-0.30	5.5	2.1	48.8	94.5	119.5
0.30-0.60	5	1.8	38.7	78.4	99.2
0.60-0.90	4.7	1.1	32.5	68.3	86.6
0.90-1.20	3.2	0.7	28.6	53.2	68.2
Mean±SD	5.34±1.86	1.82±1.04	42.46±14.11	84.22±28.10	106.14±34.11
Range	3.2-8.3	0.7-3.4	28.6-63.7	53.2-126.7	68.2-157.2

Optimum limit of available sulphur in surface soil is > 10 mg kg^{-1}

Table 5. Vertical distribution of S fractions (mg kg^{-1}) in soil profile at Arla (pH: 5.9)

Depth (m)	Available sulphur	Water Soluble Sulphur	Heat Soluble Sulphur	Organic Sulphur	Total Sulphur
0-0.15	15.3	4.8	97.4	215.7	277.5
0.15-0.30	11.5	3.9	75.5	167.8	217.1
0.30-0.60	10.7	2.5	63.4	139.8	180.5
0.60-0.90	9.6	1.2	51.2	127.9	167.2
0.90-1.20	5.0	1.1	40.3	80.4	110.6
Mean±SD	10.42±3.71	2.7±1.63	65.56±22.14	146.32±50.00	190.58±61.85
Range	5.0-15.3	1.1-4.8	40.3-97.4	80.4-215.7	110.6-277.5

Optimum limit of available sulphur in surface soil is > 10 mg kg^{-1}

surface layer (0-0.15 m) and 1.1 mg kg^{-1} in 0.90-1.20 m soil layer. Heat Soluble S which includes organic plus sulphate S ranged from 97.4 mg kg^{-1} in surface soil (0 - 0.15 m) to 40.3 mg kg^{-1} in 0.90-1.20 m soil depth.Similarly organic S and total S ranged from 215.7 mg kg^{-1} to 80.4 mg kg^{-1} and from 277.5 mg kg^{-1} to 110.6 mg kg^{-1}, in surface layer (0 – 0.15 m) and 0.90 – 1.20 m soil layer, respectively.

A close look on the vertical distribution of S in soil profiles given in table 1 to 5 revealed that all the S fractions decreased with increase in the depth of soil. All the forms of S were comparatively higher at location III (Kothi Kohar) and lowest at location IV (Mohanghati). This might be due to that at Kothi Kohar, the soil profile was under vegetable cultivation and farmers used organic manures for vegetable production, which might have increased the organic matter content of soil and ultimately the sulphur, as organic matter is the direct source of sulphur in soil. Besides this, Kothi Kohar located in the temperate region, which decreased the oxidation of organic matter and increased its accumulation. At Mohanghati, intensive cultivation of rice-wheat was carried out without any addition of organic manures, which have resulted in the mining of sulphur from the soil and this might be the reason for lower sulphur fractions. The total sulphur decreased with the depth in all the soil profiles under study might be due to the reason that the most of soil sulphur is primarily in the organic form. In general, the organic matter content decreases regularly down the profiles and total sulphur also exhibits similar trend in all the soils. These findings are similar to those reported by Singh and Sharma (1983). The organic sulphur decreased with the depth in all the soil profiles under study might be due to high content of organic matter in surface layer than subsurface layer. Also the organic matter content decreased regularly with increasing depth resulted in decreasing S fraction. Similar results were also reported by Balanagoudar and Satyanarayana (1990). Similarly, the available sulphur showed the decreasing trend with the depth in all the soil profiles under study might be due to greater plant and microbial activities and mineralization of organic matter in surface layer. Similar, results were also reported by Trivedi *et al*. (1998).

The nature and amount of soil organic matter, besides climate/altitude and soil texture, largely determined the content of sulphur forms and their distribution pattern in soil profiles. The results are in agreement with the findings of Tripathi *et al*. (1997), Trivedi *et al*. (2000), Parkash *et al*. (2003) and Ghodke *et al*. (2016).

Conclusion

All the soil profiles except at Mohanghati, were having adequate available S based on 10 mg kg^{-1} S as a limit of deficiency range. Sulphur fractions decreased with increase in the soil depth in all the soil profiles under study. Cropping system receiving higher organic matter as manures resulted in higher content of all the S fractions as compared to intensive cropping system. Organic S is higher than available S that shows the reserve of S in the soil.

Conflict of interest: The authors declare no competing interest.

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Effect of foliar spray of biostimulants on growth of transplanted rice (*Oryza sativa*)

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Abstract

A field experiment was conducted during *kharif* 2022 at the Experimental Farm of CSKHPKV, Palampur (H.P.) to study the effects of foliar spray of biostimulants on the growth of transplanted rice. The experiment which was conducted in Randomized Complete Block Design with thirteen treatments, replicated three times, revealed significant growth improvements with biostimulants *viz*., Terra-Sorb complex, *jeevamrit*, and *panchagavya*. Specifically, a double spray of Terra-Sorb complex 1250 ml ha^{-1} (T_8) increased plant height by 20.7% at 90 days after transplanting (DAT) and 20.2% at harvest compared to the control. Statistically similar results were observed with treatments of 1000 ml ha^{-1}(T_7), 1500 ml ha^{-1}(T_9), and 2000 ml ha^{-1}(T_{10}) doses. Additionally, T_8 enhanced crop growth rates (CGR) by 10.8% (CGR_1) and CGR_2 by 24.5% over the control. A double spray of Terra-Sorb complex 1000 ml ha^{-1}(T_7) is recommended as an optimal dose for improving rice growth.

Keywords: Terra-Sorb complex, *jeevamrit, panchagavya*, biostimulants, transplanted rice, growth parameters

Rice (*Oryza sativa* L.) maintains a prominent position among the world's major food crops because of its greater tolerance to edaphic, climatic, and cultural conditions. More than 100 countries produce rice worldwide, with Asia producing and consuming more than 90 % of the world's rice (Fukagawa and Ziska 2019). In India, rice is cultivated over an area of about 45.77 million ha, yielding a total of 124.37 million tonnes and an average productivity of 2717 kg ha^{-1} (Anonymous 2022a). It is a staple food for majority of the population of Himachal Pradesh (Manuja *et al*. 2015) where it covers 10 % of the total area devoted to cereal production and holds third position in terms of acreage (Anonymous 2022b).

The farmers realize much of their food security from this crop but the low production level needs urgent attention (Sharma *et al*. 2015). Further, India's population is growing rapidly; however, in order to meet the demands of this growing population, we must increase global rice production to 160 million tonnes by 2030 (Mishra *et al*. 2013). Unfortunately, this has increased the reliance of the agricultural sector on chemical fertilisers, degrading the natural resources, particularly the soil and groundwater (Sharma and Sharma 2016; Sharma *et al*. 2003). One of the strategies that can be used to boost crop production and productivity without endangering the environment, is the use of biostimulants (Bulgari *et al*. 2015).

Any microbe or substance given to plants with the intention of enhancing nutritional efficiency, providing tolerance for different types of abiotic stresses, and/or enhancing crop quality attributes is referred to as a plant biostimulant (Jardin 2015). In this quest, "Terra-Sorb complex" is a foliar based new biostimulant spray which is made of L-amino acids and can play a crucial role in plant signaling, C: N metabolism, enhancing ion transport, promoting photosynthesis and encouraging stress tolerance. This product contains a significant amount of free amino acids and a fully balanced proportion of micronutrients (Kocira 2019).

Additionally, cow- based formulations, *viz*., *jeevamrit* and *panchagavya* have been advocated to be used in various crops especially under organic/ natural farming conditions. These act as biostimulants that enhance the nutrient status of the soil and improve crop

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yields (Maity *et al*. 2020).

Materials and Methods

The experiment was conducted at the Research Farm of Department of Agronomy, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur. It is located in the North-Western Himalayas at 32°09' N latitude, 76°54' E longitude and 1290 m above mean sea level. The experiment comprised of thirteen treatments, viz., single spray of Terra-Sorb complex @ 500 ml ha^{-1}[T_1], 750 ml ha^{-1} [T_2], 1000 ml ha^{-1}[T_3] and 1250 ml ha^{-1}[T_4] each at tillering stage, double spray of Terra-Sorb complex @ 500 ml ha^{-1} [T_5], 750 ml ha^{-1} [T_6], 1000 ml ha^{-1} [T_7], 1250 ml ha^{-1} [T_8], 1500 ml ha^{-1}[T_9] and 2000 ml ha^{-1} [T_{10}]) at tillering and boot leaf stages, double spray of *jeevamrit* @ 10 % [T_{11}] at tillering and boot leaf stages, double spray of *panchagavya* @ 5 % [T_{12}] at tillering and boot leaf stages and control [water spray (T_{13})].

During the experimental period, the mean weekly maximum temperature ranged between 34.2°C in the 23rd standard week (4-10 June) and 24.2°C in the 41st standard week (8-14 October). Furthermore, the total rainfall received during the cropping season was 1852.6 mm. The physico-chemical properties of the experimental site have been presented in Table 1.

Data on various parameters were statistically analysed using the method proposed by Gomez and Gomez (1984). The critical difference (CD) was calculated for parameters whose effects were significant at the 5% confidence level.

Table 1. Physico-chemical properties of the soil (0-15 cm) of experimental site

Soil property	Average values
Texture	Silty clay loam
Organic Carbon (g kg^{-1})	7.21
pH	5.6
EC (dsm^{-1})	0.062
Available nitrogen (kg ha^{-1})	287.2
Available phosphorus (kg ha^{-1})	23.2
Available potassium (kg ha^{-1})	246.1

Results and Discussion

Plant height (cm)

A close analysis of the data presented in Table 2 reveals that administration of biostimulants did not significantly affect the plant height at 30 DAT but thereafter, the increase was significant at 60 DAT, 90 DAT and at harvest. At 60 DAT, double spray of Terra-Sorb complex @ 1250 ml ha^{-1} at tillering and boot-leaf stages (T_8) produced significantly taller plants (85.3 cm), though the treatment was at par with double spray of Terra-Sorb complex @ 1000 ml ha^{-1} (T_7), 1500 ml ha^{-1} (T_9) and 2000 ml ha^{-1} (T_{10}) at both the stages and single spray of Terra-Sorb complex @ 1250 ml ha^{-1} at tillering stage only (T_4). Significantly lower plant height was observed in control plots (T_{13}) which was statistically at par with the single spray of Terra-Sorb complex @ 500 ml ha^{-1} (T_1). At 90 DAT and at harvest significantly higher plant height (101.0 cm and 104.8 cm, respectively) was observed with the double spray of Terra-Sorb complex @ 1250 ml ha^{-1} (T_8), though this

Table 2. Effect of biostimulants on plant height (cm) of rice at periodic intervals

Treatments	30 DAT	60 DAT	90 DAT	At harvest
T_1: Terra-Sorb complex (500 ml ha^{-1})*	55.3	75.4	88.2	92.2
T_2: Terra-Sorb complex (750 ml ha^{-1})*	56.3	78.4	92.7	95.1
T_3: Terra-Sorb complex (1000 ml ha^{-1})*	56.3	81.2	93.3	96.3
T_4: Terra-Sorb complex (1250 ml ha^{-1})*	56.7	83.5	94.1	97.6
T_5: Terra-Sorb complex (500 ml ha^{-1})**	55.2	79.8	93.6	96.5
T_6: Terra-Sorb complex (750 ml ha^{-1})**	56.2	80.5	95.2	99.7
T_7: Terra-Sorb complex (1000 ml ha^{-1})**	57.3	83.5	97.8	101.4
T_8: Terra-Sorb complex (1250 ml ha^{-1})**	56.9	85.3	101.0	104.8
T_9: Terra-Sorb complex (1500 ml ha^{-1})**	56.0	83.7	98.7	102.3
T_{10}: Terra-Sorb complex (2000 ml ha^{-1})**	57.3	84.6	99.5	103.7
T_{11}: Jeevamrit (10 %)**	54.5	81.3	94.6	97.7
T_{12}: Panchagavya (5 %)**	55.8	81.4	95.2	98.1
T_{13}: Control (Water spray)	54.1	74.5	83.7	87.2
SEm±	1.1	1.3	1.5	1.6
CD (P=0.05)	NS	3.8	4.4	4.7

*Application at tillering stage ** Application at tillering and boot-leaf stages
DAT- Days after transplanting

treatment was at par with the double spray of Terra-Sorb complex @ 1000 ml ha^{-1} (T_7), 1500 ml ha^{-1} (T_9) and 2000 ml ha^{-1} (T_{10}). Control treatment showed significantly lowest plant height (83.7 cm and 87.2 cm), at 90 DAT and at harvest, respectively. No significant increase in plant height observed at 30 DAT could be attributed to the first spray of biostimulants being done at 28 DAT, which might have not reflected on plant height.

The increase in plant height at 60 and 90 DAT and at harvest could be a result of amino acids present in Terra-Sorb complex. Amino acids being building blocks of proteins, act as precursors of plant hormones like gibberellin and auxins which might have stimulated internode and cell elongation. Similarly, higher plant height in wheat was recorded when supplied with amino acids due to their possible roles in cell enlargement and division (Navarro *et al*. 2021; Ghodake *et al*. 2022).

Crop Growth Rate (g m^{-2} day^{-1})

Data pertaining to crop growth rate (CGR) have been presented in Table 3. A close perusal of data revealed that CGR values were higher between 60 and 90 DAT than between 30 and 60 DAT, indicating that greater dry matter build-up occurred between 60 and 90 DAT. Significantly higher CGR_1(11.59 g m^{-2} day^{-1}) was recorded in T_8 with spray of Terra-Sorb complex @ 1250 ml ha^{-1} each at tillering and boot-leaf stages which was, however, at par with all other treatments except T_1 (single spray of Terra-Sorb complex@ 500 ml ha^{-1} at tillering stage) and control.

Significantly lower CGR_1 was observed under control though it was at par with T_1, T_2, T_5, T_6, T_{11} and T_{12}. Similarly, significantly higher CGR_2 (18.81 g m^{-2} day^{-1}) was recorded in T_8 (spray of Terra-Sorb complex @ 1250 ml ha^{-1} each at tillering and boot-leaf stages) which was statistically at par with spray of Terra-Sorb complex @ 1000 ml ha^{-1} (T_7), 1500 ml ha^{-1} (T_9) and 2000 ml ha^{-1} (T_{10}) at both the stages. Terra-Sorb complex contains free amino acids, which not only provided the plants with organic nitrogen but also promoted nitrogen absorption and assimilation. This enhanced leaf nitrogen content may have increased photosynthesis and photosynthate translocation, resulting in higher plant biomass (Colla *et al*. 2014) and growth, therefore, resulting in higher crop growth rate. Similar results were obtained with the application of seaweed based biostimulants by Nayak *et al*. (2020).

Table 3. Effect of biostimulants on CGR of rice at periodic intervals

Treatments	CGR_1(g m^{-2} day^{-1})	CGR_2(g m^{-2} day^{-1})
T_1: Terra-Sorb complex (500 ml ha^{-1})*	10.88	14.97
T_2: Terra-Sorb complex (750 ml ha^{-1})*	11.04	15.52
T_3: Terra-Sorb complex (1000 ml ha^{-1})*	11.29	15.59
T_4: Terra-Sorb complex (1250 ml ha^{-1})*	11.48	15.70
T_5: Terra-Sorb complex (500 ml ha^{-1})**	10.96	16.86
T_6: Terra-Sorb complex (750 ml ha^{-1})**	11.03	17.00
T_7: Terra-Sorb complex (1000 ml ha^{-1})**	11.38	18.68
T_8: Terra-Sorb complex (1250 ml ha^{-1})**	11.59	18.81
T_9: Terra-Sorb complex (1500 ml ha^{-1})**	11.54	18.42
T_{10}: Terra-Sorb complex (2000 ml ha^{-1})**	11.56	18.59
T_{11}: *Jeevamrit* (10 %)**	10.97	16.86
T_{12}: *Panchagavya* (5 %)**	11.07	17.04
T_{13}: Control (Water spray)	10.46	15.11
SEm±	0.22	0.39
CD (P=0.05)	0.63	1.13

*Application at tillering stage ** Application at tillering and boot-leaf stages

CGR_1: Crop growth rate from 30 to 60 DAT, CGR_2: Crop growth rate from 60 to 90 DAT

Relative Growth Rate (mg g^{-1} day^{-1})

A close perusal of data (Table 4) revealed that the applied biostimulants did not significantly affect the relative growth rate from 30 to 60 DAT (RGR_1) but thereafter, the effect was significant from 60 to 90 DAT. Significantly higher RGR_2 was obtained with double spray of Terra-Sorb complex @ 750 ml ha^{-1} (28.8 mg g^{-1} day^{-1}) which was at par with T_5, T_6, T_8, T_9, T_{10}, T_{11} and T_{12}. The reason for this increase could be the enhancement of photosynthetic efficiency, as biostimulants have been shown to improve chlorophyll content and photosynthetic rate (Calvo *et al*. 2014).

Conclusion

The field experiment demonstrated that the administration of biostimulants, particularly the double spray of Terra-Sorb complex at 1250 $mlha^{-1}$, significantly improved plant height and crop growth rate (CGR) of rice, especially from 60 DAT onwards. At 60, 90 DAT, and harvest, Terra-Sorb complex treatments resulted in significantly taller plants and higher CGR compared to control. The increase in plant height and CGR was attributed to the amino acids in Terra-Sorb complex, which promote hormone production and enhance nitrogen absorption and assimilation, thereby improving photosynthesis and biomass accumulation. Relative growth rate (RGR) also improved significantly from 60 to 90 DAT with the application of Terra-Sorb complex, highlighting its effectiveness. Consequently, a double spray of Terra-Sorb complex at 1000 $mlha^{-1}$is recommended for optimal rice growth.

Table 4. Effect of biostimulants on RGR of rice at periodic intervals

Treatments	RGR_1 (mg g^{-1} day^{-1})	RGR_2 (mg g^{-1} day^{-1})
T_1: Terra-Sorb complex (500 ml ha^{-1})*	60.38	25.56
T_2: Terra-Sorb complex (750 ml ha^{-1})*	60.81	25.94
T_3: Terra-Sorb complex (1000 ml ha^{-1})*	60.85	25.64
T_4: Terra-Sorb complex (1250 ml ha^{-1})*	61.14	25.49
T_5: Terra-Sorb complex (500 ml ha^{-1})**	59.98	27.54
T_6: Terra-Sorb complex (750 ml ha^{-1})**	59.65	27.52
T_7: Terra-Sorb complex (1000 ml ha^{-1})**	60.76	28.80
T_8: Terra-Sorb complex (1250 ml ha^{-1})**	61.43	28.69
T_9: Terra-Sorb complex (1500 ml ha^{-1})**	61.24	28.35
T_{10}: Terra-Sorb complex (2000 ml ha^{-1})**	62.25	28.61
T_{11}: *Jeevamrit* (10 %)**	60.15	27.55
T_{12}: *Panchagavya* (5 %)**	61.07	27.66
T_{13}: Control (Water spray)	59.93	26.38
SEm±	1.81	0.62
CD (P=0.05)	NS	1.81

*Application at tillering stage ** Application at tillering and boot-leaf stages

RGR_1: Relative growth rate from 30 to 60 DAT, RGR_2: Relative growth rate from 60 to 90 DAT

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Short Communication

Effect of biofertilizers and fertility levels on phenology, agronomic efficiency and crop recovery efficiency of gobhi sarson (*Brassica napus* L.)

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Abstract

A field experiment was conducted during *rabi* 2022-23 at CSKHPKV Shivalik Agricultural Research and Extension Centre, Kangra (H.P.) to study the influence of fertility levels viz. control, 75% of recommended dose of fertilizer (RDF) and 100% RDF in main plots and seed inoculations with six liquid biofertilizers *viz. Azotobacter*, Phosphate solubilizing microorganism (PSMO), Potassium mobilizing biofertilizer (KMB), NPK consortia + Zinc solubilizing biofertilizer (ZSB), ZSB and control (no biofertilizer) as subplots of split plot design in gobhi sarson "GSC-7" on phenological stages and agronomic as well as crop recovery efficiency. Seed inoculation with liquid biofertilizers was done by soaking the seeds for 30 minutes in liquid biofertilizers procured from IFFCO. Application of 100% RDF recorded early flowering (79.6) and physiological maturity (165.5) being at par with 75% RDF compared to control. The higher values of agronomic efficiency and crop recovery efficiency were recorded in75% RDF and 100%RDF, respectively. Among biofertilizers, PSMO and *Azotobacter* recorded higher agronomic efficiency whereas crop recovery efficiency for total NPK was more with *Azotobacter* seed inoculation. The results indicated that seed inoculation with biofertilizers showed positive influence on phonological characteristics, agronomic as well as crop recovery efficiency. Amongst biofertilizers, *Azotobactor* was found to be the most efficient followed by PSMO. Thus, *gobhi sarson* seed treatment with liquid biofertilizer*Azotobacter*/PSMO and application of 120 kg N, 60 kg P_2O_5 and 40 kg K_2O/ha is advisable.

Keywords: Agronomic efficiency, *Azotobacter*, Biofertilizers, Crop recovery efficiency, *Gobhi sarson*, Phenology

India's population is projected to be 1.48 billion in 2030. Moreover, per capita edible oil consumption is also increasing. India's demand for edible oil is expected to grow at an annual rate of 3.54% between 2011 and 2030, resulting in a projected increase in per capita consumption from 13.4 kg/annum to 23.1 kg/annum by 2030 (DRMR, 2011). During 2020-21 area, production and productivity of rapeseed-mustard in the world was 34.89 million hectares, 69.23 million tonnes and 1980 kg/ha while in India it was 6.69 million hectares,10.11 million tonnes and 1511 kg/ha, respectively. Globally, India account for 19.8 % and 9.8% of the total acreage and production. In Himachal Pradesh, the average productivity of rapeseed-mustard crop is 650 kg/ha (Anonymous 2019 and Anonymous 2021).

Rapeseed-mustard crops have high nutrient requirements, but they are mainly grown by small and marginal farmers who struggle to access necessary resources. These crops are generally grown on marginal and poor fertility soils. Consequently, the growth potential of rapeseed-mustard is constrained, despite its high nutrient demands. Integrated nutrient management is crucial for overcoming limitations and enhancing production in rapeseed-mustard crops (Shekhawat *et al*.2012; Kumar 2012).

Biofertilizers are the potential source for the supply of nutrients at low cost and may prove as an important component of Integrated Nutrient Management (INM) system in oilseed crops. Biofertilizers play a vital role

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in enhancing plant growth by converting unavailable plant nutrients into accessible forms through various mechanisms. They facilitate nitrogen supply through biological nitrogen fixation (BNF), solubilize micro and macro elements, mobilize nutrients and synthesize plant growth-promoting hormones (Sharma *et al* 2016). Moreover, they provide protection against soil-borne diseases. Due to their eco-friendly nature, lack of hazards and cost-effectiveness, biofertilizers have gained popularity as an excellent supplement to chemical fertilizers in modern agriculture (Kumawat 2017).

A field experiment was conducted during *rabi* 2022-23 at CSKHPKV Shivalik Agricultural Research and Extension Centre (SAREC), Kangra, India. The soil of field experimentation was clay loam in texture having pH 5.61. The soil sample taken prior to experiment was low in available nitrogen (275.7 kg/ha) whereas medium in available phosphorus (18.3 kg/ha) and available potassium (227.4 kg/ha).The experiment was laid out in split plot design allocating fertility level in main plots, microbial consortia in subplots and replicated three times. The experiment consisted eighteen combinations of three main plot treatments *viz*. control, 75% recommended dose of fertilizer (RDF) and 100% RDF, and six subplot treatments (*Azotobacter*, Phosphate solubilizing microorganism (PSMO), Potassium mobilizing biofertilizer (KMB), Zinc solubilizing biofertilizer (ZSB), NPK consortia + ZSB and control). Seed inoculation with liquid biofertilizers was done by soaking the seeds for 30 minutes in liquid biofertilizers and then dried in shade for half an hour before sowing in field plots of gross plot area of 11.76 cm^2. The nitrogen was supplied by IFFCO (12:32:16) and urea whereas the source of potash was the muriate of potash (MOP). As per main plot treatments, full dose of phosphorus and potassium along with one third dose of nitrogen was applied as basal dressing. The remaining dose of nitrogen was given by urea at vegetative and flowering stage. The recommended dose of fertilizer was 120 kg N, 60 kg P_2O_5 and 40 kg K_2O/ha. The manual sowing of the 'GSC-7' variety was conducted using the *kera* method, with row spacing of 30 cm and plant-plant spacing of 10 cm. The seed rate was 6 kg per hectare. The date on which 50 per cent plants in the net plot had at least one open flower was recorded and number of days taken to 50 per cent flowering was calculated from the date of sowing. Physiological maturity was considered when stem of selected plants turned yellow and siliquae were ripe from sowing to maturity. Agronomic efficiency indicates kg crop yield increase per kg nutrient applied. During study, agronomic efficiency calculated by adopting the following formula (López-Bellido and López-Bellido 2001) and mathematically this can be expressed as:

Agronomic efficiency

$$= \frac{\text{Yield (kg/ha) in fertilized soil - Yield (kg/ha) in unfertilized soil}}{\text{Quantity of fertilizer supplied (kg/ha)}}$$

Crop recovery efficiency indicates that kg increase in nutrient uptake per kg nutrient applied. It was calculated for each primary nutrient and computed by using following formula:

Crop recovery efficiency

$$= \frac{\text{Nutrient uptake (kg/ha) in fertilized soil - Nutrient uptake (kg/ha) in unfertilized soil}}{\text{Amount of nutrients applied (kg/ha)}}$$

The experimental data were analyzed by using the ANOVA (Analysis of variance) techniques as explained by Cochran and Cox (1957) using t-test at a significance level of 5%. The critical difference (CD) method was used to determine the significant difference between the treatments. Data analysis was undertaken in OPSTAT http://14.139.232.166/opstat/twofactor.html?flavor=Two+Factors+Analysis software.

The application of 100% recommended dose of fertilizer (RDF) hastened days to attain 50% flowering (79.6) and physiological maturity (165.5) being at par with 75% RDF compared to control (Table 1). The reason for early flowering and physiological maturity with100% RD Fmight be attributed to the adequate supply of essential nutrients, particularly N, P and K which play crucial roles in various physiological processes within the plant including cell division, flower bud initiation and overall plant development (Sharma *et al*. 2018). The results are in conformity with findings of Rana *et al*. (2021), Mankotia *et al*. (2022) and Shilpa *et al*. (2022).

Seed inoculation with Azotobacter showed earlier 50% flowering (79.1) and physiological maturity (165.1) being at par with PSMO treatment. Biofertilizers promote early physiological maturity in

Table 1: Effect of fertility levels and microbial consortia on phonological stages of *gobhi sarson*

	Treatment	Days to 50% flowering	Days taken to physiological maturity
Fertility levels			
F_1	Control (no fertilizer)	84.3	169.4
F_2	75% RDF	80.7	166.7
F_3	100% RDF	79.6	165.5
	SE m±	0.3	0.6
	CD (P=0.05)	1.4	2.6
Microbial consortia			
T_1	*Azotobacter*	79.1	165.1
T_2	PSMO	80.1	166.2
T_3	KMB	82.5	168.1
T_4	ZSB	82.5	168.0
T_5	NPK consortia + ZSB	81.3	166.9
T_6	Control (no biofertilizer)	83.6	168.7
	SE m±	0.4	0.6
	CD (P=0.05)	1.1	1.7

RDF: Recommended dose of fertilizer; PSMO: Phosphate solubilizing microorganism; KMB: Potassium mobilizing biofertilizer; ZSB: Zinc solubilizing

plants by enhancing nitrogen fixation (*Azotobacter*), phosphorus solubilization (PSMO), hormonal regulation and nutrient uptake efficiency. These beneficial effects contribute to accelerated growth, development and reproductive processes, ultimately leading to early maturation of the plants. Rekha (2020) also reported that early physiological maturity was observed in biofertilizer treatments over control (no biofertilizer).

Agronomic efficiency at 100% RDF (8.5 kg seed/kg N, 17.1 kg seed/kgP, 25.6 kg seed/kg K applied) was significantly less than that at 75% RDF which can be ascribed to the law of diminishing returns *i.e.* less response at higher level (Chandel *et al*. 2023). Similar results were found by Karim and Ramasamy (2002). Among different biofertilizers,

Table 2: Effect of fertility levels and microbial consortia on agronomic efficiency of gobhi sarson

	Treatment	Agronomic efficiency (kg/kg)			
		Nitrogen	Phosphorus	Potassium	Total NPK
Fertility levels					
F_1	75 % RDF	10.17	20.34	30.51	5.55
F_2	100 % RDF	8.54	17.07	25.61	4.66
	SE m±	0.10	0.18	0.27	0.05
	CD (P=0.05)	0.60	1.18	1.77	0.32
Microbial consortia					
T_1	Azotobacter	9.48	18.96	28.44	5.17
T_2	PSMO	10.12	20.25	30.37	5.52
T_3	KMB	9.01	18.03	27.04	4.92
T_4	ZSB	9.00	18.00	27.00	4.91
T_5	NPK consortia + ZSB	9.21	17.42	27.63	5.02
T_6	Control (no biofertilizer)	9.30	18.60	27.90	5.07
SE m±	0.38	0.75	1.13	0.20	
CD (P=0.05)	NS	NS	NS	NS	

RDF: Recommended dose of fertilizer; PSMO: Phosphate solubilizing microorganism; KMB: Potassium mobilizing biofertilizer; ZSB: Zinc solubilizing biofertilizer

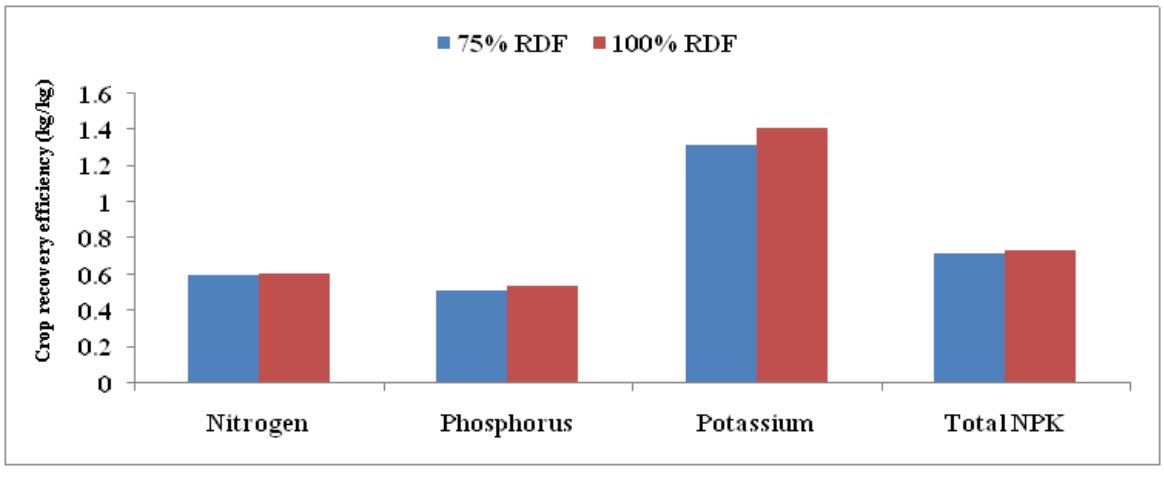

Fig. 1: Effect of fertility levels on crop recovery efficiency

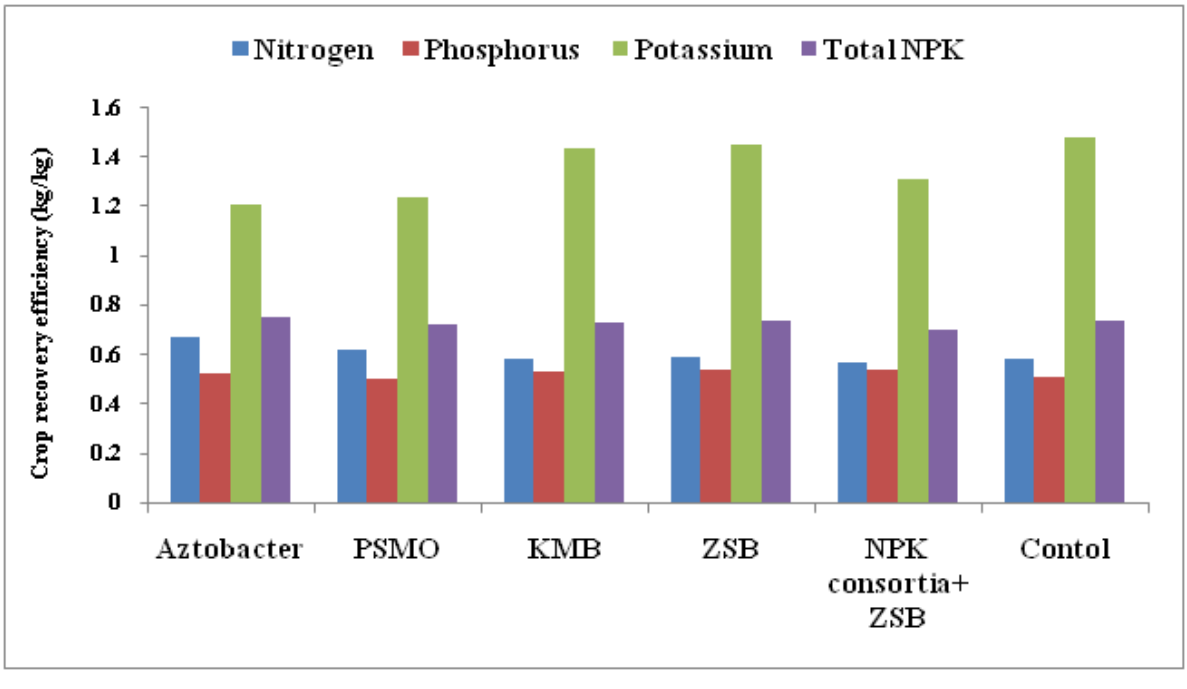

Fig. 2: Effect of fertility levels and microbial

Azotobacter and PSMO recorded numerically higher values of Agronomic efficiency than that in control (no inoculation) indicating their better efficiency in fertilized plots.

Crop recovery efficiency in plants refers to the ability of a crop to effectively utilize applied nutrients, particularly fertilizer nutrients, for optimal growth and yield production. Their higher values were observed with 100% RDF than 75% RDF. In biofertilizer treatments, the value ranged for nitrogen between 0.57-0.67 and phosphorus 0.50-0.54 whereas for total NPK, value ranged between 0.70-0.75.

Based on the findings of the investigation it may be concluded that application of 100% RDF took less days to achieve 50% flowering, physiological maturity and numerically higher crop recovery efficiency where as agronomic efficiency with 100% RDF was lower than 75% RDF. Seed inoculation with biofertilizers

recorded earlier flowering & physiological maturity of *gobhi sarson* where as, no significant influence on agronomic as well as crop recovery efficiency. Among biofertilizers, *Azotobacter* and PSMO were found to be most efficient. Hence, application of *Azotobacter* (biofertilizer) along with 100% RDF may be more preferable and can be recommended.

Conflict of interest: There is no conflict of interest in this research paper.

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Diversity and abundance of Chrysomelids associated with rice under natural farming in mid hill conditions of Himachal Pradesh

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Abstract

The study on diversity and abundance of chrysomelids associated with rice was conducted in mid-hill regions of Himachal Pradesh during *kharif* 2022 and 2023, identified eight chrysomelid species, their abundance and diversity using various indices under natural farming. Diversity of chrysomelid fauna was found higher at Palampur region with all the eight species recorded during the crop growth period as compared to Jogindernagar, where only five species were found associated with rice ecosystem during both the cropping years. Among these identified species, *Chaetocnema gracilis* and *C. nigrica* were found to be predominant in both the locations surveyed. Findings revealed seasonal variations in chrysomelid abundance, with peak population in the month of July and August, emphasizing critical periods for pest management interventions. Correlation analysis indicates intriguing patterns, such as positive influences of rainfall on certain pest species and varying correlations with maximum temperature. This study contributes essential insights into chrysomelid beetles under natural farming conditions in Himachal Pradesh and their relation to the abiotic factors prevailing in particular area.

Key words: Rice, chrysomelids, abundance, diversity, correlation, temperature, rainfall

Rice (*Oryza sativa* L.) is one of the most important cereal crops and staple food for about half of the world's population. Asia covers 90 per cent of global rice production and consumption in World. Therefore, rice production in Asia is the key for global food security. India is the second largest producer of rice in world after China, with a cultivated area of about 46.38 million hectares and a production of 130.29 million tonnes (Anonymous 2023).

Rice is the source of income and employment for many states of India and other countries. So, there is a need to increase the rice production to meet the requirement of ever-increasing population of the world (Miao *et al*. 2011). In Himachal Pradesh rice is mostly cultivated under lowland conditions, however in hilly areas of the state rice is being produced in upland conditions (Garkoti and Pandey 2022). The quality and production of rice crop is considerably affected by an array of insect- pests infesting at various stages of crop growth. The dynamics of insect pests in rice ecosystem have undergone significant changes in recent times, with several previously considered minor pests now emerging as major threats. One such example is the rice hispa, *Dicladispa armigera* (Olivier) (Coleoptera: Chrysomelidae), which has emerged as a significant concern due to its amplified impact on rice cultivation in India (Sharma *et al*. 2014). This pest's prevalence has notably risen across various rice-growing regions, with Kangra valley of Himachal Pradesh experiencing recurrent outbreaks in recent years (Sharma *et al.* 2012). Other than rice hispa, various flea beetle species of family Chrysomelidae infest rice mainly during the vegetative stage. A significant number of adults tend to congregate on rice plants causing long and narrow scrapings on the leaves, mostly observed in non-flooded rice. While these tiny beetles cause low levels of defoliation, hence are not generally regarded as a major pest in most rice-growing regions (Shepard 1995). However, certain flea beetles such as

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Chaetocnema spp. are important vector of viruses like rice yellow mottle virus. This viral infection can lead to severe consequences, causing up to 80-100 per cent yield losses in rice (Iannella *et al*. 2021).

The changing climate is known to have profound effects on the survival, development and distribution of insect-pests, thus influencing population dynamics of the same. Therefore, thorough understanding of correlation between insect pests and climatic factors is crucial for effective weather-based pest forecasting. Farming system tends to affect the diversity of insects and natural enemies in a crop ecosystem (Gallo and Pekar 1999). To encompass the adverse effects of conventional farming, recently a farming system known as "natural farming: a climate resilient type of farming" is being practiced. A plenty of information is available on the insect-pests of rice throughout the country but very little is known about the diversity of chrysomelids under natural farming. Therefore, this study aims to examine the abundance and establish correlations between key weather factors (temperature, humidity, rainfall) and chrysomelids associated with rice under natural farming in the state.

Materials and Methods

A survey was conducted in farmer's fields growing rice in mid hill regions (Zone II) of Himachal Pradesh to record the seasonal abundance of chrysomelid beetles of family Chrysomelidae under natural farming during *kharif* seasons of 2022 and 2023. Two locations namely, Palampur and Jogindernagar were surveyed and at each location, different localities namely Holta, Batolu, Chatter, Masoli and Majharnu were selected to record the abundance of chrysomelid fauna associated with rice at various crop growth stages. The data were recorded with the help of sweep net sampling method in each locality at fortnightly intervals starting from the vegetative stage and was continued up to maturity. The incidence of pests was recorded on 10 hills of each five quadrats ($1m^2$) selected in each locality. The collected insect fauna was preserved in 70 per cent ethyl alcohol and were got identified from Kerala Agricultural University, Thrissur (Plate 1). The weather data were obtained from the Agro-meteorological observatory of the Department of Agronomy, CSK HPKV, Palampur to work out the correlation between the insect-pests and abiotic factors of Palampur region.

For estimating the diversity of chrysomelid specimens under natural farming, the data of all the localities for each location were combined together. The following formulae was used:

$$\text{Relative proportion of } i^{th} \text{ species} = \frac{\text{Total number of individuals of } i^{th} \text{ species}}{\text{Total number of individuals of all the species}} \times 100$$

The species diversity on rice was evaluated at fortnightly intervals across both seasons. The assessment utilized multiple indices, including Shannon diversity index (H), Evenness index (J) and Simpson index (D) as determined by the respective formulas provided by Shannon (1948) as under:

Shannon diversity index (H) = $- \sum p_i \log_e p_i$;

where p_i = fraction of i^{th} species

Species evenness (J) = $\frac{H}{H_{max.}}$

Species dominance (D)= $1/\sum pi^2$

Results and Discussion

Distribution of chrysomelids associated with rice under natural farming

A total of eight species of insects belonging to family Chrysomelidae of order Coleoptera were recorded and identified from five localities namely, Holta, Batolu, Chatter, Majharnu and Masoli (Table 1)

Table 1. Distribution of chrysomelids recorded in rice ecosystem under mid hill conditions of Himachal Pradesh

Sr. No.	Species	Locations surveyed				
		Palampur		Jogindernagar		
		Holta	Batolu	Chatter	Majharnu	Masoli
1	*Dicladispa armigera*	+	-	+	-	+
2	*Rhadinosa* sp.	+	-	-	-	+
3	*Monolepta signata*	+	+	+	-	+
4	*Chaetocnema gracilis*	+	+	+	-	+
5	*Chaetocnema nigrica*	+	+	+	-	+
6	*Chaetocnema cognate*	+	-	-	-	-
7	*Monolepta* sp.	+	-	-	-	-
8	*Medythia suturalis*	+	-	-	-	-

+ Indicates presence -Indicates absence

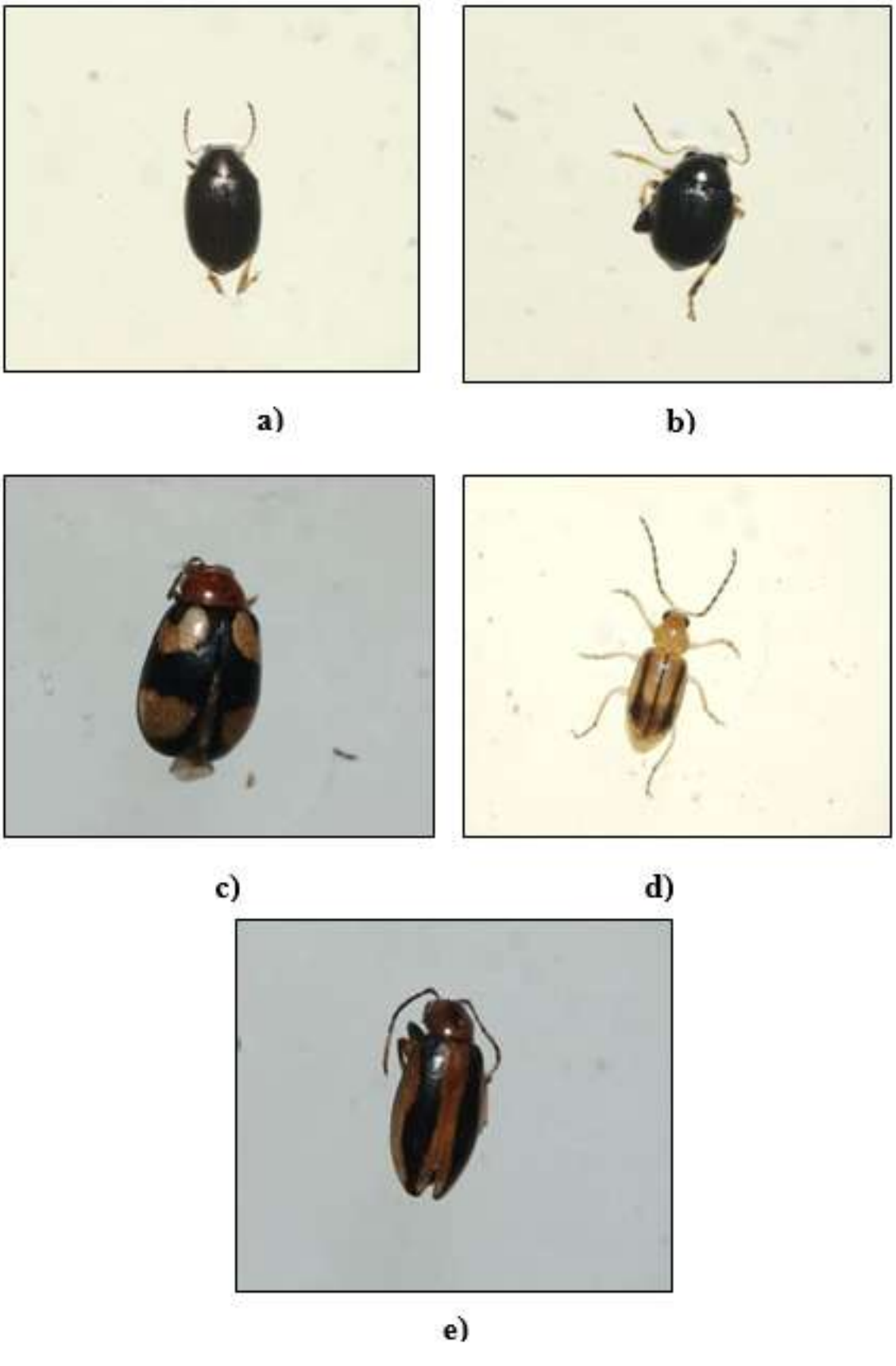

Plate 1. Chrysomelid fauna a) ***Chaetocnema gracilis*** **b)** ***Chatocnema nigrica*** **c)** ***Monolepta signata*** **d)** ***Monolepta*** **sp. e)** ***Medythia suturalis***

under natural farming conditions. All the species were recorded throughout the crop growth period at Holta, however no chrysomelid was observed at Majharnu. *Dicladispa armigera* was observed at Holta, Chatter and Masoli, whereas *Rhadinosa* sp. was present only at Holta and Masoli. *Monolepta signata*, *Chaetocnema gracilis* and *C. nigrica* were present at all the localities except Majharnu. However, *C. cognate, Monolepta* sp. and Medythia suturalis were observed only at Holta.

Diversity and abundance of chrysomelids associated with rice under natural farming

Palampur

Chaetocnema gracilis and *C. nigrica* were dominant among the eight chrysomelids recorded from rice ecosystem, with relative proportion of 42.53 and 31.39 during 2022 and 42.12 and 30.16 per cent during 2023, respectively which started appearing from 2nd fortnight of June during both the years (Table 2 and 3). The activity of chrysomelids was observed from 2nd fortnight of June to 1st fortnight of October with peak population density of 90 adults during 2022 and 78 adults / 15 sweeps during 2023 in the 2nd fortnight of July.

Data pertaining to diversity indices during 2022 and 2023 at Palampur revealed that value of Shannon (H) and Simpson index (D) was recorded maximum during 1st fortnight of August (1.77 and 4.85), during 2022 and 1.74 and 4.94, during 2023 which indicates maximum diversity and dominance of insect-pests, respectively. The chrysomelid species were more evenly distributed during 2nd fortnight of July in 2022 and 2nd fortnight of September in 2023.

Jogindernagar

The perusal of data presented in Table 4 and Table 5 revealed the presence of five chrysomelid species encountered from three localities of Jogindernagar. The adult population of chrysomelids commenced during 2nd fortnight of August with peak density level of 35 adults/ 15 sweeps during 2022 (Table 4). Similarly, in 2023 the activity of chrysomelids was observed from August to October with maximum population of 40 adults/ 15 sweeps during 2nd fortnight of July, which declined towards maturity (Table 5). Relative proportion of adult catch was recorded higher for *C. gracilis* with the values of 39.29 and 41.04 per cent followed by *C. nigrica* with 27.38 and 30.60 per cent relative proportion during 2022 and 2023,

Table 2. Diversity and abundance of chrysomelids in rice ecosystem at Palampur during 2022 under natural farming

S. No.	Species	Adults caught per 15 sweeps											
		June I	June II	July I	July II	Aug I	Aug II	Sept I	Sept II	Oct I	Oct II	Total	Relative proportion (%)
1	*Dicladispa armigera*	0.00	0.00	2.00	0.00	3.00	5.00	6.00	3.00	3.0	0.0	22.0	5.57
2	*Rhadinosa* sp.	0.00	0.00	6.00	0.00	3.00	6.00	0.00	3.00	0	0	18.0	4.56
3	*Monolepta signata*	0.00	3.00	0.00	0.00	12.0	9.00	9.00	0.00	0	0	33.0	8.35
4	*Chaetocnema gracilis*	0.00	39.0	18.0	48.0	21.0	15.0	18.0	9.00	0	0	168.0	42.53
5	*Chaetocnema nigrica*	0.00	22.0	12.0	42.0	18.0	15.0	12.0	3.00	0	0	124.0	31.39
6	*Chaetocnema cognate*	0.00	0.00	0.00	0.0	6.00	0.00	3.00	3.00	0	0	12.0	3.04
7	*Monolepta* sp.	0.00	0.00	6.00	0.0	3.00	0.00	0.00	3.00	0	0	12.0	3.04
8	*Medythia suturalis*	0.00	3.00	0.00	0.0	3.00	0.00	0.00	0.00	0	0	6.0	1.52
	Total	**0.00**	**67.0**	**44.0**	**90.0**	**69.0**	**50.0**	**48.0**	**24.0**	**3.0**	**0.0**	**395**	**100.00**
	Shannon index (H)	0.00	0.00	1.40	0.69	1.77	1.52	1.46	1.67	0.0	0.0		
	Evenness (J)	0.00	0.00	0.87	1.00	0.85	0.94	0.91	0.93	0.0	0.0		
	Dominance (D)	0.00	2.22	3.56	1.99	4.85	4.22	3.88	4.57	1.0	0.0		

I: First fortnight II: Second fortnight

Table 3. Diversity and abundance of chrysomelids in rice ecosystem at Palampur during 2023 under natural farming

S. No.	Species	Adults caught per 15 sweeps											
		Jun I	Jun II	Jul I	Jul II	Aug I	Aug II	Sep I	Sep II	Oct I	Oct II	Total	Relative proportion (%)
1	*Dicladispa armigera*	0	0	0	0	9	0	3	9	0	0	21	5.71
2	*Rhadinosa* sp.	0	0	0	0	6	3	0	0	3	0	12	3.26
3	*Monolepta signata*	0	0	0	6	9	24	6	0	0	0	45	12.23
4	*Chaetocnema gracilis*	0	41	18	42	21	15	12	6	0	0	155	42.12
5	*Chaetocnema nigrica*	0	36	12	24	15	12	6	3	3	0	111	30.16
6	*Chaetocnema cognate*	0	0	0	0	3	0	3	3	0	0	9	2.45
7	*Monolepta* sp.	0	0	3	0	3	0	0	3	0	0	9	2.45
8	*Medythia suturalis*	0	0	0	6	0	0	0	0	0	0	6	1.63
Total		**0**	**77**	**33**	**78**	**66**	**54**	**30**	**24**	**6**	**0**	**368**	**100.00**
	Shannon index (H)	0	0	0.9	1.1	1.7	1.2	1.5	1.5	0	0		
	Evenness (J)	0	0	0.8	0.8	0.9	0.9	0.9	0.9	0	0		
	Dominance (D)	0	2	2.9	2.5	4.9	3.1	3.9	4.0	2	0		

I: First fortnight II: Second fortnight

Table 4. Diversity and abundance of chrysomelids in rice ecosystem at Jogindernagar during 2022 under natural farming

Sr. No.	Species	Adults caught per 15 sweeps*					
		Aug II	Sept I	Sept II	Oct I	Total	Relative proportion (%)
1	*Dicladispa armigera*	3.00	3.00	2.00	2.00	10.00	11.90
2	*Rhadinosa* sp.	2.00	0.00	1.00	0.00	3.00	3.57
3	*Monolepta signata*	7.00	8.00	0.00	0.00	15.00	17.86
4	*Chaetocnema gracilis*	12.00	14.00	7.00	0.00	33.00	39.29
5	*Chaetocnema nigrica*	11.00	9.00	3.00	0.00	23.00	27.38
6	*Chaetocnema cognate*	0.00	0.00	0.00	0.00	0.00	0.00
7	*Monolepta* sp.	0.00	0.00	0.00	0.00	0.00	0.00
8	*Medythia suturalis*	0.00	0.00	0.00	0.00	0.00	0.00
	Total	**35.00**	**34.00**	**13.00**	**2.00**	**84**	**100.00**
	Shannon index (H)	0.00	1.27	1.16	0.00		
	Evenness (J)	0.00	0.92	0.83	0.00		
	Dominance (D)	3.75	3.30	2.68	1.00		

I: First fortnight II: Second fortnight *Observations are mean of four localities

Table 5. Diversity and abundance of chrysomelids in rice ecosystem at Jogindernagar during 2023 under natural farming

Sr. No.	Species	Adults caught per 15 sweeps*							
		July II	Aug I	Aug II	Sept I	Sept II	Oct I	Total	Relative proportion (%)
1	*Dicladispa armigera*	1.00	2.00	0.00	1.00	3.00	0.00	7.00	5.22
2	*Rhadinosa* sp.	0.00	1.00	1.00	0.00	0.00	0.00	2.00	1.49
3	*Monolepta signata*	3.00	6.00	17.00	3.00	0.00	0.00	29.00	21.64
4	*Chaetocnema gracilis*	21.00	14.00	10.00	7.00	3.00	0.00	55.00	41.04
5	*Chaetocnema nigrica*	15.00	11.00	8.00	4.00	2.00	1.00	41.00	30.60
6	*Chaetocnema cognate*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7	*Monolepta* sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8	*Medythia suturalis*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Total	**40.00**	**34.00**	**36.00**	**15.00**	**8.00**	**1.00**	**134**	**100.00**
	Shannon index (H)	0.00	1.31	1.14	1.21	1.08	0.00		
	Evenness (J)	0.00	0.81	0.83	0.87	0.99	0.00		
	Dominance (D)	2.37	3.23	2.85	3.00	2.91	1.00		

I: First fortnight II: Second fortnight *Observations are mean of four localities

respectively.

The data on diversity of chrysomelids associated with rice under natural farming at Jogindernagar during 2022 revealed the maximum value of Shannon index (H) at 1st fortnight of September (1.27), whereas during 2023 diversity was highest during 1st fortnight of August (1.31) (Table 4 and 5). Dominance of chrysomelids was highest at 2nd fortnight of August (3.75) in 2022 and at 1st fortnight of August (3.23) in 2023. However, species were more evenly distributed during the month of September.

The present findings on population buildup of rice hispa are strongly supported by the findings of Choudhary *et al*. (2001) from Himachal Pradesh who reported maximum population of hispa in the month of August. The current observations are in proximity with those of Bhattacharjee and Ray (2010) and Chakraborty and Deb (2012), who reported the higher abundance of hispa in Assam and West Bengal in September. Incidence of *M. signata* in the present findings corroborates to the earlier findings of Kumar *et al*. (2018) from Nepal, who reported that population initiated during July and reached to its maximum during the month of August.

Correlation between incidence of chrysomelid adults and various abiotic factors during 2022-23

Chysomelid insect-pests associated with rice were correlated with various weather parameters prevailing at Palampur during 2022 and 2023 and have been presented in Table 6. The data revealed that the pest

Table 6. Correlation between incidence of chrysomelid adults and various abiotic factors during 2022-23

Weather parameters	Year	Correlation coefficient (r)							
		Dicladispa armigera	*Rhadinosa* sp.	*Monolepta signata*	*Chaetocnema gracilis*	*Chaetocnema nigrica*	*Chaetocnema cognate*	*Monolepta* sp.	*Medythia suturalis*
Max. Temperature (ºC)	2022	-0.30	-0.06	-0.10	-0.08	-0.06	-0.16	-0.01	-0.01
	2023	0.27	-0.11	0.44	0.11	0.08	0.47	-0.05	-0.12
Min. Temperature (ºC)	2022	0.04	0.33	0.27	0.50	0.52	0.12	0.25	0.19
	2023	0.32	0.09	0.55	0.47	0.40	0.44	0.21	0.11
Rainfall (mm)	2022	0.30	0.60	0.66*	0.56	0.63	0.48	0.44	0.45
	2023	0.02	0.21	-0.01	0.67*	0.54	-0.10	0.30	0.67*
Relative Humidity (%)	2022	0.57	0.47	0.42	0.50	0.54	0.38	0.36	0.09
	2023	-0.04	0.37	0.09	0.72*	0.68*	-0.15	0.27	0.47

*Significant at P=0.05

population was non-significant and negatively correlated with maximum temperature for all the pests during 2022. However, *D. armigera*, *M. signata, C gracilis, C. nigrica* and *C. cognate* showed positive correlation with the maximum temperature with r value of 0.27, 0.44, 0.11, 0.08 and 0.47, respectively during 2023. Minimum temperature had positive and non-significant effect on population buildup of all pest species during both the cropping years (Table 6). Furthermore, rainfall positively influenced the pest's appearance and structure, registering significant r values of 0.66 for *M. signata* during 2022 and 0.67 for *C. gracilis* during 2023. Average relative humidity mostly appeared favourable for the growth and multiplication of most of the species during both the years, with *C. gracilis* and *C. nigrica* displaying a positive and significant correlation for adult populations during 2023, with r values of 0.72 and 0.68, respectively. However, *D. armigera* (r= -0.04) and *C. cognate* (r= -0.15) were negatively correlated with relative humidity during 2023. Present studies drew support from the findings of Adhikari *et al*. (2021) who have also reported positive and non-significant correlation of *D. armigera* population with minimum temperature and rainfall. A negative and non-significant correlation of *M. signata* with maximum temperature was also reported by Kumar *et al*. (2018).

Conclusion

The current study provided the valuable insights into the diversity and abundance of chrysomelid beetles associated with rice under natural farming in mid hill conditions of H.P. Diversity and abundance was found highest in Palampur, with eight chrysomelid species present during the crop growth period. Notably, *C. gracilis, C. nigrica* and *C. cognate* were reported for the first time from Himachal Pradesh. However, at Jogindernagar, comparatively lower diversity was recorded with only five species encountered in different localities. Among them, *C. gracilis* and *C. nigrica* were found to be widespread in Holta, Batolu, Chatter and Masoli. The seasonal abundance analysis revealed distinct patterns in the population dynamics, recording the highest diversity and dominance of insect pests in the month of July and August suggesting a critical period for pest management interventions.

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Conflict of interest: All authors declare that they do not have any conflict of interest.

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Seasonal abundance of invasive leaf miner, *Phthorimaea absoluta* (Meyrick) on tomato under mid hill conditions of Himachal Pradesh

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Abstract

The South American tomato leaf miner, *Phthorimaea absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is an invasive pest which has been causing havoc both in greenhouse and open field. The study was conducted in mid-hill region of Himachal Pradesh during summer of 2022 and 2023 to record the seasonal abundance of the pest under field conditions. The per cent leaf and fruit infestation varied from 2.38 to 32.68 and 1.49 to 12.33 during 2022 and 3.70 to 29.71 and 1.30 to 9.09 during 2023, respectively. The number of mines, leaf infestation (%) and fruit infestation (%) had a significant positive correlation with relative humidity and minimum temperature. Adult trap catch showed a negative non-significant correlation with rainfall. Based on the results it is inferred that, monitoring and pest management interventions should be initiated in the early growth period of the crop to avoid build-up of the pest.

Key words: Tomato, leaf miner, abundance, correlation, temperature, rainfall

Invasive pest species are a major threat in agricultural landscapes. Increased globalization and international trade facilitate the spread and establishment of invasive species (Hulme 2009; Paini *et al*. 2016). The South American tomato leaf miner, *Phthorimaea absoluta* (Meyrick) (Lepidoptera: Gelechiidae), native to South America (Barrientos *et al*. 1998), is a potentially invasive and key pest of tomato, *Solanum lycopersicum* L. in different parts of the world (Biondi *et al*. 2018). Outside its native home, it was detected for the first time in Spain in 2006 (Urbaneja *et al*. 2007), and since then, it has invaded many countries including India (CABI 2019). At present, the pest has spread to almost all the tomato growing parts of India (Sridhar *et al*. 2014; Ballal *et al*. 2016, Sankarganesh *et al*. 2017). In Himachal Pradesh, the first case of *P. absoluta* infestation on tomato was reported in the mid-hills of Solan in 2015 (Sharma and Gavkare 2017). Later, the pest was found in an epidemic form under protected conditions in Mandi district (Sood and Yadav 2017). It has become one of the most devastating pests of tomato crop all over the world both in greenhouse and open fields (Sapkal *et al*. 2018). Tomato is known to be the main host of *P. absoluta*, but it also feeds, develops and reproduces on other solanaceous plants and related weeds (Pereyra and Sanchez 2006). The larvae cause damage by mining into the leaves, producing large galleries that later become necrotic. The larvae also burrow inside stalks and consume apical buds and fruits. The pest cause damage during all growth stages and has a potential of causing a yield loss of 50 to 100 per cent (Medeiros et al. 2009; Desneux *et al*. 2010). It has a high reproductive potential (Gebremariam 2015), with 10–12 generations per year, depending on the favourable environmental conditions. It can overwinter in the egg, pupa, or adult stage (EPPO 2005). Considering the damage potential and threat of this invasive pest, the study was carried out to know the seasonal abundance and damage level of *P. absoluta* under mid hill conditions of Himachal Pradesh.

Materials and Methods

Investigations on the seasonal abundance of tomato leaf miner were carried out at Experimental Farm of Department of Entomology, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur (H.P.) during the summer of 2022 and 2023.

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The extent of damage caused by the pest was studied by transplanting tomato variety Avatar on 13th April in 2022 and 29th March in 2023 in an area of 150 m^2. The crop was raised following recommended package of practices except the use of insecticides (Anonymous 2018).

P. absoluta moth population was monitored on tomato crop starting from transplanting until final harvest at weekly intervals by using sex pheromone traps (WOTA open pan water traps). Sex pheromone dispensers were renewed every 15 days and the numbers of moths captured in the trap were recorded weekly during the cropping seasons of 2022 and 2023. The observations on number of eggs, larvae and mines per 3 leaves (top, middle and bottom) per plant were recorded on 30 number of randomly selected plants at weekly intervals starting from the appearance of the pest on the crop till harvest of the crop. Data on leaf and fruit infestation were also recorded to work out per cent leaf/fruit infestation. The population build up data were correlated with weather parameters to establish relationship with abiotic factors (max temp, min temp, rainfall, humidity) through simple correlation analysis (Chandel 1993). The weather data were collected from the Agro-meteorological observatory of the Department of Agronomy, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur.

$$\text{Leaf infestation (\%)} = \frac{\text{Number of mined leaves}}{\text{Total number of leaves}} \times 100$$

$$\text{Fruit infestation (\%)} = \frac{\text{Number of infested fruits}}{\text{Total number of fruits}} \times 100$$

Results and Discussion

Seasonal incidence of *P. absoluta* during summer 2022

The tomato leaf miner started appearing during 21st standard meteorological week (SMW) in the year 2022. Number of eggs per plant varied from 0.27 to 5.87 with highest density during the 27th standard week (5.87) (Table 1). *P. absoluta* larvae appeared during 21st SMW i.e., 3rd week of May with maximum number of mines made during 27th SMW (19.59). With the advancement of the season, the larval density increased to reach a peak of 12.67 larvae per plant during the 28th standard week (2nd week of July). Leaf infestation was recorded from 21st SMW, which increased with the crop maturity, to reach a maximum of 32.68 per cent during 31st SMW. The mean per cent leaf damage and mean number of larvae recorded were 17.84±2.73 and 4.73±1.23, respectively. The per cent fruit infestation was observed from 26th SMW to 31st SMW with mean infestation of 3.75±1.38 per cent during 2022. The moth catch began from 21st SMW and continued throughout the season (Fig. 1). Highest moth catch was noticed during 26th SMW (25.00) while the least number of moths trapped were 2.00 during 31st SMW. The mean moth catch per week was 10.73±2.09.

Seasonal incidence of *P. absoluta* during summer 2023

The incidence of the pest was recorded during 19th SMW in the year 2023. The eggs were first noticed on

Table 1. Incidence of *Phthorimaea absoluta* during summer 2022

SMW	No. of eggs*	No. of larvae*	No. of mines*	Leaf infestation (%)	Fruit infestation (%)
21	0.00	0.33	3.72	2.38	0.00
22	0.27	0.60	5.39	7.08	0.00
23	0.87	1.53	6.81	10.43	0.00
24	1.20	1.80	8.28	14.52	0.00
25	3.13	5.60	9.13	15.32	0.00
26	5.27	8.07	15.63	18.00	1.49
27	5.87	9.60	19.59	20.84	3.70
28	3.73	12.67	14.56	23.19	7.50
29	1.67	6.47	14.81	24.46	6.25
30	1.33	3.67	15.45	27.40	10
31	0.13	1.73	17.16	32.68	12.33
Mean±SE	2.13±0.62	4.73±1.23	11.87±1.61	17.84±2.73	3.75±1.38

*Average per 3 leaves per plant

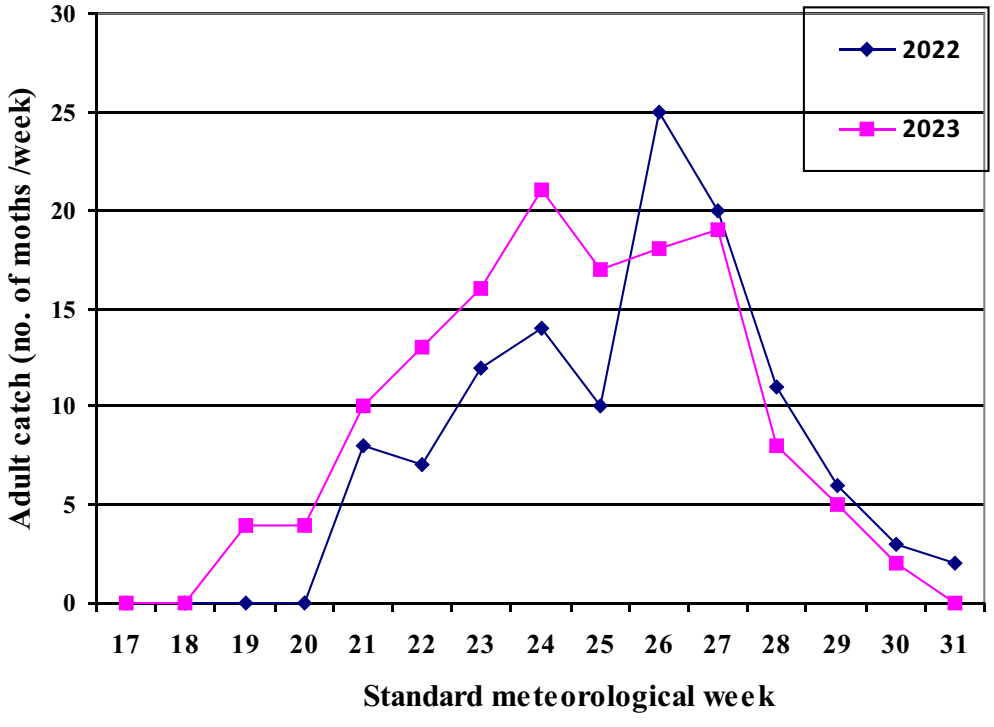

Fig. 1 Pheromone trap catch of *P. absoluta* during summer 2022 and 2023

the crop during 21st SMW and attained a peak of 6.07 eggs per plant during 27th SMW (Table 2). The larvae, mines and per cent leaf damage on the crop started appearing from 19th SMW. Highest numbers of larvae recorded per plant were 9.13 during 26th SMW. The number of mines per plant varied from 0.47 to 14.07 and the highest mine count was observed during 28th SMW. The mean number of eggs and mines per plant were 2.61±0.65 and 9.21±1.45, respectively. Per cent leaf infestation progressed with the crop maturity, with slight fluctuations, and recorded highest during 30th SMW (29.71). Fruiting started from 24th SMW and fruit infestation was noticed right from the first week of fruiting stage which ranged from 1.30 to 9.09 per cent. The mean per cent leaf and fruit infestation was 17.13±2.36 and 2.78±0.94, respectively. Pheromone

Table 2. Incidence of *Phthorimaea absoluta* during summer 2023

SMW	No. of eggs*	No. of larvae*	No. of mines*	Leaf infestation (%)	Fruit infestation (%)
19	0.00	0.13	0.47	3.70	0.00
20	0.00	0.53	1.80	6.67	0.00
21	0.60	1.07	3.53	8.68	0.00
22	1.00	3.47	5.93	11.96	0.00
23	1.53	4.73	8.27	15.32	0.00
24	2.47	7.00	11.07	18.04	1.30
25	3.73	6.00	14.00	17.49	1.85
26	5.73	9.13	12.47	19.96	2.78
27	6.07	8.40	12.40	22.42	5.00
28	5.47	5.20	14.07	25.15	7.14
29	3.40	2.13	13.00	26.42	6.25
30	1.27	1.33	13.53	29.71	9.09
Mean±SE	2.61±0.65	4.09±0.90	9.21±1.45	17.13±2.36	2.78±0.94

*Average per 3 leaves per plant

trap catch reveal that the highest number of moths were trapped during 24th SMW (21.00) (Fig. 1). Adult trap catch continued throughout the season. The mean number of moths trapped were 11.42±1.95.

The present findings are in agreement with the earlier workers (Lietti *et al*. 2005; Karut *et al*. 2011; Portakaldali *et al*. 2013; Ata and Megahed 2014) who have reported relatively low pest density in the early stage, increasing towards middle of the crop cycle and then declining thereafter. The incidence of *P. absoluta* increased with the crop growth stage and the results are in accordance with the findings of earlier workers (Ramesh 2016; Nayana *et al*. 2018, Shiberu and Getu 2018; Negi 2019). The current finding is supported by Leite *et al*. (2001) who have reported comparatively lesser number of eggs and larvae towards the end of season. The damage on fruits was lesser compared to that on leaves during both the years which find support from the studies conducted by Kumari *et al*. (2018). Higher number of mines per leaf than the corresponding larval population was reported in the present study which is in accordance with the findings of Lee *et al*. (2014) and Nitin *et al*. (2017). The adult catch in pheromone traps also varied (2-25 adults) during both the years of study; being higher towards the middle of the season and lower towards the beginning and the end of the cropping season.

Correlation between incidence of *P. absoluta* and various abiotic factors during 2022-23

Data on the the incidence of *P. absoluta* were correlated with different weather parameters prevailing at Palampur during 2022 and 2023 (Table 3). Maximum temperature showed negative correlation with eggs, larvae, mines, per cent leaf and per cent fruit infestation. Minimum temperature revealed positive correlation during both the seasons with number of eggs (r=0.67), number of mines (r=0.85), per cent leaf infestation (r=0.79) and per cent fruit infestation (r=0.68) during 2023. Present studies drew support from the findings of Negi (2019) and Chaudhary *et al*. (2022) and who have also reported negative correlation of maximum temperature with the pest incidence and a positive and significant correlation of minimum temperature with the number of mines and per cent leaf infestation. Rainfall had significant influence on the number of mines, per cent leaf and fruit infestation with r=0.67, r=0.72 and 0.84 during 2022, respectively. Adult catch was negatively influenced by rainfall during both the cropping years with r=-0.22 and r=-0.34, respectively. A positive and non-significant correlation of adult catch with minimum and maximum temperature was observed, which was also reported by Venkataramanaiah *et al*. (2021). Relative humidity had a positive significant correlation with the number of larvae (r=0.65), number of mines (r=0.91), per cent leaf (r=0.91) and per cent fruit (r=0.79) infestation during 2022.

Conclusion

Minimum temperature and relative humidity had a significant positive influence on the pest population which led to increase in infestation level. The pest density was initially low during the early stages of crop

Table 3. Correlation between incidence of *Phthorimaea absoluta* and various abiotic factors during 2022-23

Weather Factor	Year	Correlation coefficient (r)					
		No. of eggs	No. of larvae	No. of mines	Leaf infestation (%)	Fruit infestation (%)	Pheromone trap catch per week
Max. Temperature (ºC)	2022	-0.24	-0.39	-0.50	-0.53	-0.57*	0.30
	2023	-0.09	0.02	-0.01	-0.15	-0.25	0.20
Min. Temperature (ºC)	2022	0.08	0.15	0.30	0.27	0.20	0.17
	2023	0.67*	0.45	0.85**	0.79**	0.68*	0.18
Rainfall (mm)	2022	0.14	0.27	0.67*	0.72*	0.84**	-0.22
	2023	-0.01	-0.29	0.00	0.11	0.19	-0.34
Relative Humidity (%)	2022	0.47	0.65*	0.91**	0.91**	0.79**	-0.02
	2023	0.76**	0.47	0.90**	0.93**	0.87**	0.09

*Significant at P=0.05
**Significant at P=0.01

growth which progressed with the advancement of the crop and thereafter declined. Based on these results, pest monitoring should be initiated right from the transplanting of crop in the field and thereafter management practices should be followed to avoid build-up of the pest.

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Impact of storage conditions on seed-borne mycoflora and seed health parameters of soybean [*Glycine max* (L.) Merrill]

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Abstract

The study was conducted to investigate the changes in soybean seed health parameters that may occur during storage in different types of commonly used containers i.e., polythene bag, cloth bag and metallic bin. Seeds of two soybean varieties *viz*., Shivalik and Hara Soya were stored in these containers during December, 2022 to June, 2023, in the Department of Plant Pathology, CSKHPKV, Palampur. Lowest incidence of mycoflora as well as highest germination percentage, seedling shoot length, root length and seedling vigour index were obtained from sterilized seeds of Hara Soya stored in polythene bags for two months from fungicide Tebuconazole 1ml/L sprayed plots while highest mycoflora along with lowest germination percentage, seedling shoot length, root length and seedling vigour index were recorded in seeds of variety Shivalik stored in cloth bags for 6 months from unsprayed plots.

Key words: Soybean, seedling vigour index, storage, storage period, storage container

Soybean [*Glycine max* (L.) Merrill] is an important source of oil and protein. Earlier it was known as a pulse crop but due to high oil content soybean has now been placed in oilseed crop. Due to presence of high protein content, soybean is also known as 'Poor man's meat'. Lack of high seed vigour at the time of sowing is one of the main obstacles to soybean cultivation as it cannot remain viable for longer periods of time (Priestley *et al*. 1985). The genotype as well as storage conditions, particularly length of time, have an impact on the lifespan of seeds affecting seed viability (Shelar *et al*. 2008). Hamman *et al*. (1996) found that seed-borne infections had a negative impact on germination percentage and resulted in death of seeds, malformed seedlings and damaged seed coats. The seed moisture content, storage period, prevailing temperature and degree of invasion influence the development of seed borne diseases (Sharma *et al.* 2015). To preserve the seeds quality for a longer period of time, it is preferable to keep them in moisture and vapour-proof containers like polythene bags, aluminium foil, tins or any other sealed containers (Raikar *et al*. 2008). The objective of this research was to determine the effects of storage period and different types of storage containers on seed mycoflora and germination parameters of different soybean cultivars.

Materials and Methods

Collection of seed sample

To obtain seed samples of soybean, seeds of different varieties *viz*., Shivalik and Hara Soya were multiplied during the month of June 2021 at the experimental farm of the Department of Plant Pathology, CSK HPKV, Palampur. To assess the impact of fungicide on seed health, one spray of tebuconazole (1ml/L) was applied to various soybean cultivars sown in the field at pod initiation stage. At the time of harvest, seed samples were collected and labelled with the variety name, sample type (sprayed or unsprayed) and harvesting time. These samples were brought to the laboratory of the department where they were kept in different storage containers including metallic bins, polythene and cloth bags at room temperature until further investigations. The observations were recorded on the frequency of mycoflora and seed health parameters at an interval of 60 days. Seed samples were taken randomly from each storage container and were tested at the end of 2, 4 and

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6 months of storage period. Quality of soybean seeds was evaluated by determining seed germination percentage, root length, shoot length and seedling vigour index through blotter paper method as mentioned below:

Blotter paper method

In blotter paper method, three pieces of filter paper were soaked in sterilized distilled water and placed at the bottom of 9cm dia. Petri dish. Four hundred seeds of soybean from each sample of seed lot were used in this method. Sterilization of seeds was done by immersing seeds in sodium hypochlorite solution (1%) for 3 min. followed by three subsequent washings with distilled water. Seeds were then placed on the moist filter paper at the rate of 10 seeds per Petri dish. The Petri dishes were then incubated at 25+1°C for seven days and data pertaining to frequency of mycoflora, seed germination percentage, root and shoot lengths were recorded from random seedling samples. Fungal frequency and germination percentage and seedling vigour index were calculated by using formula as given below:

Fungal frequency

Fungal colonies were calculated and accessed by using formula:

$$\text{Total fungal colonies (\%)} = \frac{\text{No. of seeds colonized in each plate by a particular species}}{\text{Total no. of seeds in each plate}} \times 100$$

Germination percentage

$$\text{Seed germination \%} = \frac{\text{Germinated seed}}{\text{Total Seed}} \times 100$$

Seedling vigour index

Vigour index was calculated by the following formula given by Baki and Anderson (1973):

Vigour index = % seed germination x (mean root length + mean shoot length)

Results and Discussion

Effect of storage conditions on seed mycoflora

Data on detection of associated mycoflora in soybean seeds stored in separate containers for different durations is given in Table 1. Perusal of the data revealed that frequency of fungal species *viz*., *A. niger, A. flavus, Rhizopus* sp., *Penicillium* sp., *Trichoderma* sp., *Cladosporium* sp. increased as the storage period increased from 2 to 6 months while reduction in the frequency of *A. alternata, C. truncatum, Curvularia* sp., *F. proliferatum, F. equiseti, Phoma* sp. and *Pestalotiopsis* sp. was observed at the end of six months of storage period.

After two months of storage maximum frequency of fungal species was recorded in unsterilized seeds stored in cloth bags obtained from unsprayed plots of variety Shivalik (Table 1), wherein maximum frequency was of *A. niger* (35.75%) followed by *A. alternata* (30.25%) and *C. truncatum* (28.00%). However, minimum prevalence of mycoflora was in seeds stored in polythene bags obtained from fungicide sprayed plot of variety Hara Soya where, predominantly occurring seed mycoflora was *A. niger* (12.75%) followed by *A. alternata* (8.00%). After six months of storage period, maximum frequency of fungal species was recorded in seeds stored in cloth bags obtained from unsprayed plot of variety Shivalik (Table 1). Fungal frequency was highest for *A. niger* (38.75%) followed by *Cladosporium* sp. (36.25%) and *A. alternata* (25.75%). However, minimum prevalence of mycoflora was in sterilized seeds stored in polythene bags obtained from fungicide sprayed plot of variety Hara Soya (Table 1), where predominantly occurring seed mycoflora was *A. niger* (22.00%) followed by *A. alternata* (5.75%). Many other workers have also reported that as storage period advanced, field frequency of fungi *viz., A. alternata, Cladosporium* sp., *Fusarium* sp. declined whereas storage fungi *viz., A. niger, A. flavus, Penicillium* sp. and *Rhizopus* sp., increased up to 6 to 12 months afterwards their population also declined (Srivastava and Gupta 1980; Dwivedi and Shukla 1990; Lambat *et al*. 2015). Lambat *et al*. (2017) also reported that increase in the number of infected seeds was due to increase in mycoflora. They also advocated storage of seeds in polyethylene bag to preserve greater germinability and lesser seed invasion by the fungal flora during storage.

Effect on seed health parameters

Interaction between soybean cultivars and storage periods

The results (Table 2) clearly indicated that highest germination percentage, root length, shoot length and seedling vigour index were recorded after two months of storage of seeds of Hara Soya cultivar obtained from fungicide sprayed plots followed by four months of storage in the same seed sample. The lowest final germination percentage, root length, shoot length and

Table 1. Effect of storage container and storage duration on seed mycoflora of soybean varieties by blotter paper method

Sample	Storage Container	Storage Period (months)	*Aspergillus niger*	*Aspergillus flavus*	*Rhizopus* sp.	*Alternaria alternata*	*Penicillium* sp.	*Colletotrichum truncatum*	*Trichoderma* sp.	*Curvularia* sp.	*Fusarium proliferatum*	*Fusarium equiseti*	*Phoma* sp.	*Cladosporium* sp.	*Pestalotiopsis* sp.
Shivalik (seeds obtained from sprayed plot)	Metallic bin	2	18.75	5.50	0.00	24.00	6.75	17.50	0.25	0.00	0.00	0.00	13.00	8.00	0.00
		4	26.50	7.75	0.00	10.25	8.00	15.75	0.25	0.00	0.00	0.00	11.25	10.25	0.00
		6	30.75	9.00	0.00	6.25	16.75	12.00	0.75	0.00	0.00	0.00	9.75	13.00	0.00
	Polythene bag	2	13.00	4.25	0.00	16.25	4.25	13.75	0.00	0.00	0.00	0.00	10.75	6.75	0.00
		4	18.75	7.50	0.00	8.00	6.00	9.00	0.00	0.00	0.00	0.00	7.00	10.00	0.00
		6	23.25	10.75	0.00	4.25	8.50	6.25	0.00	0.00	0.00	0.00	2.50	12.75	0.00
	Cloth bag	2	24.50	7.50	0.00	34.00	8.50	24.00	6.75	8.75	7.25	0.00	18.50	10.25	12.00
		4	28.75	15.25	0.00	24.75	13.75	20.75	8.00	5.50	0.00	0.00	15.75	13.00	8.75
		6	35.00	18.75	0.00	22.50	16.00	14.00	13.25	0.00	0.00	0.00	14.25	17.25	6.50
Shivalik (seeds obtained from unsprayed plot)	Metallic bin	2	24.50	8.25	0.00	32.00	14.50	26.75	4.00	6.75	0.00	6.00	18.25	17.00	0.00
		4	27.75	13.75	0.00	28.25	16.00	24.25	7.25	0.00	0.00	0.00	15.50	20.75	0.00
		6	36.50	11.00	0.00	25.50	18.50	23.50	11.75	0.00	0.00	0.00	12.75	27.50	0.00
	Polythene bag	2	14.50	4.25	0.00	27.00	8.75	24.25	2.00	0.00	7.75	0.00	18.25	15.00	0.00
		4	26.25	6.75	0.00	24.25	11.00	17.50	5.00	0.00	0.00	0.00	15.75	17.50	0.00
		6	35.75	9.00	0.00	20.75	13.50	15.75	7.75	0.00	0.00	0.00	12.00	23.25	0.00
	Cloth bag	2	23.75	11.75	15.00	35.00	15.00	28.25	13.00	17.25	14.75	10.00	28.00	32.50	18.50
		4	28.50	13.25	17.00	32.25	17.25	25.50	15.25	14.00	12.50	8.50	26.50	33.00	15.50
		6	38.75	21.50	20.50	25.75	20.50	18.00	17.00	12.75	9.75	6.75	23.25	36.25	12.25
Hara Soya (seeds obtained from sprayed plot)	Metallic bin	2	12.75	0.00	0.00	14.00	2.50	15.75	0.25	0.00	0.00	0.00	5.50	4.00	0.00
		4	16.00	0.00	0.00	12.75	5.75	12.00	0.00	0.00	0.00	0.00	3.75	6.75	0.00
		6	25.75	0.00	0.00	8.25	8.75	8.75	0.00	0.00	0.00	0.00	0.00	9.50	0.00
	Polythene bag	2	12.75	0.00	0.00	8.00	0.00	6.75	0.00	0.00	0.00	0.00	4.75	0.00	0.00
		4	18.75	0.00	0.00	7.00	0.00	2.75	0.00	0.00	0.00	0.00	3.50	0.00	0.00
		6	22.00	0.00	0.00	5.75	0.25	0.50	0.00	0.00	0.00	0.00	0.00	4.00	0.00
	Cloth bag	2	32.50	2.75	0.00	24.00	4.50	18.75	0.00	0.00	0.00	0.00	7.25	5.25	0.00
		4	35.75	6.25	0.00	21.50	7.25	14.50	0.00	0.00	0.00	0.00	4.50	8.00	0.00
		6	37.50	8.00	0.00	18.75	10.75	10.00	0.00	0.00	0.00	0.00	2.75	12.50	0.00
Hara Soya (seeds obtained from unsprayed plot)	Metallic bin	2	15.00	0.00	0.00	18.75	5.75	16.25	0.00	0.00	0.00	0.00	11.00	12.25	0.00
		4	24.25	0.00	0.00	15.75	7.75	14.50	0.00	0.00	0.00	0.00	10.00	15.50	0.00
		6	27.25	0.00	0.00	12.00	10.25	11.75	0.00	0.00	0.00	0.00	8.25	23.75	0.00
	Polythene bag	2	10.50	0.00	0.00	16.75	0.00	12.75	0.00	0.00	2.50	0.00	7.25	8.50	0.00
		4	15.25	0.00	0.00	12.00	0.00	10.25	0.00	0.00	0.00	0.00	5.00	12.75	0.00
		6	18.75	0.00	0.00	10.25	6.50	7.50	0.00	0.00	0.00	0.00	3.25	15.00	0.00
	Cloth bag	2	16.50	6.75	0.00	28.00	8.50	18.25	8.50	12.00	10.00	3.25	13.75	19.00	10.50
		4	23.25	9.25	0.00	26.25	9.00	15.75	10.25	9.75	7.75	0.00	10.25	22.75	6.25
		6	28.75	14.75	10.75	22.00	12.75	12.00	13.50	7.00	5.00	0.00	7.00	25.50	4.50

Table 2. Effect of storage container and storage duration on seed health of soybean varieties by blotter paper method

Seed samples	Storage container	Germination (%) * Time interval (months)			Root length (cm) Time interval (months)			Shoot length (cm) Time interval (months)			Seedling Vigour Index Time interval (months)		
		2	4	6	2	4	6	2	4	6	2	4	6
Shivalik (seeds obtained from sprayed plot)	Metallic bin	92.33 (74.23)	88.75 (70.54)	86.33 (68.28)	10.32	10.27	10.24	3.75	3.72	3.70	1299	1242	1204
	Polythene bag	94.50 (76.63)	92.33 (74.05)	89.75 (71.37)	10.35	10.32	10.29	3.76	3.75	3.73	1333	1299	1258
	Cloth bag	90.50 (72.13)	87.00 (68.86)	85.33 (67.54)	10.34	10.31	10.28	3.77	3.74	3.72	1277	1222	1194
Shivalik (seeds obtained from unsprayed plot)	Metallic bin	88.33 (70.18)	85.00 (67.19)	85.00 (67.21)	9.23	9.20	9.17	1.81	1.78	1.76	975	933	929
	Polythene bag	91.33 (73.10)	87.67 (69.44)	85.33 (67.50)	9.25	9.22	9.19	1.82	1.80	1.78	1011	966	936
	Cloth bag	90.33 (72.03)	87.50 (69.29)	82.67 (65.40)	9.19	9.18	9.15	1.78	1.75	1.74	991	956	900
Hara Soya (seeds obtained from sprayed plot)	Metallic bin	95.00 (77.39)	94.67 (77.24)	91.33 (72.89)	10.54	10.52	10.47	4.06	4.03	4.01	1387	1378	1323
	Polythene bag	97.50 (81.61)	95.00 (77.29)	92.33 (74.04)	10.58	10.55	10.52	4.10	4.07	4.05	1432	1389	1345
	Cloth bag	93.33 (75.01)	89.67 (71.23)	85.33 (67.53)	10.51	10.48	10.45	4.03	4.98	4.67	1357	1386	1290
Hara Soya (seeds obtained from unsprayed plot)	Metallic bin	94.33 (76.39)	92.67 (74.26)	90.50 (72.21)	10.41	10.38	10.35	3.93	3.99	4.07	1352	1332	1305
	Polythene bag	96.67 (79.73)	94.33 (76.78)	92.00 (73.55)	10.44	10.42	10.38	4.06	4.05	4.02	1402	1365	1325
	Cloth bag	91.33 (73.00)	87.50 (69.41)	83.67 (66.14)	10.40	10.37	10.34	3.95	3.94	2.61	1311	1252	1084
CD (P = 0.05)													
Seed sample(A)		1.52			0.13			0.22					
Storage container (B)		1.32			NS			NS					
Interaction (A x B)		2.63			NS			0.37					
Storage condition (C)		1.32			NS			NS					
Interaction (A x C)		NS			NS			NS					
Interaction (B x C)		NS			NS			NS					
Interaction (A x B x C)		NS			NS			NS					

*Figures within parentheses are arc sine transformed values, NS= Non significant

seedling vigour index were recorded from seeds of cv. Shivalik obtained from unsprayed plots after 6 months.

Interaction between storage period and storage containers

The results also clearly indicated that germination percentage, root length, shoot length and seedling vigour index were significantly affected due to the interaction between storage periods and storage containers. It was noticed that highest germination percentage, root length, shoot length and seedling vigour index were obtained after two months of storage in polythene bags followed by soybean seeds stored in polythene bags for 4 months. However, the lowest germination percentage was obtained from soybean seeds stored in cloth bags for 6 months.

Interaction between soybean cultivars and storage containers

The results (Table 2) showed that highest germination percentage, root length, shoot length and seedling vigour index were observed in seeds of Hara Soya stored in polythene bags obtained from fungicide sprayed plots. The lowest germination percentage, root length, shoot length and seedling vigour index were obtained from seeds of cv. Shivalik stored in cloth bags obtained from unsprayed plots.

Interaction between storage periods, soybean cultivars and storage containers

The results clearly indicated that highest germination percentage (97.50%) root length (10.58 cm), shoot length (4.10 cm) and seedling vigour index (1432) were recorded in seeds of Hara Soya after 2 months of storage in polythene bags obtained from fungicide sprayed plots. The data revealed that at the end of 6 months of storage period, highest seed germination (92.33%), root length (10.52 cm), shoot length (4.05 cm) and seedling vigour index (1345) was given by seeds of variety Hara Soya obtained from fungicide sprayed plot stored in polythene bag followed by seeds of variety Hara Soya obtained from unsprayed plot stored in polythene bag with seed germination (92.00%), root length (10.38 cm), shoot length (4.02 cm) and seedling vigour index (1325) and differed statistically from each other. However, the lowest germination percentage (82.67%), root length (9.15cm), shoot length (1.74cm) and seedling vigour index (900) were obtained from seeds of cv. Shivalik stored in cloth bags for 6 months obtained from unsprayed plots.

Similar results were observed by Ram *et al*. (2020), who reported that during storage, the highest germination percentage was maintained by using polythene bags. Verma and Verma (2014) also reported that after 8 months of storage, the seeds kept in polythene bags exhibited significantly higher germination rates and vigour indices compared to seeds stored in cloth bags. Singh and Dadlani (2003) reported that soybean seeds stored in cloth bags maintained satisfactory germination for only up to 4 months of storage. The decline in germination percentage observed during the storage period, however, can be attributed to the process of seed aging and the depletion of food reserves. Additionally, factors such as reduced synthetic activity of the embryo, fungal invasion, insect damage, decreased dry matter accumulation in seedlings, fluctuations in temperature and relative humidity, and the choice of storage containers can all contribute to the deterioration of seed health during storage, as discussed by Beedi *et al*. (2018).

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Occurrence of angular leaf spot of common bean in major growing areas of Himachal Pradesh and effect of leaf wetness durations on its development

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Abstract

Angular leaf spot (ALS) caused by the hemibiotrophic fungus, *Pseudocercospora griseola* is one of the most widely distributed and damaging disease of common bean particularly in humid conditions. In Himachal Pradesh, common bean is grown during *Kharif* season and affected by ALS. Information on the occurrence and distribution of disease is important to develop management methods. Therefore, present study was carried out to assess the occurrence and distribution of angular leaf spot disease in major common bean growing areas of Himachal Pradesh and effect of leaf wetness durations on disease development was also evaluated under *in-vivo* conditions. The disease was observed in all the areas with different levels of disease severity ranging from 21.3 to 76.2 per cent. The highest disease severity was recorded in Kullu district *i.e.* 61.28 per cent and lowest disease severity of 43.50 per cent was recorded in Kinnaur district. The effect of leaf wetness durations on disease development studied under *in-vitro* conditions on susceptible variety Jwala showed a progressive increase in lesion number per leaf with the increase in wetness duration up to 24 hours.

Keywords: Angular leaf spot, Severity, leaf wetness, *Pseudocercospora griseola*

Common bean (*Phaseolus vulgaris* L.; 2n= 2x= 22), a member of family Leguminosae is the globally valued relished legume crop including India, for its green pods as well as dry beans (Sharma *et al*. 2021), having its origin in South Mexico and Central America (Vavilov 1979). In Himachal Pradesh, dry bean, locally called as 'Rajmash' and green bean as 'French bean' are among the premier pulse crops being grown in areas which are confined to 900 meters to 3000 meter above mean sea level in Shimla, Chamba, Sirmour, Kullu, Kinnaur, Mandi and Lahual & Spiti districts (Katoch *et al*. 2019). Environmental conditions which favour the growth of common bean plants, also predispose them to the attack of various fungal, bacterial and viral diseases (Dhiman *et al*. 2020). Among fungal diseases, angular leaf spot (ALS) caused by the hemibiotrophic fungus, *Pseudocercospora griseola* (Sacc.) Crous & U. Braun (*Phaeoisariopsis griseola* (Sacc.) Ferraris) is one of the most widely distributed and damaging disease of common bean particularly in humid conditions (Allorent and Savary, 2005). The optimum conditions for infection include 80-90 per cent humidity and a temperature of $\geq$ 24°C (Librelon *et al*. 2022). According to Alvarez and Schwartz (1979), infection might develop after a period of 24-48 hours of leaf wetness. To allow for effective infection and subsequent synnemata development, high relative humidity, rainfall, or dew periods are required (Lianos, 1957). The global impact of the disease has led to significant agricultural losses worldwide. The fungus infects most aerial plant parts and resulted in yield loss up to 80 per cent (Ponnappa *et al*. 1976; Shukla and Sharma 2006). In India, the disease has resulted in estimated losses ranging from 40 to 70 per cent, including damaged and unmarketable pods (Singh and Saini, 1980). In this study, disease survey was carried to know the prevalence of disease in different regions of Himachal Pradesh and effect of leaf wetness durations on development of disease, so that effective control measures can be adopted to combat the disease.

Materials and Methods

Disease survey

A disease survey was conducted during Kharif 2022 covering different locations of Kangra, Mandi,

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Kullu, Shimla, Chamba and Kinnaur districts of Himachal Pradesh (3 Zones) to record the prevalence and distribution of angular leaf spot of common bean. Observations were made by randomly selecting plants from the farmer's fields of each location with quadrant of 1.0 m^2 area and disease score was given on the basis of 1-9 disease rating scale (CIAT 1987) (Table 1). Further, disease severity was calculated by Townsend and Heuberger's (1943) formula as below

Disease severity index (DSI) was calculated as per following formula:

$$\text{Disease severity (\%)} = \frac{\text{(No. of leaves at each scale} \times \text{Scale value)}}{\text{Highest scale value} \times \text{Total no. of leaves}} \times 100$$

Isolation of pathogen and pathogenicity test

Isolation of pathogen (*Pseudocercospora griseola*) was done by picking up synnemata from diseased leaves, on V8 juice agar medium. In this method, a diseased leaf was carefully placed under a stereo microscope and microscope was focused on the synnemata present on the diseased portion of the leaf. Using a sterilized blade, the synnemata were delicately collected. Subsequently, these collected synnemata were transferred onto fresh V8 juice agar plates and the plates were kept in an incubator at temperature of 25±1 ºC. After 7 days of incubation, Petri plates showing small olive green or grey coloured colony of *P. griseola* were selected and maintained for further studies. Rezene *et al*. (2018) isolated the pathogen by picking up synnemata from diseased leaf with small piece of agar.

Following purification, Petri plates carrying the best growth of pathogen were selected. Sterilized distilled water was then added to the selected fungal growths and then surfaces of isolates were scraped with a sterilized spatula to detach the conidia. This suspension was subjected to microscopic examination using a heamocytometer to establish a conidial concentration of 2 x 10^4 conidia/ml. For inoculation, three seeds of the susceptible cultivar (Jwala) were planted in sterilized 15 cm pots containing sterilized soil. The seeds were sterilized using a 2.0 per cent sodium hypochlorite solution. The conidial suspension was applied to the plants at the 3-leaf stage and covered with plastic bags that had small aeration holes. These inoculated plants were then placed in a controlled glasshouse environment with a temperature of 25±3ºC and high humidity was maintained by spraying water seven times for four consecutive days. Daily observations were recorded until the first appearance of symptoms. Infected leaves were removed, examined under a microscope by slide preparations and the pathogen was re-isolated using the afore mentioned method. The morpho-cultural characteristics (colony color, colony growth, dimensions and shape of conidia) of the re-isolated pathogen were compared to the originally isolated pathogen. Isolates were maintained for further studies.

Effect of leaf wetness durations on disease development

In order to find out the most optimum leaf wetness duration for infection by *Pseudocercospora griseola*, potted common bean plants of cultivar Jwala were inoculated by spraying conidial suspension on both surfaces of the leaf. Water was sprayed for different time intervals *i.e*., 3, 6, 9, 12, 18, 24 and 48 hours to maintain leaf wetness. After 48 hours of wetness, the water spray was stopped and the plants were kept in glasshouse to wait for symptom development. All the treatments were replicated three times. The data on number of lesions per leaf and incubation period were recorded.

Effect of intermittent leaf wetness on infection efficiency

To study the influence of intermittent leaf wetness on infection efficiency, 21 days old potted plants of common bean cv. Jwala were subjected to total wetness periods ranging from 12 to 48 hours each alternating

Table 1: Disease scale for common bean against angular leaf spot under field conditions (CIAT 1987)

Scale	Type of lesion
1	No visible disease symptoms (0% infection)
3	Plants with 5 -10% leaf area having lesions
5	Plants with 20% leaf area having lesions and sporulating
7	Plants with up to 60% leaf area having lesions associated with chlorosis and necrosis,
9	Plants with 90% leaf area having lesions associated with early leaf fall and plant death

with 12 hours dryness in each 24-hour period. The plants were inoculated by spraying conidial suspension on both surfaces of the leaf. Wetting of plants was started after 12 hours of inoculation. Water was sprayed on plants to keep them wet for required period. Dry periods were achieved by stopping the water spray. Three pots in each treatment were allocated for lesion counts and average number of lesions in each treatment was worked out.

Results and discussion

Occurrence of angular leaf spot of common bean in major growing areas of Himachal Pradesh

To assess the occurrence and distribution of angular leaf spot disease, major common bean growing areas in six districts of Himachal Pradesh *viz*., Kangra, Mandi, Kullu, Shimla, Chamba and Kinnaur were surveyed during *Kharif* 2022. The data recorded on disease severity is presented in Table 2. The disease was observed in all the areas with different levels of disease severity ranging from 21.3 to 76.2 per cent. Highest disease severity of 76.2 per cent was recorded in district Kullu while lowest disease severity of 21.3 per cent was recorded in district Mandi. However, highest mean disease severity of 61.28 per cent was recorded in district Kullu followed by Shimla (60.85%), Kangra (59.77%), Chamba (58.05%), Mandi (54.12%) and lowest mean disease severity of 43.5 per cent was recorded in district Kinnaur (Table 2).

The findings from the survey conducted in six districts of Himachal Pradesh during the *Kharif* season of 2022 reveal significant insights into the occurrence and distribution of angular leaf spot disease in common bean cultivation. The data presented in Table 2 demonstrates that the disease was pervasive across

Table 2: Prevalence of angular leaf spot of common bean in different districts of Himachal Pradesh

District	Number of Locations	Disease Severity (%)	
		Range	Mean
Mandi	15	21.3-70.2	54.12
Kullu	17	30.5-76.2	61.28
Kangra	8	43.2-73.1	59.77
Kinnaur	6	32.6-51.4	43.50
Chamba	2	51.0-65.1	58.05
Shimla	2	60.4-61.3	60.85

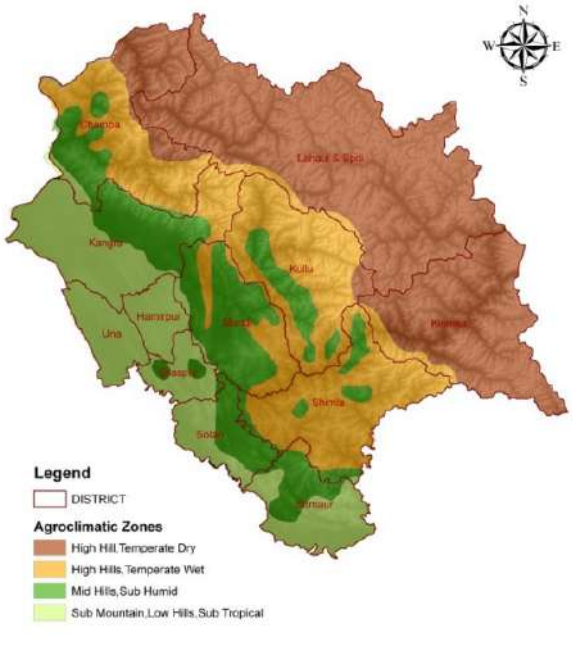

Figure 1 Different agro-climatic zones of Himachal Pradesh

all surveyed areas, with varying degrees of severity. This suggests that angular leaf spot poses a notable threat to bean crops throughout the region.

The significant variation in disease severity observed among the different districts and villages. The highest recorded severity of 76.2% in Kullu district, highlights the potential for significant economic losses in areas heavily impacted by the disease. Conversely, the lowest severity of 21.3% observed in Mandi district, highlights the existence of local factors or practices that may contribute to disease suppression. The geographical distribution of angular leaf spot is influenced by various factors, including climate conditions, bean production practices and the movement of infected plant material. Climatic conditions play a crucial role in the prevalence and severity of the disease. Regions with high humidity, moderate temperatures and frequent rainfall create favorable conditions for the growth and spread of the pathogen. Due to these factors, district Kullu emerges as the most severely affected, followed closely by Shimla. Several other researchers also conduct the disease severity survey of angular leaf spot in their regions. Pamela *et al*. (2014) surveyed different farmers field in Uganda and 21-80 per cent disease severity was recorded. Kijana *et al*. (2017) conducted field survey during two crop seasons, in two main beans growing zones of eastern Democratic Republic of Congo. Angular leaf spot in these fields had an average severity index (PSI) of 49.9%. Percent severity index was significantly different ($P<0.05$) between districts and seasons. The highest severity (PSI=59.7%) was observed in Kabare district, and the lowest in Uvira district (PSI=39.5%). Canpolat and Maden (2021) also surveyed western black sea region of Turkey during autumn and summer periods to assess the occurrence of angular leaf spot of common bean. They revealed that disease severity ranged from 66 to 82 per cent in the autumn period and from 74 to 86 per cent in the summer period, respectively.

These findings highlight the importance of proactive disease management strategies in common bean cultivation. Furthermore, continued surveillance and research efforts are essential to monitor disease dynamics and develop effective control measures in response to evolving challenges posed by plant pathogens.

Isolation and maintenance of *Pseudocercospora griseola* isolate

Isolations were made by picking up synnemata from diseased leaves, on V8 juice agar medium.

After successful isolation of pathogen, pathogenicity of *Pseudocercospora griseola* isolate was proved under *in-vivo* conditions on variety "Jwala" by inoculating pathogen at 3 leaf stage plants. The initial symptoms of the disease appear as grey spots undersides of leaves, delimited by veins and veinlets on trifoliate leaves. Subsequently, characteristic symptom development occurred between 10-14 days after inoculation as grey spots undersides of leaves turn into brown and dark grey to black, synnemata bearing conidia produced on all types of lesions. It had exhibited identical symptoms as observed from collected disease samples. The pathogen was re-isolated from leaves that showed characteristics symptoms in pathogenicity test. Pure culture obtained by single spore showed cent per cent similarity with the inoculated test pathogen, like olive-green to grey colored colony with irregular growth. Conidia were solitary, subcylindrical, fusiform to obclavate, straight to somewhat curved with length and width 27.5-55.1×4.13-10.62 m, respectively having 1-4 septations, hence pathogenicity of *P. griseola* was proved and isolate was maintained to conduct further experiments.

Effect of leaf wetness duration on disease development

The effect of leaf wetness duration on disease development was studied under *in-vivo* conditions on common bean cultivar Jwala and the data regarding incubation period and number of lesions per leaf were recorded and presented in Table 3. Effect of different wetness durations revealed that increase in leaf wetness duration from 3 to 12 hours showed a corresponding decrease in incubation period from 12 to 10 days while lesion number increased from 8.22 to 35.22. However, further increase in leaf wetness did not exert any effect on incubation period whereas there was a progressive increase in lesion number per leaf with the increase in wetness duration up to 24 hours (48.50 lesions/leaf) after which lesion number per leaf declined with the increase in wetness duration. Mathew (1999) also reported that 24-hour leaf wetness duration increase the rate of disease development. Alvarez and

Table 3 Effect of different leaf wetness durations on disease development

Leaf wetness period (hours)	Incubation period (days)	Lesions/Leaf
3	12.33	8.22
6	12.00	23.25
9	10.33	29.25
12	10.00	35.22
18	9.00	39.22
24	9.00	48.50
48	9.00	43.25
C.D.(p=0.05)	-	2.16

Schwartz (1979) also revealed that infection might develop after a period of 24-48 hours of leaf wetness

Effect of intermittent leaf wetness durations on disease development

The study on the role of intermittent leaf wetness on disease development was done on Jwala plants. The data on the effect of intermittent leaf wetness (Table 4) revealed that the number of lesions increased with the interruption of leaf wetness with dry periods up to 3 cycles (40.25 lesions) and further extension of wet and dry periods resulted decrease in lesion number. Mathew (1999) also reported that 3 cycles of leaf wetness with dry periods increase the efficiency of disease.

The study investigating the impact of leaf wetness duration on angular leaf spot development in the common bean reveals a clear relationship between moisture availability and disease progression. Increasing leaf wetness duration from 3 to 12 hours correlates with a decrease in the incubation period and a significant rise in lesion number per leaf, highlighting the critical role of moisture in facilitating pathogen germination and infection. While further extension of wetness duration beyond 12 hours does not affect the incubation period, lesion formation continues to escalate, peaking at 48.50 lesions per leaf after 24 hours. Additionally, the examination of intermittent leaf wetness demonstrates a non-linear relationship between wet and dry cycles and lesion development, emphasizing the complex interplay between moisture management and disease severity in common bean cultivation. These findings highlight the importance of implementing targeted management strategies to mitigate the impact of angular leaf spot and safeguard crop productivity.

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Conflict of interest: The authors declare that there is no conflict of interest in this research paper.

Table 4 Effect of intermittent leaf wetness durations on disease development

Duration of wet and dry period (hours)	Wet/dry sequence (hours)								Lesions/leaf
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	*Mean
24	12	12	-	-	-	-	-	-	32.25
48	12	12	12	12	-	-	-	-	36.50
72	12	12	12	12	12	12	-	-	40.25
96	12	12	12	12	12	12	12	12	38.97
C.D.=(p=0.05)									0.68

*Average of five replications

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Endophytic and pathogenic fungal root communities associated with pea in sub-humid and dry temperate regions of Himachal Pradesh

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Abstract

Pea root rot/wilt incited by multitude of pathogens is often considered as a major pitfall and yield limiting factor to pea production across the world. Considering the importance of crop and disease in state, major pea growing locations of sub-humid and dry temperate zones were surveyed during the year 2021-2022 to record the prevalence of pea root rot complex and to trace the beneficial indigenous fungi associated with healthy plants. Overall, the incidence of disease complex in all the surveyed locations found ranged between 15.83 to 58.17 per cent. Out of 45 pathogens isolated 27 isolates were obtained from subhumid while 18 were recovered from dry temperate regions of Himachal Pradesh. Based on morpho-cultural features, 15 isolates were identified as *Fusarium oxysporum,* 13 of *Fusarium solani,* 6 of *Rhizoctonia solani,* 3 of *Pythium* spp., 2 each of *Phytophthora* sp. and *Fusarium equiseti,* and one each of *Didymella* sp., *Aphanomyces* and *Sclerotinia sclerotiorum.* Among all these isolates *Fusarium oxysporum* was recorded as most frequently isolated genera. In addition, endophytic fungi harboured in pea plants were also figured out, in which maximum fungal endophytic isolates (17) were obtained from Kangra district.

Keywords: Pea root rot complex, survey, fungal endophytes, symptoms, disease incidence

Garden pea (*Pisum sativum* L.; Family Leguminosae) is an economically important and oldest cultivated cool season legume across the world. The crop holds key role in sustainable agricultural system because it can fix atmospheric nitrogen and reduce the demand of external chemical fertilizer (Sharma *et al*. 2007). Owing to its high protein content (20–30%) and overall high nutritional status, pea has become a major contributor to the plant-derived protein market (Wei *et al*. 2020). The crop is rich source of vitamins and minerals like Ca and Mg (Sekhon *et al.* 2019). It also has a high quantity of fiber that improves bowel health. Pea also contains Vitamin B complex (Niacin) that helps in the reduction of triglycerides, thereby resulting in less cholesterol. Garden pea is quite palatable and excellent food for human consumption, which is eaten as fresh, canned, frozen and in dehydrated forms (Sharma *et al.* 2022). Green peas are planted over an area of around 0.549 million ha in India, with an annual yield of 5.68 MT and a productivity of 10.34 mt/ha (FAO 2021). Uttar Pradesh, Madhya Pradesh, Jharkhand, Punjab, Himachal Pradesh, West Bengal, Haryana, Bihar, Uttarakhand, Orissa, and Karnataka are the major pea-growing states. Himachal Pradesh is India's fifth largest pea-growing state, producing 294.96 thousand metric tonnes in 2017-18 (Anonymous 2018).

During cultivation process, the plants are challenged by a numerous interaction of soilborne fungal and oomycete pathogens. Among them, root rot complex in few past years has posed serious menace in the successful cultivation of pea crops and contribute to the quantitative and qualitative reduction in the production. More than 20 different pathogens have been reported to be associated with the disease from different parts of the world. These include various fungal pathogens such as several *Fusarium* spp., *Didymella pinodes*., *Didymella pinodella* and *Rhizoctonia solani*, as well as the oomycetes *Pythium* spp. and *Aphanomyces euteiches* (Zitnick-Anderson *et al*. 2018).

PRR-complex poses a serious threat to profitable

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cultivation of pea among all the pea growing localities of Himachal Pradesh. Available literature however, revealed only limited work on occurrence of disease complex however, to date there is no report on fungal endophytic communities associated with pea crops in sub-humid and dry temperate zones of Himachal Pradesh. Therefore, looking into the high importance of the crop and the disease in state, the present investigation was planned to record the prevalence and pathogens linked with PRR complex and additionally, to explore the inherent potential of plant by isolating the beneficial indigenous fungi for future management practices.

Materials and Methods

Disease survey

Considering the importance of pea root rot complex (PRRC) in Himachal Pradesh, an extensive survey of major pea growing areas in the sub-humid (representing Mandi and Kangra districts) and dry-temperate zones (representing Kinnaur and Lahaul Spiti districts) was conducted during the year 2021-2022. Owing to different crop growth period of pea in the above-mentioned regions, the localities of sub-humid and dry temperate were surveyed in winter (November-June) and summer (April-June) seasons, respectively. The altitude of the surveyed areas ranged from 733 to 4270 meters above sea level (masl). Three pea fields each comprising of 100 plants at one location were randomly selected and observed for pea root root/wilt symptoms. The per cent disease incidence (PDI) was calculated as below:

$$\text{Disease incidence (\%)} = \frac{\text{Number of diseased plants}}{\text{Total number of plants observed}} \times 100$$

During the surveys, healthy samples as well as pea root rot/wilt infected inflicted samples were collected in Khakhi envelopes to determine the population count of fungal endophytes and causative pathogen (s). To get pure cultures of isolates using the single hyphal tip approach, isolations were performed on PDA. The identification of pathogens was carried out using pathogenicity tests after microscopic examinations for cultural and morphological characteristics.

Sample collection, isolation and purification of the microbial culture (s)

During survey, healthy and symptomatic pea roots exhibiting typical above ground symptoms of pea root rot/wilt complex in the field were collected and were used to isolate fungal endophytes and the pathogen (s) respectively. The roots of healthy and diseased pea plants were up-rooted carefully with the help of a spade, placed in polythene zip bags, immediately brought to the laboratory, and processed within 24-48 hours of collection for enumeration of endophytic fungi and causative pathogen (s) prevalent in major pea growing areas of sub-humid and dry temperate regions of Himachal Pradesh.

Isolation and purification of pathogen (s)

To isolate the causative pathogen(s), the infected roots were washed thoroughly under running tap water to eliminate all adhering soil particles. With the assistance of a sharp sterilized blade, small bits (0.5 cm size) of the symptomatic root tissue were dissected lengthwise from the junction of the diseased and healthy sections exhibiting typical vascular discolouration. Selected root fragments were superficially disinfected for 5 minutes in a 1 per cent sodium hypochlorite (NaOCl) solution, rinsed three times with sterile distilled water to remove any remaining traces of NaOCl. The tissue fragments were subsequently placed on sterilized filter paper to remove excess moisture and then transferred aseptically to the Petri plates (four pieces/plate) containing Potato Dextrose Agar (PDA) medium. The inoculated Petri plates were incubated in BOD at 25 ± 2^0C and periodically examined for growth. The axenic culture of the pathogen(s) was obtained via hyphal tip method and scrutinized under a compound microscope (Olympus BX50), and identified based on its morphological and cultural characteristics, as documented in a standard authentic description as well as with the help of available literature (Booth 1971; Ellis 1976; Nelson *et al.* 1983). The culture was maintained on PDA slants at 4^0C in a refrigerator and sub-cultured as required for future research endeavours. Out of total isolates obtained, most prevalent pathogen was subjected further to pathogenicity test and microscopic observations for

their pathogenic confirmation and identification. Pure cultures of obtained isolates were observed for their morphological, cultural and pathogenic characteristics.

Isolation, enumeration and purification of fungal endophytes

Fungal endophytes were isolated from asymptomatic pea roots according to the protocol used by Pal *et al*. (2020). In brief, the healthy root samples were reduced to small fragments (0.5 cm size) and washed under running tap water for a period of 10-15 minutes to remove dirt and adhering soil particles. Following this, the samples were air-dried and weighed to precisely one gram and were then immersed in distilled water and drained. The root fragments were then subjected to surface sterilization by being submerged in 70 per cent ethanol for a duration of one minute and in 4 per cent sodium hypochlorite (NaOCl) for five minutes. Subsequently, the samples were treated with 70 per cent ethanol for 30 seconds and finally rinsed five times in sterilized distilled water. The surface sterilized samples were carefully dried on sterile blotting paper and were then macerated in 1 ml of sterile distilled water using a sterilized pestle and mortar. Serial dilutions were produced for each macerated sample up to 10^{-5} dilutions. 100µl from each dilution of the respective sample was poured into the corresponding Petri plates (marked from 10^{-1} to 10^{-5}), containing Potato Dextrose Agar (PDA) medium, and spread with the help of spreader. The plating was conducted in triplicate for each dilution. The plates were promptly incubated at 25 ± 2^{0} for two weeks to allow the growth of fungal cultures. Observation of these plates for mycelial growth was done on a regular basis.

To obtain a pure culture, outgrowing hyphal tips were aseptically transferred onto new Petri plate containing PDA medium. The purified fungal cultures were maintained on PDA slants at a temperature of 4^{0}C in a refrigerator for further studies. Purified endophytic cultures were designated as JPE1, JPE2, JPE3 and so on. These slants were appropriately labelled with the designated name of the endophyte and the date of culturing. Purified fungal stock cultures were sub-cultured every 9 to 10 weeks. Population of fungal root endophytes were enumerated at dilution (10^{-2}) employing plate count technique (Agarwal and Hasija 1986). The colony count of fungal endophytes was performed in triplicate and was calculated as:

$$\text{Cfu/g} = \frac{\text{No. of colonies} \times \text{Dilution factor}}{\text{Weight of root sample taken (g)}}$$

Morphological identification of predominant pathogen (s)

The cultural and morphological characteristics (after 7 days of incubation) of the isolated pathogens were studied by raising them on the PDA media under *in vitro* conditions. Characteristic morphological features such as septation of hyphae and conidia, shape of spores, pigmentation, appearance, shape of the colony and formation of chlamydospores were taken into consideration. The observed morphological and cultural characteristics were compared with the standard identification keys as described for *Fusarium* sp. (Booth 1971; Nelson *et al*. 1983), *Rhizoctonia* sp. (Parmeter 1970), *Pythium* sp. (Waterhouse 1967).

Results and Discussion

Disease survey

It is evident from the data embedded in Table 1 that the PRRC was widespread in all the pea growing localities of sub-humid and dry temperate regions of Himachal Pradesh. The maximum disease incidence (58.17%) of pea root rot/wilt was observed in Kuther area of Mandi district followed by Zamaanaabad (51.17%), and Trilokinath (22.50%) of district Kangra and Lahaul Spiti, respectively. However, the least incidence (15.83%) was recorded in Nako area of Kinnaur district. The locations surveyed in Lahaul Spiti district, in general, had very high mean incidence (38.80%) of PRRC which was followed by district Mandi (34.67%). In contrast, the minimum (24.87%) mean incidence of pea root rot/wilt complex was noted in district Kinnaur. Overall, the incidence of disease complex found ranged between 15.83 to 58.17 per cent in different locations, and none of field surveyed was free from the disease which reflects an endemic situation of PRRC in the state. The drastic emergence of the disease in all major pea growing localities of the state imposed the necessity of reliable and effective management practices.

During disease survey, it was noticed that the sites where mono-culturing are being utilized continuously by the pea growers for raising the pea crops had high

Table 1. Survey of pea root rot complex in sub-humid and dry temperate zones of Himachal Pradesh during 2021-2022

Zone	District	Location	Disease incidence (%)
Sub-humid	Kangra	Palampur	27.50
		Nagrota	22.50
		Zamaanaabad	51.17
		Mundla	20.67
		Sunehar	24.33
		Mean	**29.13**
	Mandi	Behna	25.17
		Kuther	58.17
		Movi Seri	27.00
		Naun	38.33
		Kot	24.67
		Mean	**34.67**
Dry temperate	Kinnaur	Sangla	49.00
		Kalpa	18.00
		Leo	19.83
		Chango	21.67
		Nako	15.83
		Mean	**24.87**
	Lahaul and Spiti	Lari	28.33
		Poh	25.67
		Shichling	42.67
		Trilokinath	50.33
		Kukumseri	47.00
		Mean	**38.80**

infection of PRR-complex. In Mandi district, maximum incidence of root rot in Kuther area may be due to warm and moist soil conditions particularly in the vicinity of root zone. The presence of slightly acidic pH, clay loam soil, and comparatively more rainfall could be other conducive factors. High incidence of disease in Lahaul Spiti district may be attributed to the presence of sandy loam soil and warm as well as wet moist conditions, slightly acidic pH and comparatively more rainfall (100-130 cm) in crop growth season in comparison to previous years.

Wide spread occurrence of pea root rot/wilt complex has been reported previously by various workers across the globe such as Chatterton *et al*. (2019) in Canada, Bodah *et al*. (2016) and Williamson-Benavides *et al*. (2020) in US. In India, Sharma *et al*. (2005) observed 93 per cent crop loss due to pea root rot and wilt complex. Kumari *et al*. (2016) recorded highest incidence of PRRC in HAREC region of Kukumseri (54.7%) in Himachal Pradesh representing the agroclimatic zone IV, similarly highest incidence of PRRC in agroclimatic Zone II of Himachal Pradesh was observed in Palampur, Department farm of CSKHPKV (35.30%).

Isolation of the pathogens associated with pea root rot complex

During survey, diseased samples exhibiting characteristic symptoms of pea root rot/wilt were collected and processed under laboratory conditions. A total of 45 isolates of the pathogens associated with PRRC were obtained, out of which 27 isolates were obtained from sub-humid regions while 18 isolates were recovered from the dry-temperate regions of Himachal Pradesh. Based on morpho-cultural features of these isolates, the fungal cultures were identified as 15 isolates of *Fusarium oxysporum* 13 isolates of *Fusarium solani,* 6 isolates of *Rhizoctonia solani*, 3 isolates of *Pythium* spp., 2 isolates of *Phythopthora*

sp., 2 isolates of *Fusarium equiseti* and one isolate each of *Didymella* sp., *Aphanomyces* and *Sclerotinia sclerotiorum*. This is the first instance when extensive survey was conducted in sub-humid and dry temperate regions to gather data on occurrence and pathogen communities associated with pea root rot/wilt complex. Our observations are in close agreement with earlier researchers who demonstrated that among *Fusarium* spp, *F. oxysporum f.* sp. pisi, *F. solani f.* sp. pisi and *F. avenaceum* are frequently detected genera due to ubiquitous nature and are reported to play a key role in the disease expression of the PRR-complex (Hamid *et al*. 2012). Recent surveys in North Dakota have also indicated that *Fusarium* spp. including *F. avenaceum, F. culmorum, F. graminearum, F. oxysporum, F. redolens, F. solaniand* F. *sporotrichioides* are the pathogens most frequently associated with pea root rots (Gregoire and Bradley 2005). Various other researchers also emphasized that geographical distribution of *Fusarium* spp. is influenced greatly by varied climatic conditions.

Isolation and enumeration of fungal root endophytes from pea plants

The data presented in Table 2 indicated that healthy pea root tissues collected from different pea growing sites in Himachal Pradesh harboured diverse population of fungal endosymbionts in their roots (Plate 1). In this, maximum fungal root endophytes were obtained from Palampur and Mundla locations of Kangra district and Movi Seri area of Mandi district ($4.67x10^2$cfu/g root) followed by Sunehar region of Kangra district and Nako region of Kinnaur district ($3.67x10^2$cfu/g root). Least population of the fungal endophytes was obtained from Leo area of Kinnaur district ($1.33x10^2$cfu/g root). Overall, maximum number of fungal endophytic isolates (17) were obtained from Kangra district followed by Mandi (13) and Lahaul Spiti (12) district. However, least fungal isolates (9) were recovered from Kinnaur district. In totality, 51 fungal endophytic isolates were obtained from different areas of sub-humid and dry temperate regions of HP. The variation in population of isolated

Table 2: Isolation and enumeration of fungal root endophytes associated with pea plants in sub-humid and dry temperate zones of Himachal Pradesh

Zone	District	Location	Endophytic fungal population (10^2cfu/g root)	Purified fungal Endophytes
Sub-humid	Kangra	Palampur	4.67	17
		Nagrota	2.00	
		Zamaanaabad	4.33	
		Mundla	4.67	
		Sunehar	3.67	
	Mandi	Behna	3.00	13
		Kuther	2.33	
		Movi Seri	4.67	
		Naun	2.67	
Drytemperate	Kinnaur	Kot	2.00	09
		Sangla	2.33	
		Kalpa	1.67	
		Leo	1.33	
		Chango	2.00	
		Nako	3.67	
	Lahaul & Spiti	Lari	3.33	12
		Poh	2.33	
		Shichling	3.00	
		Trilokinath	2.00	
		Kukumseri	2.67	
	Total			**51**

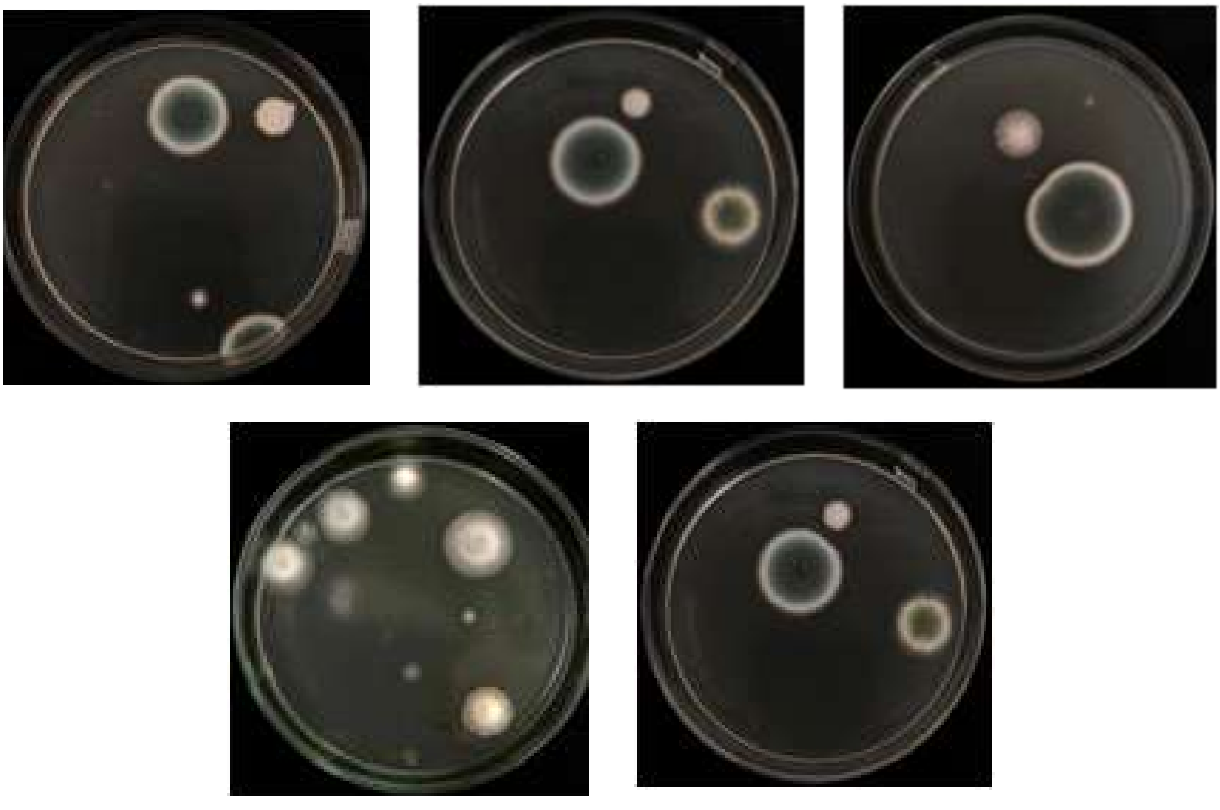

Plate 1: Isolation and enumeration of the endophytic fungi (10^2cfu/g root samples) isolated from pea plants in sub-humid and dry temperate zones of Himachal Pradesh

endophytic communities may be attributed to the climatic condition of the surveyed locations, type of variety/cultivar, sampling time, age of host plant and their interaction with different ecological niches. Our observations are similar with the findings of earlier researchers who demonstrated that diversity of endophytes is influenced greatly by the geographical locations, type and age of host plant, and physicochemical properties of soil (Adnan *et al*. 2018).

Morpho-cultural identification of the predominant pathogen (s)

Pure culture of the most predominant pathogen isolate (*Fusarium oxysporum isolate* JPP1) was obtained on PDA after 7 days of incubation at 25^0C which showed that the colour of mycelia changed from white to pink, often displayed a purple hue on reverse side of Petri plate (Plate 2 a). The mycelia were distributed sparsely or found in abundance. Microscopic examinations revealed that the fungus was observed to produce septate hyphae (Plate 2b) and three distinct types of spores, namely macroconidia, microconidia, and chlamydospores (Plate 2c). Macroconidia, which were found in varying quantities, grew on branched conidiophores or on the surface of sporodochia. They exhibited thin walls and were characterized by being three- to five septate, fusoid-subulate, and pointed at both ends. The three-septate macroconidia (Plate 2d) being more commonly observed the average measurements of which were usually within the range of 15–37.5 × 2.5–4 . On the other hand, microconidia were found in abundance and grew on simple phialides that arose laterally (Plate 2e). They were straight or curved and had an oval-ellipsoid shape. Their average measurements ranged from 2.5 to 15 × 2 to3 , and they were typically non-septate or had a single septum. Chlamydospores, which were both smooth and rough walled, were abundant and formed either terminally or on an intercalary position. They were primarily solitary in nature, although occasionally observed to form pair or chains. Based on the above morpho-cultural features, the predominantly associated pathogen with PRRC in Himachal Pradesh was preliminarily identified as *Fusarium oxysporum* accounting to 33.33 per cent of the total pathogenic isolates obtained from the different locations while

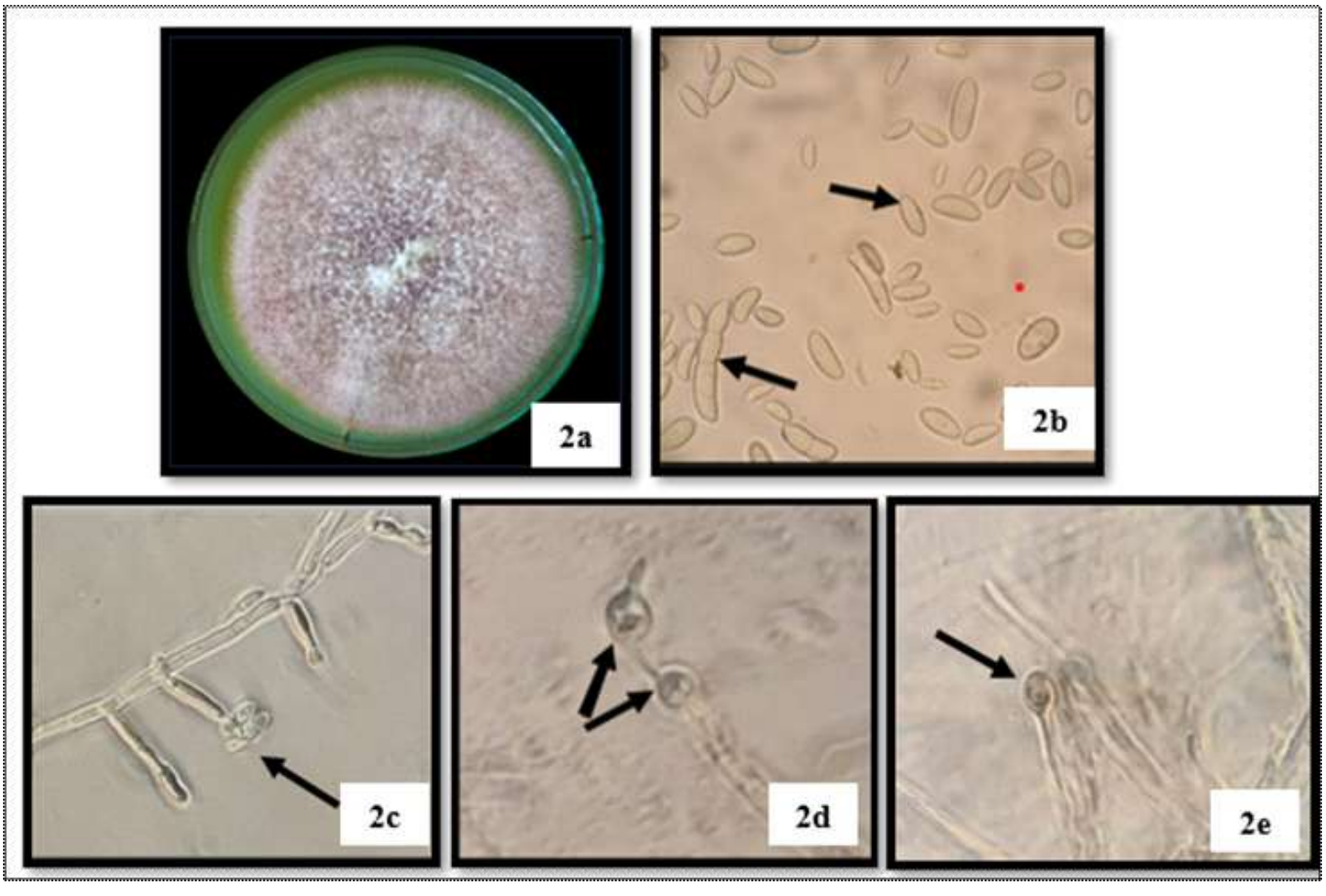

Plate 2.Morpho-cultural characteristics of most predominant pea root rot/wilt pathogen

survey. All the 15 isolates of *Fusarium oxysporum* obtained displayed a notable degree of diversity in terms of their culture and morphology. Similar to our observations, the prevalence of *Fusarium oxysporum* has been documented by Chittem *et al*. (2015), who successfully identified *F. oxysporum* f. sp. *pisi* (*Fop*) in 94.7% of the pea fields examined in North Dakota in the year 2009. Similarly, Dubey *et al*. (2010) isolated 246 isolates of *Fusarium* spp. from wilt-inflicted chickpea plants. Out of these 112 isolates were recognized morphologically as *Fusarium oxysporum* as described by Booth (1971). These isolates showed white coloured floccose to felted type mycelium with a purple hue, macroconidia (16.5-37.9 x 4.0-5.9 µm), microconidia (5.1-12.8 x 2.5-5.0 µm) with 1-5 septations. Similar morphological characters of *F. oxysporum* in pea root rot/wilt were reported by Kumari *et al*. (2016) in Himachal Pradesh.

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Viral diseases incidence and symptomatology spectrum associated with Capsicum under protected cultivation in Himachal Pradesh

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Abstract

Capsicum (*Capsicum annuum* L. var. grossum Sendt.) is popularly grown vegetable crop in the world. Diseases caused by viruses in capsicum crop affect quality and quantity of produce significantly. To know the prevalence and incidence of viral diseases in capsicum, surveys were undertaken during 2022-23 in three districts of Himachal Pradesh *viz*., Kangra, Hamirpur and Mandi. Results revealed that the highest viral mean disease incidence i.e. 32.77% was in district Kangra followed by district Hamirpur (18.75%) and district Mandi (15.74%). Varied symptomatology associated with viral diseases was observed in all surveyed the districts. Symptoms viz., mosaic, mottling, vein-thickening, vein-banding, yellowing and stunting were the most prominent and common symptoms associated with viral diseases.

Keywords: Capsicum, disease incidence, symptomatology

Capsicum annuum L. var. grossum Sendt. belongs to the Nightshade family *Solanaceae* and is originated from tropical South America (Shoemaker and Teskey 1995). It is one of the most economical and agriculturally important crop all over the world (Macneish 1964). Sweet Pepper or Bell Pepper is also popularly known as Shimla Mirch. It is high value low volume crop cultivated under natural and protected conditions in India (Nikki *et al*. 2017). In the year 2022, total annual production of capsicum in Himachal Pradesh was 48.86 thousand tonnes from an area of 2.85 thousand ha (Anonymous 2023). Himachal Pradesh is a leading supplier of capsicum to the plains during summer and rainy season. The produce becomes off-season to the plains and fetches higher price to the vegetable growers (Sreedhara *et al*. 2013).

Capsicum is attacked by numerous fungal, bacterial and viral pathogens but viral diseases are the most serious threat under protected cultivation as they affect both quantity and quality of the produce (Singh *et al*. 2020). More than 68 viruses are known to attack capsicum (Waweru *et al*. 2019). In Himachal Pradesh, viruses that are of great significance to capsicum cultivation are Cucumber mosaic virus (CMV), Pepper mild mottle virus (PMMoV), Tomato mosaic virus (TMV), Tomato spotted wilt virus (TSWV), Potato virus Y (PVY), Pepper veinal mottle virus (PVMV) and Capsicum chlorosis virus (CaCV) (Rialch *et al*. 2015, Sharma and Kulshrestha 2016).

Materials and Methods

To record the prevalence and incidence of viral diseases, surveys of major districts of Himachal Pradesh *viz*., Kangra, Mandi and Hamirpur were conducted during 2022-2023. The plants were observed for the presence of virus like symptoms *viz*., puckering, mottling, mosaic, vein-banding, vein-clearing, stunting, and yellowing etc. Data on per cent disease incidence of symptom variability was recorded during the survey. Per cent disease incidence was calculated using the following formula:

$$\text{Disease incidence (\%)} = \frac{\text{No. of infected plants}}{\text{No. of plants observed}} \times 100$$

Results and Discussion

During 2022-23, roving surveys were undertaken in twenty (20) locations of three (3) districts *viz*., Kangra, Hamirpur and Mandi to assess the prevalence

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of diseases caused by virus (es) affecting capsicum under protected cultivation (Figure 1). Surveys in three districts revealed that the presence of virus like symptoms was present in all the locations. During the survey, it was found that Kangra district had highest mean viral disease incidence *i.e.* 32.77% followed by Hamirpur and Mandi district having 18.75% and 15.74% mean viral disease incidence respectively (Figure 2). Amongst the locations surveyed in Kangra district, Dadhamb had the highest disease incidence *i.e.* 60%. Likewise, in Hamirpur and Mandi district, Choru (45%) and Siyanji (35%) had the highest disease incidence respectively. Bharmoti in the district Hamirpur, Jajraut and Dhangu in district Mandi were concluded to have least *i.e.* 5% viral disease incidence. In Kangra district, Dadhamb had the highest per cent disease incidence *i.e.* 60% followed by Vegetable farm Palampur (55%) and Rajol (50%) and least disease incidence was found in Uparla Dohbh, Bhatu Palam and Dehan *i.e.* 15%. In Hamirpur district, highest disease incidence was recorded in Choru *i.e.* 45% and the least disease incidence *i.e.* 5% was in Bharmoti. In district Mandi, Siyanji had the highest per cent disease incidence *i.e.* 35% followed by Chauntra (25%) and Palahota (20%).

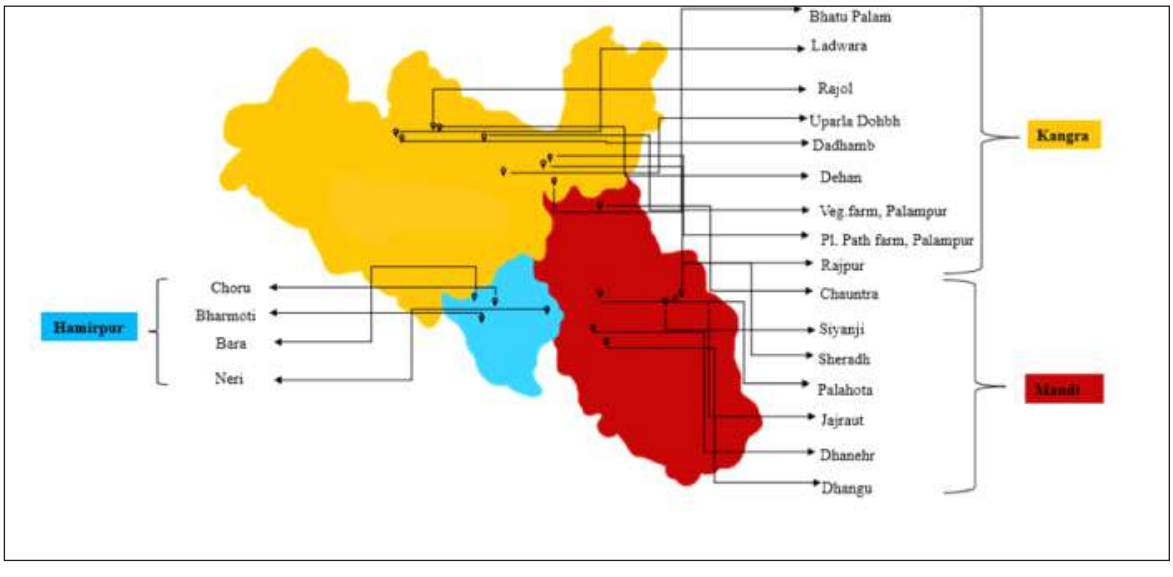

Figure 1: Map depicting different surveyed locations in Himachal Pradesh

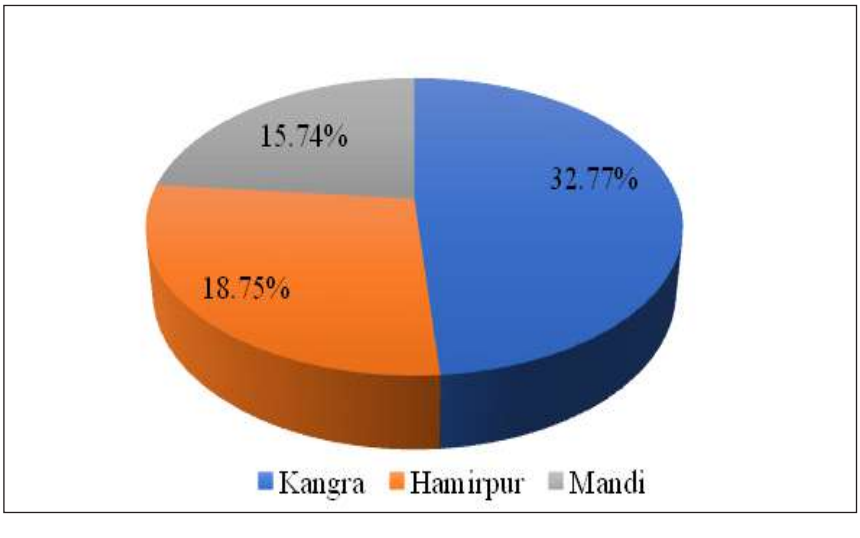

Figure 2: Average incidence of viral diseases incidence on capsicum crop in different districts of Himachal Pradesh

Varied symptomatology in capsicum was observed at different locations during the survey (Table1). The diseased plant expressed an array of symptoms like mosaic, mottling, puckering, vein-banding, vein-clearing, yellowing, stunting, chlorosis, upward or downward curling and deformation of leaves etc (Figure 3). Mottling, puckering, mosaic, leaf deformation, vein-thickening, vein-banding, upward cupping and phyllody were observed in plants suspected to be infected with virus (es).

In Kangra district, the prevalence of vein-thickening/greening disease symptoms was 17.64% followed by second highest prevalence of mosaic disease symptoms *i.e.* 16.17%. In Hamirpur district mottling/puckering were the most prevalent symptoms *i.e.* 23.68% followed by mosaic (21.05%) and leaf curling (15.78%). Likewise in Mandi district, most prevalent symptom was mosaic *i.e.* 21.21% followed by stunting/wilting and yellowing/chlorosis *i.e.* 18.88% (Table 2). Further it can be concluded that overall mean prevalence of mosaic diseases was highest *i.e.*19.47%, followed by mottling/puckering *i.e.* 16.83% and yellowing/chlorosis diseases *i.e.* 15.57% in Himachal Pradesh. The least prevalent symptoms were necrotic spots/ chlorotic ringspots *i.e.* 2.02% followed by vein-clearing/vein-banding *i.e.* 10.83% (Figure 4).

Present experiments results are in accordance with earlier workers, who also found variable incidence of virus diseases in chilli and bell pepper in different areas of Himachal Pradesh. Rialch *et al*. (2015) conducted survey to record viral disease incidence on capsicum in seven (7) districts of Himachal Pradesh. They observed that the disease incidence ranged from 12.5-40 % in surveyed districts. Highest incidence of viral diseases was observed in Bilaspur district (Berthin) with about 60% average incidence followed by Kullu and then Kangra with

Table 1: Per cent disease incidence of viral diseases in different Capsicum growing areas of Himachal Pradesh

Location (district-wise)	Samples collected (No.)	Disease incidence (%)	Geographical location		Major symptomatology observed
			Latitude	Longitude	
District Kangra					
1. Rajpur	4	35.00	32°04’34.6"N	76°32’00.1"E	Vein-thickening, shortening of internodes, lustrous greening, mosaic, mottling puckering, yellowing, wilting, stunting, vein-banding, vein-clearing, chlorotic ringspots, apical necrosis, phyllody, curling and silvering of midrib
2. Ladwara	2	20.00	32°10’39.9"N	76°13’13.4"E	
3. UparlaDohbh	3	15.00	32°11’42.1"N	76°19’28.8"E	
4. Rajol	4	50.00	32°10’10.8"N	76°14’36.7"E	
5. Dadhamb	3	60.00	32°12’57.5"N	76°10’25.8"E	
6. Bhatu Palam	4	15.00	32°04’59.2"N	76°29’26.4"E	
7. Vegetable farm, Palampur	16	55.00	32°05’38.0"N	76°32’14.4"E	
8. Pl. Path. farm, Palampur	6	30.00	32°05’54.6"N	76°32’46.1"E	
9. Dehan	3	15.00	32°04’02.9"N	76°31’01.6"E	
District Hamirpur					
10. Choru	5	45.00	32°46’47.9"N	76°25’24.0"E	Mosaic, mottling, leaf curling, necrotic lesions, vein-clearing, vein-banding and chlorosis
11. Bara	5	10.00	31°46’19.2"N	76°24’32.2"E	
12. Bharmoti	4	5.00	31°46’08.1"N	76°20’31.5"E	
13. Neri	4	7.5	31°40’41.5"N	76°29’20.0"E	
District Mandi					
14. Siyanji	6	35.00	31°29’00.8"N	76°58’41.8"E	Yellowing, wilting, vein-vclearing, mosaic, crinkling, curling, mottling, puckering and vein-thickening
15. Chauntra	2	25.00	31°00’45.7"N	76°44’56.9"E	
16. Dhanehr	3	10.00	31°34’15.4"N	76°50’59.8"E	
17. Jajruat	3	05.00	31°34’26.2"N	76°52’07.6"E	
18. Palahota	4	20.00	31°32’12.9"N	76°55’54.6"E	
19. Seradh	2	15.00	31°29’56.6"N	76°57’58.5"E	
20. Dhangu	2	5.00	31°35’44.4"N	76°55’43.0"E	

Figure 3: Symptom variability observed in Himachal Pradesh (A: Mottling and Mild puckering; B: Mosaic; C: Puckering; D: Vein-banding; E: Vein-thickening; F: Upward cupping and mosaic)

Table 2: Average prevalence of symptomatology on capsicum under protected cultivation in different districts

Districts	Symptoms prevalence (%)							
	Mottling/ puckering	Mosaic	Vein-thickening/ greening	Necrotic spots/ chlorotic ringspots	Stunting/ wilting	Yellowing/ chlorosis	Vein-clearing/ vein-banding	Leaf curling
Kangra	14.70	16.17	17.64	04.42	14.70	14.71	11.78	05.88
Mandi	23.68	21.05	10.52	00.00	02.63	13.16	13.12	15.78
Hamirpur	12.12	21.21	06.06	03.05	18.88	18.88	09.01	15.16

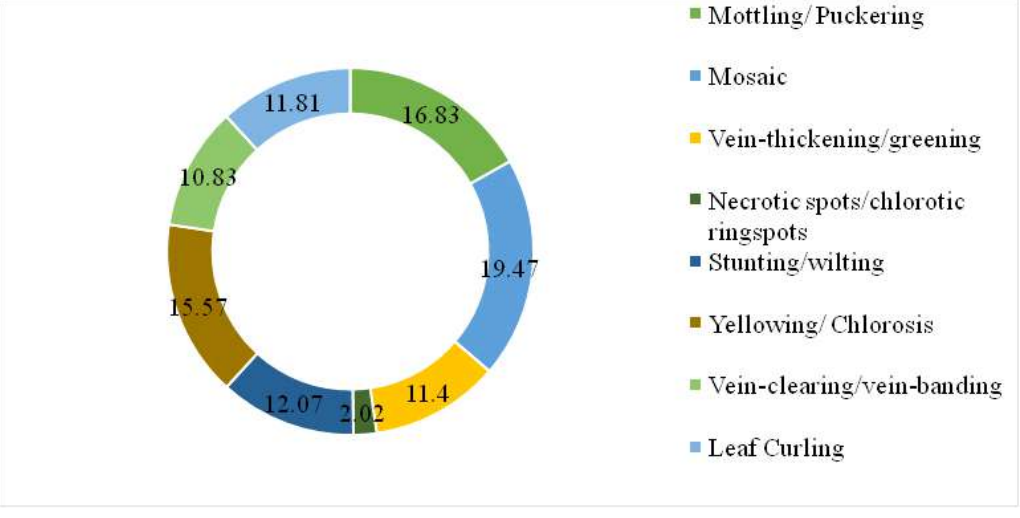

Figure 4: Pie diagram of overall mean prevalence (%) of viral symptomatology in Himachal Pradesh

24% and 20% average viral incidence, respectively. These results are in accordance with Sivaprasad *et al*. (2015) and Vinodini *et al*. (2021), who reported that viruses generally induce symptoms viz., leaf mosaic, curling, puckering, vein-banding, mottling, apical necrosis, chlorosis, yellowing and deformed fruits. Tsedaley (2015) recorded the data on viral disease incidence in capsicum crop from different locations in Nigeria, further reported that it ranged from 20-60%. Channakeshava (2019) reported that in protected cultivation of capsicum mean viral disease incidence was 27.92% in Karnataka. They also deciphered that the most prevalent disease symptom was mottling i.e 19.16% followed by leaf curling (16.83%) and mosaic (13.55%). In the face of climate change, updated information on prevalence of viral diseases under protected cultivation in Himachal Pradesh should be generated regularly. Further, characterization of variants resulting in varied symptomatology and yield losses is necessary to devise effective management strategies.

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Conflict of interest: The authors declare that there is no conflict of interest in this research paper.

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Biochemical and physiological characterization of *Ralstonia solanacearum* causing bacterial wilt of tomato

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Abstract

Bacterial wilt, caused by *Ralstonia solanacearum* (Smith) is a destructive and prevalent soilborne disease that limits tomato production in the tropics, subtropics, and warm temperate regions of the world. The present studies physiological, biochemical and pathogenicity tests were conducted to characterize *R. solanacearum*. The bacterium showed positive reaction in simple staining as purple coloured rod shaped cells, in potassium hydroxide solubility test the bacterium formed thick mucoid slime thread on reacting with KOH solution, in gelatin liquification the bacterium inoculated test tubes failed to solidify in comparison to control, among test conducted for utilization of sugars *viz*., maltose, lactose, cellobiose, sorbitol, mannitol and dulcitol the change in colour was observed from green to yellow, in dole and H_2S production test also showed positive reaction as there was turbid growth around stab and formation of black precipitates was observed. In case of starch hydrolysis, absence of colourless halo around the bacterial streak was observed and in gram staining the bacterium appeared as pink coloured rod-shaped cells which indicated the negative reaction. The pathogenicity test was conducted by three methods *viz*., soil drenching method, stem inoculation method and seedling root dip method containing bacterial suspension of 10^8 cfu/ml to study the virulent behaviour of *Ralstonia solanacearum*. Among all methods the initial symptoms of disease development were observed within 5-6 days of inoculation. The initial symptoms produced were flaccid appearance of leaves due to loss of turgidity which further progressed as drooping of leaves and complete wilting of plants. However, the drooping of leaves was observed in 15 days by stem inoculation method, followed by 14 days by seedling root dip inoculation method and 12 days by drench inoculation method.

Keywords: Bacterial wilt, Tomato, biochemical, physiological, characterization, *Ralstonia solanacearum*

Tomato (*Solanum lycopersicum* L.) is one of the most widely grown and economically significant solanaceous vegetable crop throughout the world after sweet potato and potato. In terms of nutrition, tomato plants are good source of iron, vitamins, minerals and antioxidants that are beneficial to human body. The major component of red tomatoes is lycopene, a main carotenoid in tomato. Among the pharmacological activities of lycopene and other phenolic compounds includes anticancer, anti-inflammatory, antidiabetic, antioxidant, vasodilator and cardio protective effects (Zhu *et al*., 2020).

India ranks second in the area as well as in production of tomato with production of 20.573 million grown in 8,12,000 hectares with an average yield of 25.3 MT/ha (Anonymous, 2020). In Himachal Pradesh tomato is cultivated in an area of 13,185 hectares with production of 5,39,540 tonnes (Anonymous, 2021). Bacterial wilt incited by *Ralstonia solanacearum* (synonyms *Pseudomonas solanacearum* and *Burkholderia solanacearum*) is one of the devastating diseases of tomato crop in the tropical and subtropical areas of the world (Wei *et al*., 2018).

Ralstonia solanacearum is a soil borne, rod shaped, gram negative, -proteobacterium which is pathogenic on more than 200 plant species belonging to 54 different botanical families including wide range

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of economically important crops such as eggplant, potato, tobacco, tomato and non-solanaceous crops such as peanut, banana and ginger (Genin and Denny, 2012). In India, the prevalence of bacterial wilt in tomato was first reported from Solan district of Himachal Pradesh (Gupta *et al*., 1998) where tomato is one of the major crops generally grown in summer season. Bacterial wilt was first noticed in Kangra valley of Himachal Pradesh in 1981 and remained sporadic in nature till 1985. Since then, the disease has become endemic in the mid-hill and sub-humid zone of Himachal Pradesh, comprising Kangra and Mandi districts (Sood and Singh, 1993). It causes substantial losses, varying from 2 to 90 % in different agroclimatic conditions, seasons, cultivars and strains of pathogen thus a major constraint on tomato production. Therefore, the present study was conducted to study the virulent behaviour of *Ralstonia solanacearum* based on cultural, physiological and biochemical characteristics.

Materials and Methods

The plants showing typical symptoms of vascular discoloration caused by *R. solanacearum* were collected and brought to bacteriological research laboratory in the Department of Plant Pathology. The stem of wilted plants showing discolored vascular tissues was cut into small pieces of 3-4 mm size using a sterilized scalpel blade. The infected bits were then surface sterilized in 1.0 per cent sodium hypochlorite for 30 seconds followed by three subsequent washings of sterile water to remove traces of sodium hypochlorite. The infected bits were then suspended in a test tube containing sterilized distilled water for 10 minutes. The oozing of the bacterial cells from the tissue took place, turning the water in the test tube milky.

The pathogenicity test was carried out to prove Koch's postulates. Tomato nursery was raised by sowing the seeds in pro-trays and filled with coco-peat, vermiculite and perlite in the ratio 3: 1: 1 respectively. The plastic pots (23×20×26 cm) containing sterilized soil were used for transplanting. Twenty-five to thirty days old healthy seedlings having 3-4 true leaves were used for transplanting in pots and having two seedlings per pot. The pots were filled with sand and potting medium in the ratio of 1:3 respectively. The potting mix was composed of humus and soil in the ratio 1:2 respectively. For the pathogenicity test a set of three (30 day old) tomato plants were inoculated by following soil drenching method, stem inoculation method and seedling root dip technique with the bacterial suspension containing 10^8 cfu/ml.

The pathogen *R. solanacearum* was characterized according to the guidelines described in the Bergey's Manual of Systematic Bacteriology (Garrity, 2001).

Simple staining- A drop of crystal violet (0.5% aqueous) was added to the bacterial smear and kept for 1 minute followed by rinsing in a gentle stream of running tap water. The slide was blotted dry and then microscopic observations were recorded.

Gram staining- The slide was flooded with aqueous crystal violet stain (0.5% aqueous) for 30 seconds and followed by washing with water and decolorized with 95 per cent ethanol. Then, the slide was flooded with counter stain safranin for 30 seconds and washed in a gentle stream of running tap water and decolorized with 95 per cent ethanol. The slide was rinsed with water, blotted dry and examined under the microscope.

Potassium hydroxide (KOH) solubility test- A drop of 3 per cent aqueous KOH was placed on slide and a single colony of the pathogen was removed using a cooled sterilized wire loop and mixed into KOH until an even suspension was obtained. The loop was then lifted from the slide and observations were recorded.

Starch hydrolysis-The ability of bacterium to hydrolyse starch was studied on nutrient agar media containing 1.0 per cent soluble starch. The liquefied nutrient agar was poured to Petri plates and allowed to solidify. After inoculating plates, it was incubated for seven days at 28 ± 1°C. The plates were then flooded with Lugol's iodine solution (Iodine 1g, potassium iodide 2 g and distilled water 300 ml).

Production of hydrogen sulphide- Sulphide indole motility medium was prepared and autoclaved. After autoclaving 10 ml of medium was poured in test tubes and allowed to solidify. The bacterial colony of *R. solanacearum* was picked and test tubes were inoculated followed by incubation for 24-36 hrs. Then 2 to 3 drops of Kavoc's reagent were added into inoculated test tubes to detect the H_2S production.

Gelatin liquification- Gelatin agar medium was prepared and 10 ml of medium was dispensed in test tubes. The test tubes were then allowed to solidify in upright position. The solidified test tubes were then

inoculated with a loopful of *R. solanacearum* by stabbing 4-5 times in centre of medium. The inoculated test tubes were then incubated at 28 ± 1°C for 72 hrs. along with control. After incubation test tubes were kept at 4°C until the control test tubes were solidified and observations were recorded.

Results and Discussion

The pathogen *R. solanacearum* was characterized according to the guidelines described in the Bergey's Manual of Systematic Bacteriology (Table 1). After conducting ooze test, bacterial growth was observed after streaking a loopful of bacterial cell suspension on Triphenyl Tetrazolium Chloride (TZC) medium after 24-36 hrs. The well separated irregular round, dull white, fluidal colonies, with light pink centre were observed (Plate 1) When stem pieces of such plants were suspended in distilled water, a milky white stream of ooze emerged from the tissue (Plate 1b). According to Kelman (1954), triphenyl tetrazolium chloride (TZC) medium is used to distinguish *R. solanacearum* from other bacteria during isolation. French *et al*.

Table 1: Physiological and biochemical tests for characterization of *Ralstonia solanacearum* (+) positive result, (-) negative result

Test	Reactions	Observations
a) Simple staining	+	Appearance of bacterium as purple colored rod cells
b) Gram staining	-	Appearance of bacterium as pink colored rod cells
c) KOH solubility tests	+	Appearance of thick mucoid string
d) Starch hydrolysis	-	Bacterial streak does not exhibit colorless halo
e) Gelatin liquification	+	No solidification of inoculated test tubes
f) H_2S production	+	Appearance of turbid growth around stab and formation of black precipitates
g) Utilization of sugars		
i. Maltose	+	Change of color from green to yellow
ii. Lactose	+	Change of color from green to yellow
iii. Cellobiose	+	Change of color from green to yellow
iv. Sorbitol	+	Change of color from green to yellow
v. Mannitol	+	Change of color from green to yellow
vi. Dulcitol	+	Change of color from green to yellow

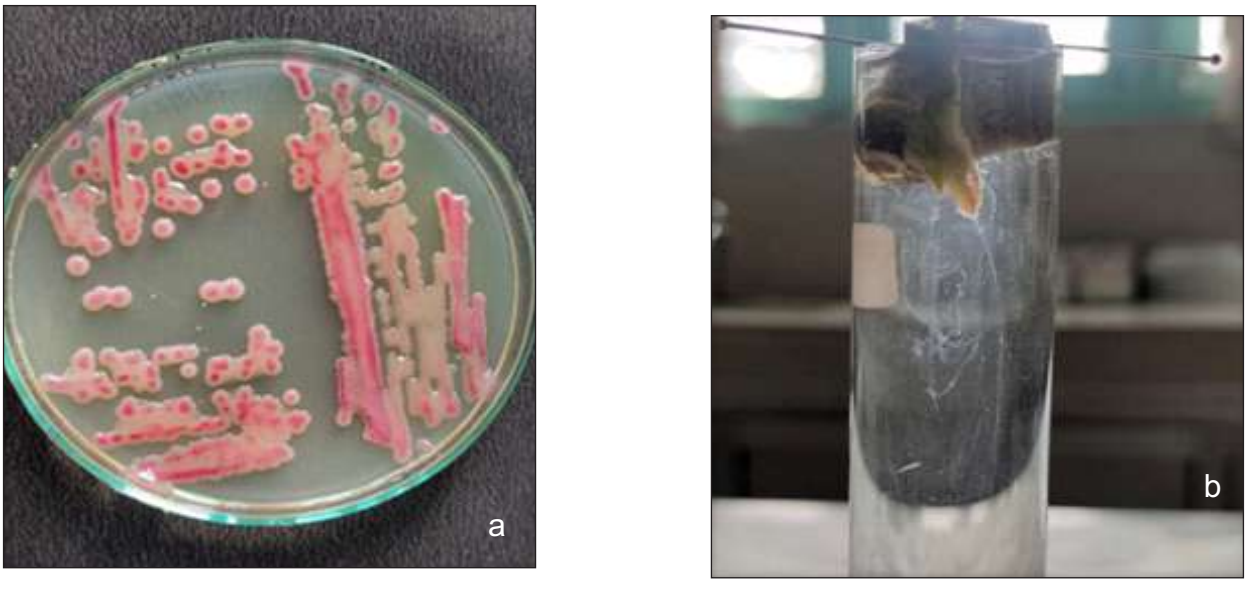

Plate 1.Isolation of R. solanacearum on TZC medium (a) and bacterial ooze test (b)

(1995) also classified the virulent coloniesof *R. solanacearum* as elevated, fluidal and either entirely white or with a pale red centre in contrary avirulent mutant colonies were butyrous and deep red. Khasabulli *et al*. (2017)and Chauhan *et al*. (2022) also reported similar results for the bacterium growth as dull white colour, fluidal, irregularly round colonies with light pink centre.

The results of gram staining reactions showed that the cells of *R. solanacearum* were small rod shaped, pink coloured when observed under microscope. The bacterium showed negative reaction for gram staining. Schaad (2001) alsotested colony of *R. solanacearum* from TTC plates and mixed with a few drops of water on a glass slide and gram stained. He observed staining results under microscope as negative reddish pink rod-shaped cells. Similar results were also reported Shahbaz *et al*. (2015). When single colony of pathogen was mixed with a drop of 3 per cent aqueous KOH solution for 5 seconds, it formed slime thread of culture suspension when loop was lifted from glass slide. It was observed that the test bacterium gave a positive result for the KOH test, as it formed thick mucoid string on reacting with KOH solution (Plate 2 a). According to Suslow *et al*. (1982) the KOH technique is far simpler and quicker than the conventional Gram strain method, which uses dyes for distinguishing between Gram-positive and Gram-negative bacteria. The results agree with those described by Vanitha *et al*. (2009).The bacterium R. solanacearum showed negative reaction in starch hydrolysis test. The results are in conformation with as reported by Zhang *et al*. (2006) and Nouri *et al*. (2009). The bacterium R. solanacearum was tested for the indole production and motility which revealed positive results for motility, cultures showed turbid growth around the stab (Plate 2 b). Tripathi (2004) reported positive results of *R. solanacearum* in the production of indole. The bacterium was tested for utilization of sugar and results revealed the change of colour from green to yellow indicating the oxidization of sugars by bacterial isolates. However, the control plates of different sugar and sugar alcohol remain unchanged. Similar results were also reported by Kumar *et al*. (1993). The results obtained from pathogenicity tests revealed that the initial symptoms of disease development were observed within 5-6 days of inoculation in drench inoculation, seedling dip inoculation and stem inoculation. The initial symptoms produced were flaccid appearance of leaves due to loss of turgidity which further progressed as drooping of leaves and complete wilting of plants. However, the drooping of leaves was observed in 15 days by stem inoculation, followed by 14 days by seedling root dip inoculation and 12 days by drench inoculation (Fig.1). *Vasse et al*. (2000) conducted two independent

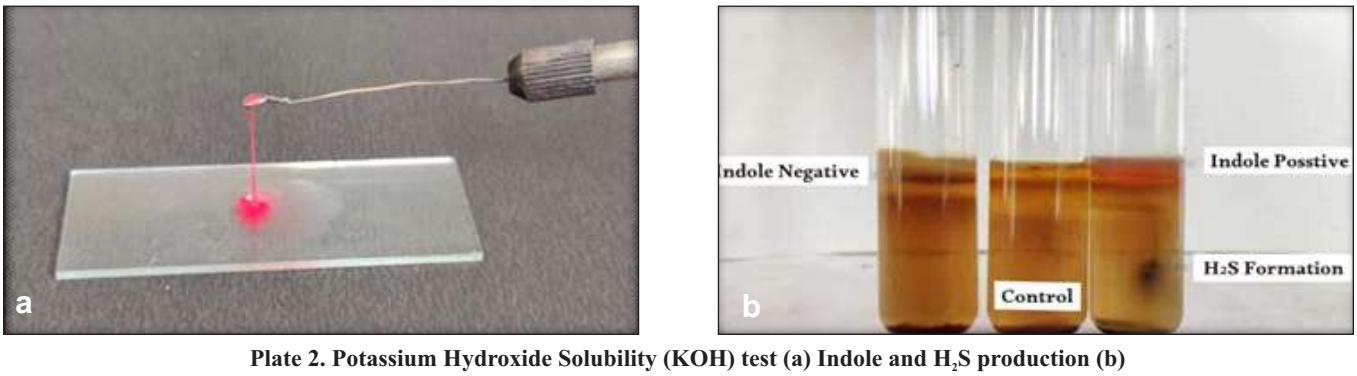

Plate 2. Potassium Hydroxide Solubility (KOH) test (a) Indole and H_2S production (b)

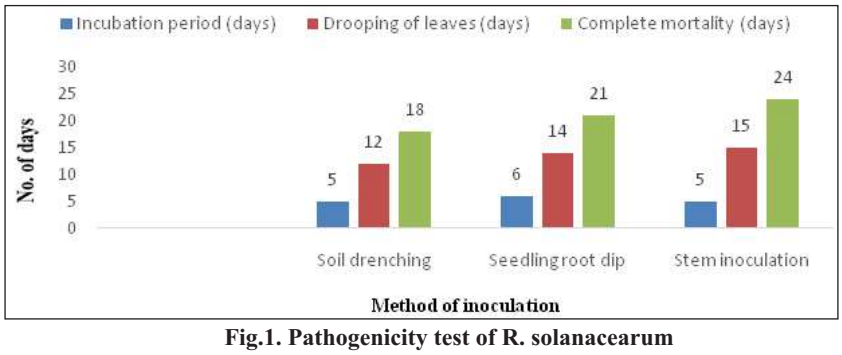

Fig.1. Pathogenicity test of R. solanacearum

pathogenicity tests, with 20 tomato plants per experiment. The wilting symptoms along with bacterial colonization were observed in tomato plants within 10 or 18 of post inoculation. Kumar and Sood (2003) also observed wilting of plants within 12 and 14 days of inoculation by suspension drenching and seedling root dip method.

Conflict of interest: The authors declare that there is no conflict of interest in this research paper.

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Studies on factors affecting the pathogenesis of *Alternaria solani* (Ell. and Mart.) Jones and Grout on Tomato *(Solanum lycopersicum* Mill*.)*

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Abstract

Development of disease is essential to study the various aspects of pathogen for the management of disease. Hence, the present study was conducted to ascertain the effect of inoculum load, age of pathogen culture, sub-culturing and host age on the development of early blight of tomato. Five conidial concentrations *viz.*, $5x10^3$, $1x10^4$, $5x10^4$, $1x10^5$ and $5x10^5$ conidia/ml were evaluated. *Alternaria solani* inoculum load of $5x10^5$ spores/ml gave maximum disease severity (34.60%) with shortest incubation period of 3.0 days followed by $1x10^5$ (31.53%) with 4.2 days of incubation period. The findings of the present study suggested that 14 days old culture of *A. solani* as compared to 7, 21, 28 and 35 days old was most virulent with highest mean disease severity (27.47%) and shortest incubation period (3.6 days). The shortest incubation period (3.0 days) was observed with first sub-culture as compared to 2^{nd}, 3^{rd}, 4^{th} and 5^{th} subcultures, which resulted in highest mean disease severity (31.13%). The results for the second sub-culture were also found to be at par. This indicated that the pathogen was most virulent up to second sub-culturing and that successive sub-cultures reduced the inoculum potential. The disease was observed more on the mature plants as compared to younger plant. It was also found that tomato plants of 50-60 days after sowing are most susceptible to early blight infection with maximum disease severity of 37.80 per cent and host plant most likely exhibited only seedling resistance against the necrotrophic pathogen *A. solani.*

Key words: Artificial inoculation, tomato, *Alternaria solani*, pathogenicity

India is one of the largest tomato growing countries in the world, occupying the first position in area and second position in production after China. Tomato is the second most important vegetable crop in the world after potato and accounts for nearly 32 per cent of the total vegetables produced in the country (Anonymous 2020). In spite of these achievements, the last couple of years, the low production of tomato in India has consequently resulted in drastic decrease in its per capita availability and has compelled India to import large quantity of tomatoes (Adhikari *et al.* 2017). The main cause of low productivity is due to various diseases and pests associated with solanaceous crops (Chauhan *et al.* 2020). Early blight is one of the important diseases of tomato responsible for average yield loss of 10 to 70 per cent in different parts of Northern India depending upon the severity (Shinde *et al.* 2018). Due to the lack of availability of the sources of resistance against *Alternaria solani*, early blight is considered the most damaging and widespread fungal disease of tomatoes (Sharma *et al.* 2021). Since early blight is increasingly destructive, disease management strategies need to be developed. The first step in any of the resistance breeding programme is to rapidly screen all the available genetic stocks, including the local land races, improved cuttings and exotic germplasms using empirical techniques in glass houses, or by field tests. For a successful screening, artificial inoculation of plants at susceptible growth stage with an adequate amount of inoculum is necessary. The artificial inoculation of host is necessary to obtain a more uniform disease; moreover, establishment of disease by artificial inoculation is also essential for studies of various aspects of plant pathology, including epidemiology, etiology, disease resistance, host-parasite interaction, and disease control (Thakur and Banyal 2022). Jie *et al*. (2009) also reported that the

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artificial inoculation method provided a foundational understanding of ecological enrichment to control banana wilt disease in future. In this report, the effect of age of pathogen culture, sub-culturing, inoculum load and host age on the development of early blight of tomato was ascertained with the objective to standardize these factors for pathogenesis studies of *A. solani* on tomato.

Materials and Methods

Alternaria solani (Berk.) Sacc. culture was derivedfrom diseased tomato leaf samples using standard leaf bit methodology (Dhingra and Sinclair 1985)at CSK HPKV Palampur. Fungal culture was purified by single spore method (Choi *et al.* 1999) and the isolate was maintained on potato dextrose agar (PDA) in the Department of Plant Pathology. To enhance the sporulation of *A. solani*on PDA medium, the plates were exposed to UV light exposure for 20 second after 4-5 days of incubation and then placed back in the incubator (Aragaki 1961; Yadav *et al*. 2015). A conidial suspension was prepared by scraping mycelia and spores from plates of actively growing fungal cultures into autoclaved water and filtering the suspension through four layers of cheese cloth to remove most of the mycelia. The filtered spore suspension was centrifuged at 2000 x g for 5 min and re suspended in deionized water. This centrifugation was repeated till a clear spore suspension was obtained. *Alternaria solani* conidia being long beaked, tend to clog together among themselves and with bits of media and mycelium. This prevented accurate calculation of the spore concentration. Therefore, a clear spore suspension was preferred. After the final wash, supernatant was discarded and spores were re suspended in water containing 0.05% Tween-20.

Inoculum load

Different inoculum concentrations (spores/ml) were evaluated to standardize the optimum inoculum concentration required for successful infection and development of early blight symptoms on tomato plants. For this, two-month-oldseedlings of susceptible tomato hybrid Avtar were spray inoculated with different conidial concentrations *viz.*, $5x10^3$, $1x10^4$, $5x10^4$, $1x10^5$ and $5x10^5$ conidia/ml. Simultaneously, control treatment by spraying sterilized water was also maintained. The inoculated plants were placed in net-house by maintaining RH > 90% for 48 hours at temperature 25-30°C and thereafter the pots were watered daily until the development of disease symptoms. Five plants were taken for each replication and the treatments were replicated thrice in CRD. The incubation period was recorded for each treatment and per cent disease index (PDI) was recorded using rating scale given by Pandey *et al.* (2003). The PDI values were expressed using the formula given by McKinney (1923). Disease progression was measured by calculating AUDPC and apparent rate of infection (r) as per logistic equation given by Vander plank (1963).

$$\text{PDI} = \sum \frac{Severity\ grade \times Number\ of\ leaves}{Maximum\ grade \times Total\ number\ of\ leaves\ scored} \times 100$$

$$\text{AUDPC} = \sum_{i=1}^{n-1} \frac{y_i + y_{i+1}}{2} \times (t_{i+1} - t_i)$$

Where, y_i= Disease severity at the i^{th} observation

t_i = time (in days) at the i^{th} observation

n = total number of observations.

$$r = \frac{2.303}{t_2 - t_1} \log 10 \frac{x_2(1 - x_1)}{x_1(1 - x_2)}$$

Where,

r = apparent infection rate per day

t_2-t_1= time interval between first and last observation

x_1 & x_2= proportion of leaf area covered by lesion at t_1 and t_2 time intervals, respectively

(1-x_1) & (1-x_2) = proportion of healthy leaf area at t_1 and t_2 time intervals, respectively

Age of culture

The isolated culture of *A. solani* from the infected tomato leaves on PDA was maintained in the laboratory for different time durations *viz.,* 7 days old culture, up to 35 days. The pathogenicity tests were carried out in polyhouse condition by spraying mycelial suspension ($1x10^5$ conidia/ml) from 7, 14, 21, 28 and 35-days old culture on two-months old seedlings of susceptible tomato hybrid Avtar grown in pots. The inoculated plants were placed in net-house by maintaining RH > 90% for 48 hours at temperature 25-30°C and thereafter the pots were watered daily until the development of disease symptoms. Five plants were taken for each replication and the treatments were replicated thrice in CRD.Observation on incubation period was recorded for each treatment.

Per cent Disease Index (PDI) was recorded at weekly interval. The AUDPC and infection rate were calculated as described earlier.

Pathogen sub-culturing

To investigate the effect of sub-culturing of pathogen on the development of early blight on tomato, the first isolated and purified colony of *A. solani* grown on PDA for 7 days was designated as the first-generation culture. For subsequent sub-culturing, mycelial plug from the center of each colony was used to establish a new growth on a Petri plate with PDA. Five plates were prepared at each sub-culturing after every 7 days up to 5 generations. The pathogenicity tests were carried out in polyhouse condition by spraying conidial suspension ($1xl0^5$ conidia/ml) from 1^{st}, 2^{nd}, 3^{rd},4^{th} and 5^{th} generation pathogen culture on two-month-oldseedlings of susceptible tomato hybrid Avtar grown in pots. Five plants were taken for each replication and each treatment was replicated thrice in pots along with an uninoculated control. The inoculated plants were placed in net-house by maintaining RH > 90% for 48 hours at temperature 25-30°C and thereafter the pots were watered daily until the development of disease symptoms. Observations on incubation period and per cent disease index (PDI) were recorded. The AUDPC and infection rate were calculated as described earlier.

Host age

Tomato seeds of susceptible hybrid Avtar sown (five seeds/pot) in pots were spray inoculated at 30, 40, 50 and 60 days after sowing. For this staggered sowing was done at 10 days interval up to 60 days. Plants were grown in controlled condition. The pathogenicity tests were carried out in polyhouse condition by spraying mycelial suspension ($1xl0^5$ conidia/ml). Five plants were taken for each replication and each treatment was replicated thrice in pots along with an uninoculated control. The inoculated plants were placed in net-house by maintaining RH > 90% for 48 hours at temperature 25-30°C and thereafter the pots were watered daily until the development of disease symptoms. Incubation period and per cent disease index (PDI) were recorded and AUDPC and infection rate were calculated as described earlier.

Results and Discussion

Inoculum load

The effect of different inoculum levels on disease development was studied and the data has been presented in Table 1. Significant variation in incubation period, disease severity, AUDPC and rate of infection was recorded at different inoculum loads. Inoculum load of $5x10^5$ spores/ml gave maximum disease severity (34.60%) with shortest incubation period of 3.0±0.71 days followed by $1x10^5$ (31.53%) with 4.2±0.45 days of incubation period. However, minimum disease severity (26.93%) was observed at inoculum level $5x10^3$ after 6.8±0.84 days of incubation period. The disease progress was also significantly high at $5x10^5$ spores/ml inoculum load which was evident by highest AUDPC (485.80) and apparent infection rate (0.07). This was followed by $1x10^5$ conidial concentrations with 448.00 AUDPC and 0.06 rate of infection. Significantly lowest disease severity was observed at $5x10^3$spores/ml with minimum AUDPC and apparent infection rate *i.e*. 377.30 and 0.01, respectively. The standardization of inoculum load helped determine that spore concentration between $1x10^5$ and $5x10^5$spores/ml can initiate the infection for successful disease development with minimum incubation period. Therefore, for all further experimentations inoculum load of $1x10^5$ spores/ml was used.

Table 1. Effect of inoculum load of *Alternaria solani* on the development of early blight of tomato

Inoculum load (spores/ml)	Incubation period days	Disease severity (%) after disease appearance				AUDPC	Apparent rate of infection (r/day)
		7	14	21	Mean		
$5x10^3$	6.8±0.84	18.20	27.00	35.60	26.93	377.30	0.01
$1x10^4$	5.4±0.55	21.80	30.00	36.20	29.33	413.00	0.02
$5x10^4$	4.4±0.55	20.00	31.20	38.20	29.80	422.10	0.04
$1x10^5$	4.2±0.45	21.20	33.40	40.00	31.53	448.00	0.06
$5x10^5$	3.0±0.71	23.40	35.00	45.40	34.60	485.80	0.07
LSD (0.05)	-	0.69	1.23	1.89	-	3.89	0.02

The results are in conformity with other workers. Coffey *et al.* (1975) reported that early blight severity gradually increased on young tomato plants with increase in *A. solani* conidial concentration from $5x10^3$ to $8x10^4$spores/ml. A positive correlation between inoculum concentration and symptom development has also been demonstrated for other *Alternaria* species by Christ and Maczuga (1989) and Vloutoglou (1994). Vloutoglou and Kalogerakis (2000) reported that as the inoculum concentration of *A. solani* increased from $6x10^3$ to $11x10^3$conidia/ml, the percentage of tomato leaf area affected and defoliation increased linearly. Bhardwaj (2018) observed $6x10^4$ as the ideal inoculum concentration for rapid advancement of *Alternaria porri*on garlic, while inoculum concentration below $2x10^4$ noticeably delayed the infection process. Adequate amount of inoculum is a pre-requisite for initiation of symptoms and disease progress as this is likely to be proportional to the amount of pathogenicity factors required for initial host invasion.

Age of pathogen culture

To study the effect of age of pathogen culture on the development of early blight of tomato, an experiment was conducted in pots with 7, 14, 21, 28 and 35-days old pathogen culture maintained on PDA. The data presented in Table 2 revealed that 14 days old culture was most virulent with highest mean disease severity (27.47%) and shortest incubation period (3.6±0.89 days) along with maximum AUDPC and infection rate of 378.70 and 0.038 r/day, respectively. The difference in incubation period was statistically insignificant in plants inoculated with 21 to 35 days old cultures. Minimum disease severity (13.87%), AUDPC (200.20) as well as infection rate (0.018) was recorded with 35 days old pathogen culture. The disease severity obtained with 7 (23.27%) and 21 (23.53%) days old culture was statistically at par with each other. Pathogen culture older than 14 days indicated successively reduced virulence as evident from delayed disease appearance and slow disease progress with low terminal disease severity. The pathogen virulence was also observed less with 7 days old culture, indicating that about 2 weeks old pathogen culture is most suitable for development of disease and sporulation. The rate of sporulation and growth rate significantly decreased after 14 days pertaining to the depleting nutrients in the culture media

Vloutoglou and Kalogerakis (2000) studied the effects of age of culture, inoculum concentration, wetness duration and plant age on development of early blight and on shedding of leaves in tomato plants and found that all these factors could affect the disease development. Koley and Mahapatra (2015) reported that maximum growth of *A. solani* was observed at 8 days after inoculation however, the growth rate consistently decreased after 3 days of inoculation. The sporulation rate also declined with increase in the age of culture. Similar observations were recorded for other fungal pathogens by Sennoi *et al.* (2013) and Anand (2019).

Pathogen sub-culturing

An experiment was conducted *in vivo* to study the effect of sub-culturing of *A. solani* on the development of early blight of tomato and the data recorded are

Table 2. Effect of age of culture of *Alternaria solani* on the development of early blight of tomato

Age of culture (days)	Incubation period (days)	Disease severity (%) days after disease appearance				AUDPC	Apparent rate of infection (r/day)
		7	14	21	Mean		
7	4.8±0.45	16.80	20.20	32.80	23.27	326.20	0.031
14	3.6±0.89	20.20	25.80	36.40	27.47	378.70	0.038
21	5.4±1.00	16.40	22.20	32.00	23.53	319.20	0.027
28	5.7±0.71	8.60	16.40	27.00	17.33	239.40	0.026
35	6.6±0.55	7.80	15.60	18.20	13.87	200.20	0.018
LSD (0.05)	-	2.09	1.55	2.92	-	4.10	0.002

presented in Table 3. The shortest incubation period (3.0±0.71 days) was observed with first sub-culture which also resulted in highest mean disease severity (31.13 per cent) with maximum AUDPC and infection rate of 437.50 and 0.051 r/day, respectively. The 2nd subculture was found to be statistically at par with 1st subculture resulting in 30.47 per cent disease severity with AUDPC and infection rate of 432.60 and 0.054 r/day, respectively. The incubation period gradually increased with subsequent sub-culturing of the pathogen and it was recorded to be maximum (7.6±0.55 days) for the 5th sub-culture. The conidial production and rate of conidial germination was reduced on sub-culturing and this indicated that the pathogen was highly virulent up to second sub-culturing and that successive sub-cultures reduced the inoculum potential leading to delayed disease appearance and lower disease progress and terminal disease severity.

Although sub-culturing is required to prolong the lifespan and/or increase the number of microbial cells in the culture, successive sub-culturing has been reported to affect the virulence, conidial yield, germination and stability of strains in various fungal pathogens (Bruslind 2021). The *in vitro* sporulation of *A. solani* requires special conditions and the conidial production tends to decrease after periodic sub-culturing (Yadav *et al.* 2015). Anand (2019) also recorded that young and early generation of pathogen culture gave maximum disease incidence of collar rot of cow pea and significant decrease in the disease incidence was observed with increase in age and sub-culturing of the pathogen.

Host age

To determine the most susceptible stage of tomato for infection of early blight, an experiment was conducted under pot condition. Susceptible tomato plants were inoculated with *A. solani* ($1x10^5$ spores/ml) at 30, 40, 50 and 60 days after sowing (DAS). Plant at 60 DAS was recorded as the most critical growth stage with highest disease severity of 37.80 per cent (Table 4). The disease progress was also significantly high at this stage which was evident by highest AUDPC (520.10) and apparent infection rate (0.06). This was followed by 50 days old plant with AUDPC and rate of infection *i.e.* 465.50 and 0.04, respectively.

Table 3. Effect of sub-culturing of *Alternaria solani* on the development of early blight of tomato

Sub-culture (SC)	Incubation period (days)	Disease severity (%) days after disease appearance				AUDPC	Apparent rate of infection (r/day)
		7	14	21	Mean		
SC1	3.0±0.71	22.00	31.60	39.80	31.13	437.50	0.051
SC2	4.2±0.45	21.80	32.20	37.40	30.47	432.60	0.054
SC3	4.4±0.55	16.40	27.80	33.40	25.87	368.90	0.046
SC4	5.4±0.55	12.40	20.40	29.40	20.73	289.10	0.033
SC5	7.6±0.55	12.20	19.00	24.00	18.40	259.70	0.033
LSD (0.05)	-	1.73	1.98	2.44	-	4.89	0.002

Table 4. Effect of host age on the development of early blight of tomato

Host age (DAS)	Incubation period (days)	Disease severity (%) after disease appearance				AUDPC	Apparent rate of infection (r/day)
		7	14	21	Mean		
30	4.0±0.71	13.60	26.60	39.20	26.47	371.00	0.03
40	4.6±0.55	16.80	30.40	42.60	29.93	420.70	0.04
50	4.0±0.71	22.00	32.00	47.00	33.67	465.50	0.04
60	4.6±0.89	26.00	35.20	52.20	37.80	520.10	0.06
LSD (0.05)	-	1.67	1.89	1.93	-	3.89	0.02

DAS- Days after sowing

Significantly lowest disease severity was observed in plants inoculated at the youngest stage (30 DAS) with minimum AUDPC and apparent infection rate *i.e.* 371.00 & 0.03, respectively. No significant difference in the incubation period was recorded between plants inoculated at 30 to 60 DAS. It was concluded that tomato plants of 50-60 DAS are most susceptible to early blight infection under suitable conditions. The host plant most likely exhibited only seedling resistance against the necrotrophic pathogen *A. solani.*

Pandey *et al.* (2003) observed that symptoms of early blight appear at 30-35 days after transplanting and the most critical stage was reported at 35 and 55 days of plant growth. Several workers have reported that older leaves are more susceptible to early blight than younger ones (Vennila *et al.* 2020). Kong *et al*. (1995) reported that the susceptibility of *Alternaria helianthi* in sunflower tissue increased with age, so that older leaves were more susceptible than young and expanding leaves. Similar observations on increased susceptibility of host with advanced growth stages have been reported in Alternaria blight on Indian mustardand *Alternaria porri* on onion and niger (Maniyar*et al*. 2018; Sharma and Ratnoo 2019).

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Morpho-cultural variability of *Fusarium solani* isolates causing root rot of okra in low and mid hills of Himachal Pradesh

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Abstract

Morphological and cultural variability was studied among the okra root rot pathogen isolates (35) of *Fusarium solani* collected from infested okra fields constituting three agroclimatic zones of Himachal Pradesh. The isolates cultured on potato dextrose agar medium showed considerable cultural variabilities in conidia dimensions, colony colour, colony diameter, and number of septa. Colony diameter among *F. solani* isolates ranged from 74-86 mm and produced white/ greyish white growth with light pink/purplish/brownish pigmentation. Aggressiveness of all the 35 isolates was tested by soil infestation method where isolates were categorized as highly, moderately, or weakly pathogenic. The isolates produced macroconidia of size ranged from 18.23-30.85×4.70-5.99 µm with 1-5 septation, microconidia of size 5.91-11.71×2.78-3.97 µm with 0-1 septation and chlamydospore of size 8-12×8-10 µm. Based on these morpho-cultural characteristics and cluster analysis using SPSS 16.0 software, all 35 isolates grown on PDA medium at 25±1 ºC were categorized into seven morpho-cultural groups and were designated as FS-MV-1 to FS-MV-7.

Key words: Root rot, morpho-cultural, variability, *Fusarium solani*

Okra [*Abelmoschus esculentus* (L.) Moench] is an important vegetable grown in almost all parts of India. The okra fruits along with its seeds are a source of variety of nutrients and therefore acquire a high position in nutritional charts. Its fruits are edible and are rich source of calcium, iron, saturated fats, carbohydrates, proteins, riboflavins and vitamin A, B, C, E and K, etc. hence provide nutritional and health benefits. Okra production has been suffering to a great extend because of many fungal diseases but root rot is one of the destructive diseases caused by many fungal pathogens like *Fusarium* spp. and *Rhizoctonia solani* where the most frequently associated pathogen with root rot was *Fusarium solani.* (Purba 2004). A deep comprehension of the populations of pathogens is important as they show variations in pathogenicity, response to management systems, environment, and host differences. Thus, population biology of the pathogen needs to be studied thoroughly. In Himachal Pradesh, root rot caused by *F. solani* has also emerged as a major disease constraint in okra production so a detailed investigation of pathogen causing okra root rot is required. In this regard, major okra growing areas were investigated for pathogens association and prevalence of major pathogen in different areas of HP besides morphological studies were carried out which revealed *Fusarium solani* to be major causal organism for okra root rot in Himachal Pradesh along with *Fusarium oxysporum* and *Rhizoctonia solani.*

Materials and methods

Isolation of pathogen and pathogenicity test:

The fungal cultures were isolated from diseased tissues using standard methodology on PDA. The diseased samples were washed with sterilized water and cut into small bits of 2-3 mm having half healthy and half diseased portion. These bits were surface sterilized by dipping in 1% sodium hypochlorite solution for 10-15 seconds followed by washing three times in sterilized distilled water under laminar air flow hood. The bits were dried in 2-folds of sterilized filter papers to remove excess moisture and transferred into PDA Petri plates under aseptic conditions and incubated in BOD at 25±1°C. After 48 hrs. of

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incubation, growing mycelium was transferred to PDA slants and purified by single spore culture. Fungal colony arising from single spore of each isolate was maintained on PDA medium and used for further studies. Pathogenicity test was performed on susceptible okra cultivar 'P-8' in pots under controlled condition by three methods *viz.,* a) seed inoculation b) soil inoculation and c) seed-cum-soil inoculation to confirm the pathogenic nature of isolate. The spore suspension of the fungus having 4.0×10^5 spores/ml was used as inoculum. All the isolates of the fungus were multiplied on sterilized corn flour and sand mixture (1:9 w/w basis) for 10-15 days in flasks. The inoculum of each isolate was separately mixed @ 100 g/kg sterilized soil and this mixture was put in the plastic pots. Ten apparent healthy and surface sterilized seeds of susceptible okra variety P-8 were sown in each sick pot (6" diameter). Seeds sown in pots having sterilized soil without inoculum served as check. The pots were transferred to the green house and watered daily and observed for the development of disease symptoms. The pathogen was re-isolated from the diseased plant and cultured by standard methods discussed previously. The characteristics of pathogen culture thus obtained was compared with that of corresponding inoculated culture of the pathogen to prove the pathogenicity.

Identification of pathogen

The identification of test pathogens was done by studying morpho-cultural parameters. The test pathogens were grown on PDA in Petri plates and incubated for 5 days at 25±1°C in BOD incubator. The mycelium was scraped by using sterilized needle and placed over the glass slide and examined under compound microscope at 10X and 40X. *Fusarium solani* was identified based on colony colour and colony growth on PDA, size of microconidia and macroconidia and compared with the morphological characters documented in taxonomic keys (Booth, 1971). *F. solani* was identified based on colony colour, diameter, and the size of macroconidia and microconidia.

Identification based on morphological characters

Collection of isolates was done and then studied by following methods for different parameters of morphological identification of the pathogen.

a) Colony diameter

Colony diameter was measured by the measuring scale after 9th day (from 3 replications).

b) Colony colour

Colour of colonied were observed visually from the front and back of the Petri plate at 9th day (from 3 replications).

b) Colony growth

Growth pattern and shape of colony was examined by observing mycelium (from 3 replications).

d) Dimensions of macro and microconidia

The length, breadth, and number of septa of macro and microconidia were observed under compound microscope at 40 x magnifications by using stage and ocular meter. Data of fifteen conidia were recorded from all 35 isolates.

Results and Discussion

Isolation of *Fusarium solani* isolates

During *Kharif* season of 2019-20, 2020-21 and 2021-22 different okra growing localities were surveyed and thirty-five isolates of pathogen associated with root rot of okra were collected from six districts of the state, belonging to three agro-climatic zones (Table 1) which included 18 isolates from district Kangra, 5 from Hamirpur, and three each from Bilaspur, Chamba, Una and Mandi districts. Majority of the isolates were collected from Kangra district.

Pathogenicity test

Pathogenicity with root rot of okra associated pathogen (*F. solani*) isolates was proved under pot culture conditions on variety 'P-8' making sick soil by adding 100 g inoculum per kg of soil. Initial symptoms appeared early at the seedling stage, mostly during two-three leaf stage of the plant. Initially the tender leaves start wilting and drooping, in advanced stages, blackening of collar regions of stem takes place, which progresses up to 1-2 cm above the soil level. On uprooting, it was found that the root system of infected plants was poorly developed, damaged, and decayed. On examining the collar region, it was noticed that the blackening and decaying progressed up to stellar region thereby disrupting the nutrient supply to the growing parts. The pathogen was recovered after isolation from plants exhibited characteristics symptoms in pathogenicity test. Pure culture obtained by single spore matched exactly with the inoculated test pathogen hence pathogenicity proved.

The pathogenicity of all the 35 isolates of *Fusarium solani* was tested by soil infestation method (Wagh 2009). Observations on root rot/wilt incidence

Table 1. *Fusarium solani* isolates collected from different locations

Isolate No./Name	Location	Agro-Climatic Zone	Latitude	Longitude	District
Fs01	Palampur	Zone-II	32.1109°N	76.5363°E	Kangra
Fs02	Nagri	Zone-II	32.1314°N	76.4708°E	Kangra
Fs03	Banuri	Zone-II	32.1018°N	76.5575°E	Kangra
Fs04	Bharmat	Zone-II	32.1071°N	76.5676°E	Kangra
Fs05	Sungal	Zone-II	32.0835°N	76.5821°E	Kangra
Fs06	Maranda	Zone-II	32.0801°N	76.5112°E	Kangra
Fs07	Bhawarna	Zone-II	32.0398°N	76.4997°E	Kangra
Fs08	Panchrukhi	Zone-II	32.0566°N	76.5647°E	Kangra
Fs09	Panapar	Zone-II	32.0441°N	76.4501°E	Kangra
Fs10	Ghalour	Zone-I	31.8365°N	76.2790°E	Kangra
Fs11	Jawalamukhi	Zone-I	31.8752°N	76.3203°E	Kangra
Fs12	Paprola	Zone-II	32.0528°N	76.6341°E	Kangra
Fs13	Alhilal	Zone-II	32.0646°N	76.6138°E	Kangra
Fs14	Kangra	Zone-I	32.1015°N	76.2731°E	Kangra
Fs15	Nagrota	Zone-II	32.1054°N	76.3789°E	Kangra
Fs16	Hatwas	Zone-II	32.1125°N	76.4016°E	Kangra
Fs17	Abdullapur	Zone-I	32.1245°N	76.2667°E	Kangra
Fs18	Zamanabad	Zone-I	32.1195°N	76.2660°E	Kangra
Fs19	Putrial	Zone-I	31.7467°N	76.4546°E	Hamirpur
Fs20	Bara	Zone-I	31.8731°N	76.2117°E	Hamirpur
Fs21	Fatehpur	Zone-I	31.3742°N	76.3156°E	Hamirpur
Fs22	Bharmoti	Zone-I	31.7629°N	76.3475°E	Hamirpur
Fs23	Sujanpur	Zone-I	31.8339°N	76.5055°E	Hamirpur
Fs24	Ghumarwin	Zone-I	31.9156°N	76.3710°E	Bilaspur
Fs25	Chhajoli	Zone-I	31.5170°N	76.6507°E	Bilaspur
Fs26	Naswal	Zone-I	31.4716°N	76.6788°E	Bilaspur
Fs27	Saru	Zone-III	32.1030°N	76.5821°E	Chamba
Fs28	Parel	Zone-III	32.5630°N	76.1187°E	Chamba
Fs29	Banota	Zone-III	31.5527°N	76.6369°E	Chamba
Fs30	Amb	Zone-I	31.6798°N	76.1175°E	Una
Fs31	Andora	Zone-I	31.6858°N	76.1143°E	Una
Fs32	Mubarikpur	Zone-I	31.7095°N	76.0827°E	Una
Fs33	Jogindernagar	Zone-II	31.9912°N	76.7899°E	Mandi
Fs34	Naun	Zone-III	31.5597°N	77.0262°E	Mandi
Fs35	Siyanji	Zone-III	31.4837°N	76.9801°E	Mandi

Zone I = Sub-Mountain and Low Hills Sub-Tropical Zone (365-914 meters amsl), Zone II = Mid Hills Sub-Humid Zone (915-1523 meters amsl), Zone III = High Hills Temperate Wet Zone (1524-2472 meters amsl)

made after 10-15 days of sowing categorized the isolates into four groups as highly pathogenic (>40% root rot/wilt incidence), moderately pathogenic (20-40% root rot/wilt incidence), weakly pathogenic (0-20% root rot/wilt incidence) and non-pathogenic (no root rot/wilt incidence) Data presented in Table 2 showed that 25 isolates *viz.,* Fs01, Fs02, Fs05, Fs10, Fs11, Fs12, Fs14, Fs15, Fs16, Fs17, Fs18, Fs19, Fs21, Fs24, Fs25, Fs26, Fs27, Fs28, Fs29, Fs30, Fs31, Fs32, Fs33, Fs34 & Fs35 showed root rot/wilt incidence above 40 per cent and categorized as highly pathogenic. Nine isolates namely Fs04, Fs06, Fs07, Fs08, Fs09, Fs13, Fs20, Fs22 & Fs23 showed root rot/wilt incidence between 20-40 per cent and those were categorized as moderately pathogenic whereas one isolate of *Fusarium solani viz.,* Fs03 showed root

Table 2. Pathogenicity test of different isolates of *Fusarium solani* causing root rot in okra in pots under net house conditions

Isolate	Disease incidence (%)		Categorization*
	10DAS	15DAS	
Fs01	13.33	66.67	Highly pathogenic
Fs02	13.33	46.67	Highly pathogenic
Fs03	00.00	13.33	Weakly pathogenic
Fs04	06.67	20.00	Moderately pathogenic
Fs05	13.33	46.67	Highly pathogenic
Fs06	20.00	33.33	Moderately pathogenic
Fs07	06.67	26.67	Moderately pathogenic
Fs08	13.33	33.33	Moderately pathogenic
Fs09	06.67	33.33	Moderately pathogenic
Fs10	13.33	40.00	Highly pathogenic
Fs11	13.33	40.00	Highly pathogenic
Fs12	20.00	40.00	Highly pathogenic
Fs13	13.33	33.33	Moderately pathogenic
Fs14	26.67	53.33	Highly pathogenic
Fs15	20.00	46.67	Highly pathogenic
Fs16	20.00	53.33	Highly pathogenic
Fs17	13.33	46.67	Highly pathogenic
Fs18	13.33	46.67	Highly pathogenic
Fs19	13.33	40.00	Highly pathogenic
Fs20	00.00	33.33	Moderately pathogenic
Fs21	06.67	46.67	Highly pathogenic
Fs22	00.00	33.33	Moderately pathogenic
Fs23	00.00	33.33	Moderately pathogenic
Fs24	33.33	80.00	Highly pathogenic
Fs25	26.67	60.00	Highly pathogenic
Fs26	26.67	66.67	Highly pathogenic
Fs27	26.67	53.33	Highly pathogenic
Fs28	13.33	60.00	Highly pathogenic
Fs29	13.33	40.00	Highly pathogenic
Fs30	20.00	46.67	Highly pathogenic
Fs31	00.00	40.00	Highly pathogenic
Fs32	13.33	46.67	Highly pathogenic
Fs33	06.67	46.67	Highly pathogenic
Fs34	13.33	53.33	Highly pathogenic
Fs35	13.33	46.67	Highly pathogenic

*0-20%- Weakly pathogenic, 20-40%- Moderately pathogenic, >40%- Highly pathogenic

rot/wilt incidence up to 13.33 per cent after 15 days of sowing and categorized as weakly pathogenic. The results of pathogenicity observed in the present investigation are in accordance with the findings of earlier workers. Association of *Fusarium solani* with root rot of okra has been established by various workers. Patel and Vala (2003) isolated *F. solani* from wilt affected okra plant and experimentally established its pathogenic association and causal nature by confirming Koch's postulates. El-Mohamedy (2004) proved pathogenicity test with *F. solani*, *R. solani* and *M. phaseolina* causal agents of okra damping off and root rot diseases by applying inoculum and found that the most aggressive fungi were *F. solani* and *R. solani*.

Purba (2004) reported that *F. solani* was most frequently associated pathogen with root rot in okra in Himachal Pradesh ranging from 8.35 to 78.94 per cent. Purba (2004) also reported pathogenicity of *F. solani* to cause root rot of okra by adding 21 days old inoculum prepared on sand: maize meal (9:1) in pot and observed symptoms of the disease as blackening of collar region of stems and decaying of roots in young seedlings. Sharma (2011) also reported that *R. solani* and *F. solani* were associated with damping off of okra in Himachal Pradesh and observed that maximum pre-emergence mortality was recorded in Hamirpur district (60.0%) and post-emergence mortality in Kangra district (20.5%).

Identification based on morphological characters

Pathogen was identified based on the characteristic symptoms produced after inoculation and morphological identification of conidia. *Fusarium solani* was identified with morphological characters like size, color, shape of conidia as well as number of septation in conidia. These parameters were studied by using compound microscope at 40 X with stage and ocular micrometres (Table 3). Macro and micro conidia were observed measuring 18.23-30.85×4.70-5.99 µm mostly with 1-5 septa and fusiform, cylindrical somewhat curved with a short blunt apical point whereas cylindrical to oval measuring 5.91-11.71×2.78-3.97 µm mostly with no septation, respectively.

Pathogen was identified on the basis of the morphological characters which were favoured with the earlier documented literature. Chattopadhyay and Basu (1957), observed that *F. solani*, the causal agent of okra wilt produced oval shaped thick-walled microconidia with rounded ends or straight with pointed ends measuring 2.8-5.5 x 5.5-6.5 µm; macroconidia with 1-3 septa and measuring 10.9-36.3 x 3.3-6.5 µm. Ravichandran and Kumar (2012) found that 13 different isolates of *F. solani* (Mart.) Sacc., collected from different parts of Andhra Pradesh showed variation in morphological character of microconidia from 3-4 X 1-2 µm to 9-10 X 1-3µm & macroconidia 13-15 X 3-4 µm to 27-29 X 4-5 µm in size they also reported the number of septa in macroconidia & microconidia 3-5 and 0-1 respectively and conidia were hyaline.

Gupta *et al*. (2011) found that 20 different isolates of *F. oxysporum* f. sp. *pisi* collected from different parts of Himachal Pradesh showed variation in morphological characteristics, microconidia varied from 3.16 x 3.16 to 9.13 x 5.44 µm and macroconidia varied from 11.77 x 3.16 to 24.60 x 5.91 µm in size.

Table 3. Morphological characteristics of *Fusarium solani* associated with root rot of okra

Character	Test pathogen (Isolated pathogen)	*Fusarium solani* (Booth, 1971 and Mycology Online)
Colour of colony on PDA	White to greyish white colonies were growing rapidly with aerial mycelium and agar typically develops a light pink/ purplish/ brownish discoloration.	White to cream (greyish white) colonies growing rapidly with aerial mycelium and agar typically develops a bluish brown discoloration.
Growth on PDA	3.8 – 4.00 cm in four days	4.5 cm in four days
Macroconidia	18.23-30.85×4.70-5.99 µm mostly with 1-5 septa and fusiform, cylindrical somewhat curved with a short blunt apical point.	28-42×4.5-6 µm mostly with 3-5 septa and fusiform, cylindrical, often moderately curved, with an indistinct pedicellate at foot cell and a short blunt apical point.
Microconidia	Cylindrical to oval measuring 5.91-11.71×2.78-3.97 µm mostly with no septation.	Cylindrical and rather broader to oval measuring 8-16×2-4 µm may become 1 septate
Chlamydospores	Smooth to rough walled chlamydospores of 8-12×8-10 µm were formed terminally or in chains on short lateral branches.	Globose to oval, smooth to rough-walled chlamydospores of 9-12×8-10 µm borne singly or in pairs on short lateral hyphal branches.

Morphological characteristics of test pathogen were found in line with the description of *F. solani*. Thus, based on morphological characteristics the pathogen causing root rot of okra was identified as *Fusarium solani.*

Morpho-cultural variability

The results of different parameters like conidia dimensions, colony color, colony diameter, and number of septa studied for morpho-cultural variability with pure-culture of all the isolates are presented in Table 4.

In general, the growth of fungus varied from 74 mm to 86 mm after 9 days of incubation on PDA medium at 25 ± 1 ºC. The isolate Fs20, Fs24, Fs25 and Fs28 were most fast growing followed by Fs26 and Fs32. Various colors *viz.,* white and greyish white colonies were observed in different isolates. Light pink, purplish to brownish pigmentation was found in

Table 4. Morphological and cultural characteristics of different isolates of *Fusarium solani*

Isolate	Average size of conidia						Colony colour	Pigmentation	Colony diameter in mm (9DAI)
	Macroconidia			Macroconidia					
	(L)× (µm)	(B)× (µm)	No. of Septa	(L)× (µm)	(B)× (µm)	No.of Septa			
Fs01	20.83	5.19	2-3	8.35	2.99	0	White	-	78
Fs02	19.91	5.01	1-3	5.65	2.78	0	White	-	78
Fs03	18.80	4.90	2-3	8.82	3.01	0	Greyish White	-	78
Fs04	20.24	5.11	2-3	8.93	3.22	0	White	-	80
Fs05	21.76	4.88	2-3	6.26	2.79	0	White	-	76
Fs06	20.48	4.77	2-3	8.74	3.07	0	White	Light pink	78
Fs07	19.60	4.74	2-3	6.60	3.05	0	White	Light pink	78
Fs08	19.09	4.72	2-3	6.13	2.94	0	White	-	80
Fs09	20.09	4.57	2-3	6.04	3.04	0	White	-	78
Fs10	21.89	5.23	2-4	8.90	2.94	0	White	Light pink	76
Fs11	21.67	5.31	2-3	8.77	2.89	0	White	Light pink	78
Fs12	20.45	4.98	2-3	8.97	2.87	0	White	-	76
Fs13	22.49	5.21	2-3	8.89	3.02	0	White	-	74
Fs14	23.67	5.18	2-3	8.74	2.89	0	White	-	78
Fs15	22.77	5.13	2-3	8.46	2.97	0	White	-	74
Fs16	22.79	5.15	2-3	8.69	2.99	0	White	-	76
Fs17	20.92	4.83	2-3	8.77	3.07	0	White	-	74
Fs18	21.92	4.87	2-3	8.72	2.87	0	White	-	80
Fs19	18.59	4.74	1-3	6.68	2.97	0	Greyish White	-	78
Fs20	18.23	4.70	1-3	6.52	2.78	0	White	-	86
Fs21	23.73	5.06	3-4	10.68	3.79	0	White	Light pink	78
Fs22	22.39	5.03	2-3	5.91	2.86	0	White	-	80
Fs23	21.22	4.82	1-3	6.83	2.92	0	White	-	82
Fs24	30.35	5.95	3-4	11.14	3.05	0	White	Purplish	86
Fs25	28.47	5.81	3-4	10.70	3.30	0	White	Purplish	86
Fs26	25.20	5.39	2-4	10.24	3.23	0	White	Light pink	84
Fs27	25.63	5.41	3-4	10.47	3.81	0-1	Greyish White	-	80
Fs28	28.26	5.62	4-5	10.76	3.45	0-1	White	-	86
Fs29	28.21	5.31	3-4	10.71	3.46	0	White	-	82
Fs30	30.95	5.99	2-3	11.71	3.56	0	White	Light pink	82
Fs31	28.68	5.83	2-3	11.04	3.97	0	White	Light pink	80
Fs32	23.77	5.18	2-3	10.70	3.09	0	White	Light pink	84
Fs33	25.86	5.57	3-4	9.26	2.91	0	White	Brownish	74
Fs34	28.39	5.72	3-4	10.44	3.95	0	White	Brownish	78
Fs35	23.54	5.15	2-3	8.62	3.27	0	White	Brownish	76
Range	18.23-30.95	4.57-5.99	1-5	5.65-11.71	2.78-3.97	0-1			74-86

the isolates of *F. solani.* Length of macroconidia varied from 18.23-30.85 µm with an average of 23.17 µm and width varied from 4.70-5.99 µm with an average value of 5.17 µm with a range of 1-5 septa. Length of microconidia varied from 5.91-11.71 µm with an average of 8.77 µm and width varied from 2.78-3.97 µm with an average value of 3.14 µm with a range of 0-1 septa. Thus, on the basis of these morpho-cultural characteristics, isolates grown on PDA medium at 25±1 ºC were categorized into 7 morpho-cultural groups (FS-MV-1 to FS-MV-35) as presented in Table 5.

FS-MV: *Fusarium solani* morpho variability

These morpho-cultural groups were designated as FS-MV-1- FS-MV-7 (Table 5, Fig. 1). FS-MV-1 comprised of 5 isolates (Fs-02, Fs-07, Fs-08, Fs-09 and Fs-19) with white to greyish white colony having slightly light pink pigmentation, macroconidia

Table 5. Grouping of 35 isolates of *Fusarium solani* based on morpho-cultural characteristics

Group No.	Isolate	Morpho-cultural characteristics
FS-MV-1	Fs-02, Fs-07, Fs-08, Fs-09, Fs-19	Colony is white to greyish white in colour having slightly light pink pigmentation, macroconidia measuring 19.91-21.89 x 4.57-5.01µm with 1-3 septa whereas microconidia measuring 5.65-6.68 x 2.78-3.04 µm with no septation and around 78-80 mm growth on PDA at 9DAI.
FS-MV-2	Fs-01, Fs-03, Fs-04, Fs-06, Fs-11, Fs-18, Fs-22, Fs-23	Colony is white to greyish white in colour having light pink pigmentation, macroconidia measuring 18.80-22.39 x 4.77-5.31 µm with 1-3 septa whereas microconidia measuring 5.91-8.93 x 2.86-3.22 µm with no septation and around 78-82 mm growth on PDA at 9DAI.
FS-MV-3	Fs-14, Fs-21, Fs-27	Colony is white to greyish white in colour having light pink pigmentation, macroconidia measuring 23.67-25.63 x 5.06-5.41 µm with 2-4 septa whereas microconidia measuring 8.74-10.68 x 2.89-3.81 µm with 0-1 septa and around 78-80 mm growth on PDA at 9DAI.
FS-MV-4	Fs-05, Fs-10, Fs-12, Fs-13, Fs-15, Fs-16, Fs-17, Fs-33, Fs-35	Colony is white in colour having light pink to purplish/ brownish pigmentation, macroconidia measuring 20.45-25.86 x 4.83-5.57 µm with 2-4 septation whereas microconidia measuring 6.26-9.26 x 2.79-3.27 µm with no septation and around 74-76 mm growth on PDA at 9DAI.
FS-MV-5	Fs-20	Colony is white in colour having no pigmentation, macroconidia measuring 18.23 x 4.70 µm with 1-3 septa whereas microconidia measuring 6.52 x 2.78 µm with no septation and around 86 mm growth on PDA at 9DAI.
FS-MV-6	Fs-29, Fs-30, Fs-31, Fs-34	Colony is white in colour having light pink to brownish pigmentation, macroconidia measuring 28.21-30.95 x 5.31-5.99 µm with 2-4 septation whereas microconidia measuring 10.44-11.71 x 3.46-3.99 µm with no septation and around 78-82 mm growth on PDA at 9DAI.
FS-MV-7	Fs-24, Fs-25, Fs-26, Fs-28, Fs-32	Colony is white in colour having light pink to purplish pigmentation, macroconidia measuring 23.77-30.35 x 5.18-5.95 µm with 2-5 septa whereas microconidia measuring 10.24-11.14 x 3.05-3.45 µm with 0-1 septation and around 84-86 mm growth on PDA at 9DAI.

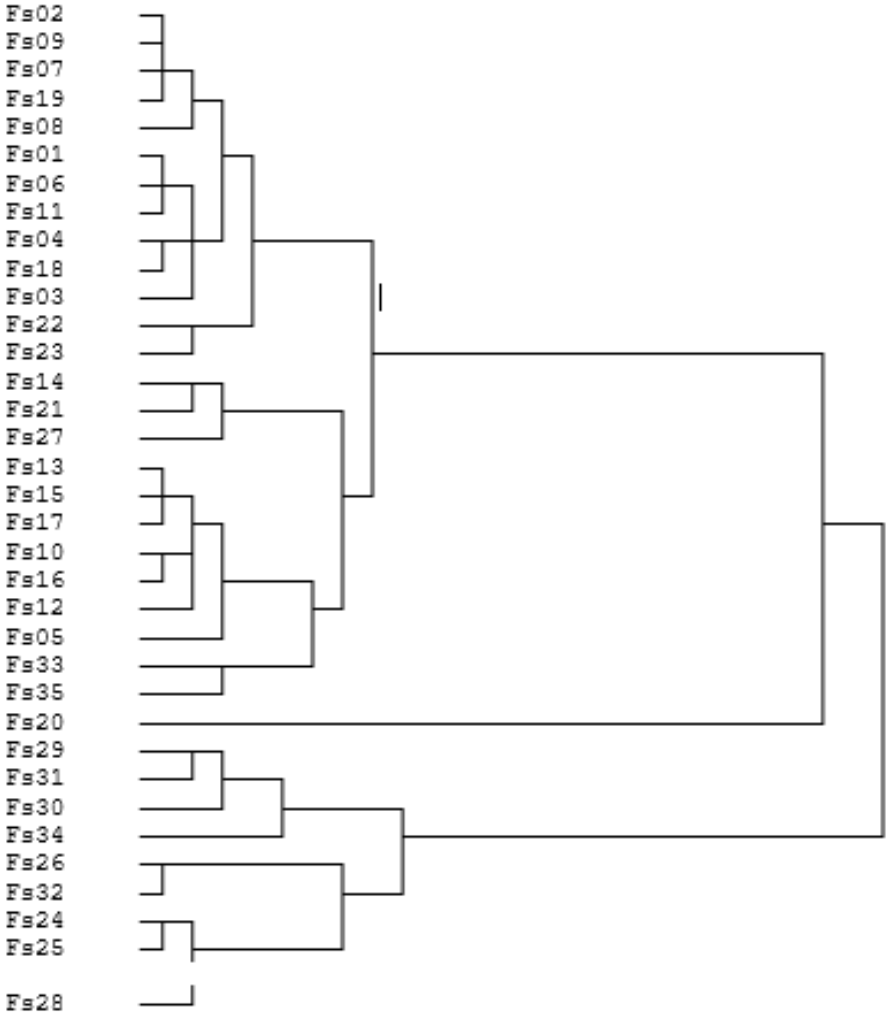

Figure 1: Cluster analysis indicating diversity within 35 isolates of *Fusarium solani* using SPSS 16.0 software

measuring 19.91-21.89 x 4.57-5.01µm with 1-3 septa whereas microconidia measuring 5.65-6.68 x 2.78-3.04 µm with no septation and around 78-80 mm growth on PDA at 9DAI. Isolates Fs-01, Fs-03, Fs-04, Fs-06, Fs-11, Fs-18, Fs-22, and Fs-23 with white to greyish white colony having light pink pigmentation, macroconidia measuring 18.80-22.39 x 4.77-5.31 µm with 1-3 septa whereas microconidia measuring 5.91-8.93 x 2.86-3.22 µm with no septation and around 78-82 mm growth on PDA at 9DAI were grouped under FS-MV-2 group. Group FS-MV-3 constitute three isolates (Fs-14, Fs-21 and Fs-27) with white to greyish white colony colour having light pink pigmentation, macroconidia measuring 23.67-25.63 x 5.06-5.41 µm with 2-4 septa whereas microconidia measuring 8.74-10.68 x 2.89-3.81 µm with 0-1 septa and around 78-80 mm growth on PDA at 9DAI. Group 4 had nine isolates (Fs-05, Fs-10, Fs-12, Fs-13, Fs-15, Fs-16, Fs-17, Fs-33 and Fs-35) had white colony colour having light pink to purplish/brownish pigmentation, macroconidia measuring 20.45-25.86 x 4.83-5.57 µm with 2-4 septation whereas microconidia measuring 6.26-9.26 x 2.79-3.27 µm with no septation and around 74-76 mm growth on PDA at 9DAI. Group 5 includes only one isolate (Fs20) which had white colony colour having no pigmentation, macroconidia measuring 18.23 x 4.70 µm with 1-3 septa whereas microconidia measuring 6.52 x 2.78 µm with no septation and around 86 mm growth on PDA at 9DAI. Group FS-MV-6 (Fs-29, Fs-30, Fs-31 and Fs-34) had white colony colour having light pink to brownish pigmentation, macroconidia measuring 28.21-30.95 x 5.31-5.99 µm with 2-4 septation whereas microconidia measuring 10.44-11.71 x 3.46-3.99 µm with no septation and around 78-82 mm growth on PDA at 9DAI. Isolate Fs-24, Fs-25, Fs-26, Fs-28 and Fs-32 were clubbed in group FS-MV-7 which had white colony colour having light pink to purplish

pigmentation, macroconidia measuring 23.77-30.35 x 5.18-5.95 µm with 2-5 septa whereas microconidia measuring 10.24-11.14 x 3.05-3.45 µm with 0-1 septation and around 84-86 mm growth on PDA at 9DAI.

Present findings are in conformity with the results of Ali *et al*. (2013) who studied the biology of five different isolates of *Fusarium solani*, causing root rot of okra in Peshawar and reported variation in the fungal colony colour. Colony colour was reported to be white to off white, creamy, and chocolate colour or bright or silver coloured. Chavan *et al*. (2011) also reported variation in cultural characteristics of eight isolates of *F. solani* infecting patchouli. They observed maximum colony diameter in isolate Fs1 (90.00 mm) whereas minimum colony diameter in isolate Fs7 (84.00 mm) on PDA medium. Wagh *et al*. (2010) reported that six isolates of *F. oxysporum* f. sp. *lini* varied significantly in their cultural characteristics on PDA. Isolate Fol-6 was recorded as fast growing (82.00 mm) while remaining isolates showed moderate mycelium growth ranging from 71.6mm to78.7mm. They also reported variations with respect to colony pigmentation where isolates Fol-2, Fol-3 and Fol6 produced a bright white mycelium, while isolates Fol-1 and Fol-4 produced slight white mycelium and isolate Fol-5 produced violet coloured mycelial pigmentation. Dubey *et al*. (2010) also studied 112 isolates of *F. oxysporum* f. sp. *ciceri* causing chickpea wilt and reported them to be highly variable in their colony growth pattern, size of colony and pigmentation. White coloured mycelium was observed in most of isolates but other pigmentations such as violet, yellow, and prominent grey were also reported.

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Short communication

Seasonal incidence of insect pests of cauliflower in Kullu valley of Himachal Pradesh

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Abstract

Seasonal incidence of insect pests of cauliflower was studied by carrying out a field trial at experimental farm of Krishi Vigyan Kendra, Bajaura, Kullu in the main season crop grown during February-June, 2023. The data revealed that major activity period of the cabbage aphid was observed from 8th to 22nd Standard meteorological week with peak incidence in 12th SMW and the cabbage butterfly was observed from 10th to 22nd SMW with peak incidence in 15th SMW, while the diamondback moth appeared from 9th to 21st SMW with its peak activity in 13th SMW. The cabbage semilooper was observed from 15th to 21st SMW with peak incidence in 19th SMW. The correlation between insect pest population and weather parameters (maximum, minimum temperature, morning, evening relative humidity and rainfall) was found to be non-significant.

Key words: Cauliflower, seasonal incidence, insect pests, correlation

Cole crops are important group of winter vegetables consumed worldwide belonging to genus *Brassica* in family Brassicaceae. However, cauliflower (*Brassica oleracea* var. *botrytis*) and cabbage (*Brassica oleracea* var. *capitata*) are the two main crops grown in this group (Mishra *et al*. 2018). In Himachal Pradesh, cauliflower is grown as a main season as well as an off-season crop in mid and high hills, providing profitable return to the growers (Ghosh 2017). The ability of state to produce cauliflower is impeded by a number of insect pests, the most significant of which are the cabbage aphid, *Brevicoryne brassicae* (L.), the cabbage butterfly, *Pieris brassicae* (L.), the diamondback moth, *Plutella xylostella* (L.), the tobacco caterpillar, *Spodoptera litura* (F.), the painted bug, *Bagrada cruciferarum* (K.) and the cabbage head borer, *Hellula undalis* (F.) (Bhatia and Gupta 2003; Kumar *et al*. 2014; Meghna *et al*. (2018). In Himachal Pradesh, *B. brassicae* was reported as a key pest of cauliflower and cabbage along with *P. Brassicae* and *P. xylostella* as major and minor pest, respectively (Bhalla 1990; Barwal 1997). The quality and quantity of the crop are both negatively impacted by insect pest damage. The incidence of insect pest varies from region to region due to variation in cropping season and climatic conditions. Understanding the seasonal occurrence of insect pests at various cauliflower crop growth stages will help in development of an effective management schedule as it gives an idea of their peak activity period.

The field experiment was conducted in the main season (February-June) during, 2023 at Krishi Vigyan Kendra, Bajaura (Kullu). Geographically, it is situated between 31.83p North latitude and 77.17p East longitude an altitude of 1090 meter above mean sea level (AMSL). The seedlings of variety Madhuri (Clause company) were transplanted in 100 m^2 area having a plot size 4.5m × 2.25m with a row to row and plant to plant spacing of 45cm × 45cm. The observations were recorded at weekly intervals during the morning hours starting from 10 days after transplanting till the maturity of the crop. The population of aphid was recorded from random row, plant and leaf coordinates to determine aphid density per plant by counting the number of nymphs and adults per three leaves (one each from top, middle and bottom)/ plant, by visual count method using a magnifying lens. Thirty leaves were taken for counting aphids from 10 randomly selected plants and expressed

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as number of aphids per plant. The population of lepidopteran insects was recorded by counting the actual number of larvae per plant from 10 randomly selected plants. Simple correlation was worked out between population of major insect pests and abiotic factors. For recording plant infestation at weekly intervals, 20 plants were randomly selected and data on infested and healthy plants were recorded. The per cent plant infestation was worked out by using the following formula:

$$\text{Per cent infestation} = \frac{\text{Number of infested plants}}{\text{Total number of plants observed}} \times 100$$

Infestation index was calculated by using the following formula given by Bhalla and Verma (1991).

$$\text{Infestation index} = \log \left(\frac{\text{Mean larval population} \times \text{Per cent plant infested}}{100} + 1 \right)$$

The seasonal abundance of population (Individual insects/ plant), plant infestation (%) and infestation index have been given in Table 1 and Table 2.

(i) Cabbage aphid (*Brevicoryne brassicae*)

The mean population of cabbage aphid varied from 6.8-101.9 aphids per plant and the damage of the

Table 1. Seasonal abundance of different insect pests associated with cauliflower in main season during 2023

SMW	*Brevicornye brassiace*	*Pieris brassicae*	*Plutella xylostella*	*Thysanoplusia orichalcea*
8	6.8±4.6	0.0±0.0	0.0±0.0	0.0±0.0
9	11±3.2	0.0±0.0	0.2±0.1	0.0±0.0
10	49.2±7.6	2.2±1.2	1.9±0.7	0.0±0.0
11	67.9±6.6	6.8±2.7	3.4±0.9	0.0±0.0
12	101.9±5.2	8.4±2.4	5.5±1.1	0.0±0.0
13	91.2±5.7	10.5±3.6	8.3±1.1	0.0±0.0
14	48.7±7.6	12.6±3.7	7.2±0.9	0.0±0.0
15	50.6±7.0	15.7±4.4	7.8±0.9	0.1±0.1
16	21.2±6.9	12.6±3.0	4.3±1.4	0.1±0.1
17	36.7±6.4	12.1±2.9	3.1±0.7	0.3±0.2
18	24.9±5.9	11.9±2.6	1.4±0.4	0.2±0.2
19	21.2±7.1	10.2±2.4	1.0±0.3	0.4±0.1
20	19.6±6.1	9.8±2.0	0.7±0.3	0.2±0.2
21	17.2±3.5	5.5±2.0	0.1±0.1	0.1±0.1
22	14.8±4.6	2.9±1.1	0.0±0.0	0±0.0

SMW- Standard meteorological week

Table 2. Plant infestation (%) and infestation index of different insect pests associated with cauliflower in main season during 2023

SMW	Plant infestation (%)				Infestation index			
	B. brassicae	*P. brassicae*	*P. xylostella*	*T. orichalcea*	*B. brassicae*	*P. brassicae*	*P. xylostella*	*T. orichalcea*
8	10	0.0	0.0	0.0	0.23	0.00	0.00	0.00
9	25	0.0	5.0	0.0	0.57	0.00	0.00	0.00
10	45	10.0	15.0	0.0	1.36	0.09	0.11	0.00
11	55	10.0	20.0	0.0	1.58	0.23	0.23	0.00
12	70	15.0	35.0	0.0	1.86	0.35	0.47	0.00
13	50	25.0	45.0	0.0	1.67	0.56	0.68	0.00
14	40	40.0	40.0	0.0	1.31	0.78	0.59	0.00
15	35	60.0	45.0	5.0	1.27	1.02	0.65	0.00
16	25	40.0	35.0	5.0	0.80	0.78	0.40	0.00
17	15	40.0	30.0	10.0	0.81	0.77	0.29	0.01
18	15	35.0	25.0	10.0	0.68	0.71	0.13	0.01
19	20	30.0	20.0	20.0	0.72	0.61	0.08	0.03
20	15	15.0	15.0	15.0	0.60	0.39	0.04	0.01
21	10	15.0	5.0	5.0	0.43	0.26	0.00	0.00
22	10	5.0	0.0	0.0	0.39	0.06	0.00	0.00

SMW- Standard meteorological week

pest was noticed from seedling stage to maturity of the crop. The cabbage aphid population emerged during the 8th Standard Meteorological Week (SMW), initially recorded at 6.8 aphids per plant with 10 per cent plant infestation and an infestation index of 0.23. Over time, the population steadily increased, and reached its peak during the 12th SMW at 101.9 aphids per plant, with 70 per cent plant infestation and an infestation index of 1.86. However, by the 13th SMW, the population experienced a decline to 91.2 aphids per plant, marking the beginning of a downward trend. Rainfall played a significant role in reducing the aphid population by washing them off. Present observations are more or less similar with the results of earlier workers Pal and Singh (2012) who reported that aphid population initiated in the 7th SMW and reached their peak mean population during 11th SMW at Uttar Pradesh. In contrary, Kishore *et al*. (2024) found that aphid population initiated in the 45th SMW and reached its peak in 48th SMW at Telangana, India. The differences in the findings may be due to variation in growing season of cauliflower.

(ii) Cabbage butterfly (*Pieris brassicae*)

The population of *P. brassicae* larvae ranged from 2.2-15.7 larvae per plant, during crop production. The pest was first recorded in the 10th SMW with larval mean of 2.2 larvae per plant with corresponding 10 per cent plant infestation and infestation index of 0.09. The pest was found active from March-May. The population showed an increasing trend and reached its peak in the 15th SMW with 15.7 larvae per plant, 60 per cent plant infestation and 1.02 infestation index. The population dwindled to 12.6 larvae per plant in the 16th SMW and then started to decline. The results are in conformity with Sood (2007) who also recorded peak incidence of cabbage butterfly in the month of April and May at Palampur and Sangla valley (H.P.) respectively.

(iii) Diamondback moth (*Plutella xylostella)*

The population count of *P. xylostella* fluctuated between 0.1-8.3 larvae per plant during main season cauliflower crop. The pest initially appeared in the 9th SMW with 0.2 larvae per plant and 5 per cent plant infestation which further showed a gradual increase in number and reached its peak in the 13th SMW with 8.3 larvae per plant and corresponding plant infestation of 45 per cent. The population thereafter fluctuated and finally declined to zero till harvesting of the crop. The present findings are more or less in conformity with Kumar *et al*. (2023) & Anitha and Kalasariya (2024) who also reported diamondback moth as a major pest of cauliflower. Venugopal *et al*. (2017) observed that diamondback moth is active throughout the year with a varying degree of infestation at Allahabad. The pest initially appeared in the 6th SMW and reached its peak in 13th SMW.

(iv) Semilooper (*Thysanoplusia orichalcea)*

The population density of *T. orichalcea* remained very low (0.1-0.4 larvae per plant) in the main season cauliflower crop. The maximum population was recorded in 19th SMW with 0.4 larvae per plant, 20% plant infestation and 0.03 infestation index. No larva of this pest was found in the 22nd SMW. In present investigation the mean population of *T. orichalcea* remained low throughout the growing season whereas Bhat (2018) reported that semilooper is present in abundance in Srinagar of J&K, India which contradicts the present findings. The results may vary due to different weather conditions, locations and growing of the crop at different time of the year.

Conclusion

From the present studies it can be concluded that cauliflower crop was found abundantly and majorly infested with *B. brassicae* followed by *P. brassicae*, *P. xylostella* and *T. orichalcea* throughout the growing season and reached their peak population in 12th SMW, 15th SMW, 13th SMW and 19th SMW, respectively. The correlation between insect pest population and weather parameters (maximum, minimum temperature, morning, evening relative humidity and rainfall) was found statistically non-significant. The study of seasonal incidence will be helpful in planning effective management strategies against major insect pests of this crop.

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Short Communication

Seasonal abundance and population fluctuation of pea leaf miner, *Chromatomyiahorticola* (Goureau) infesting pea

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Abstract

Seasonal abundance and population fluctuation of pea leaf miner was studied by carrying out survey at experimental farms of Department of Entomology, CSKHPKV, Palampur and in farmer's fields at different locations *viz.,* Kangra, Kullu and Karsog during *Rabi* 2022-23. The data revealed highest mean leaf infestation (60.38%, 51.23%) and mean larval & pupal population (65.12, 54.16 larvae & pupae/ plant)during 13th SMW, 2023 at Kangra and Palampur, respectively. At Kullu the highest mean infestation of 60.13% and 65.37 mean larval & pupal population were recorded during 14th SMW, whereas at Karsog it was recorded during 16th SMW, 2023 with 60.13% mean infestation and 65.37 larvae & pupae/ plant. Correlation coefficients between pea leaf miner infestation, larval & pupal population and weather parameters indicated that temperature (max. and min.) exerted significant positive correlation in all surveyed locations except Karsog, where maximum temperature exerted non- significant positive correlation. Relative humidity showed non- significant positive correlation in all the surveyed locations except Kangra. Rainfall resulted in non- significant positive correlation in all surveyed locations except Kullu.

Key words: Pea leaf miner, *Chromatomyia horticola,* Seasonal abundance

Garden pea (*Pisum sativum* L.) is one of the most important legume crops belonging to leguminaceae family and grown on commercial scale around the world (Sharma *et al*. 2022). The crop is cultivated for use as vegetable and mature dry pods to use as a pulse. It provides diverse nutrient profile of health building substances like vitamins, minerals and lysine, a limiting amino acid in cereals (Sharma *et al*. 2007). The productivity of pea is low because of numerous biotic and abiotic causes. Among the biotic constraints,large numbers of insect pests attack different parts of the plant at different growth stages, from seedling to harvest. As many as 24 insect species have been reported infesting the pea crop at different stages (Bijjur and Verma 1995). Among themagromyzid leaf miner*, Chromatomyia horticola* (Goureau) is the most serious pests in both the temperate and tropical regions. The activity and population dynamics of different insect pests are also closely related to a number of abiotic environmental conditions. Abundance of different insect-pests depends on various climatic conditions of the area. Singh and Saravanan (2008) noted that infestation of pea leaf miner increased with increase in maximum temperature. It has been reported that weather parameters were the major regulatory factors for the leaf miner infestation under field conditions. Venkateshwarlu *et al*. (2011) observed that green and succulent foliage combined with moderate temperature and humidity range were favourable for the infestation of the pest. Leaf miner is also known to transmit plant diseases (Parrella *et al.* 1985; Parrella 1987). The larvae of pea leaf miner feed on mesophyll of leaf by making mines between the leaf surfaces that reduce photosynthetic ability of plants, hereby reducing flower production and seriously affecting crop quality. In case of severe attack 86-93 per cent of the leaves are found affected by leaf minerTariq *et al.*

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1991). Keeping in view these facts an attempt was made here to study seasonal abundance and population fluctuation of pea leaf miner.

Pea growing areas of Palampur, Kangra, Kullu and Karsog were surveyed after 10-15 days of crop emergence till harvesting of the crop in the farmer's fields of these areas to know about the incidence and larval & pupal population of pea leaf miner in these locations. Five villages from each location and three fields from each village were selected to record the seasonal incidence of the pest except Palampur where three experimental farms were selected with two replications each. Twenty plants were randomly selected in each field. For leaf infestation and population count, five compound leaves-one terminal, two each from middle and lower canopy were examined from each selected plant. The infested as well as total number of leaflets were counted and per cent leaf infestation was worked out using the following formula:

$$\text{Per cent leaf infestation} = \frac{\text{Number of infested leaflets}}{\text{Total number of leaflets}} \times 100$$

To establish the relationship between leaf infestation, larval and pupal population and weather parameters, the leaf infestation and population (larvae and pupae) of a particular period was correlated with weather parameters like maximum temperature, minimum temperature, rainfall and relative humidity, prevailing during same period. The meteorological data were obtained from the Department of Agronomy, CSKHPKV, Palampur and data for different surveyed locations were obtained from the agro met observatories installed at KVK's of surveyed locations.Correlation coefficients were worked out between weekly averages of pest incidence and averages of weather parameters prevailing during preceding week.

It is evident from Table 1 that the pest appeared in the fields during 52nd standard meteorological week (SMW) *i.e.*, last week of December, 2022 both at Kangra and Palampur. The infestation commenced from the lower leaves of the plants, thereafter the leaf infestation and larval and pupal population started increasing and reached at its peak after that followed a decreasing trend. The mean leaf infestation varied from 0.91 to 60.38 per cent at Kangra and 0.47 to 51.23 per cent at Palampur. The pooled mean larval & pupal population at both these locations varied from 0.14 to 65.12 and from 0.19 to 65.37, respectively during entire crop period. The highest mean leaf infestation of 60.38 and 51.23 per cent and pooled mean larval &

Table 1. Population build-up of *Chromatomyia horticola* on pea at Kangra and Palampur (*Rabi* 2022-23)

SMW	Mean leaf infestation (%)		Mean population (larvae & pupae/ plant)	
	Kangra	Palampur	Kangra	Palampur
52	0.91	0.47	0.14	0.06
1	2.52	1.64	0.36	0.27
2	3.49	2.78	0.57	0.59
3	5.23	3.99	0.97	1.17
4	6.78	4.67	1.65	1.86
5	9.02	6.41	3.39	2.21
6	11.35	8.40	5.11	3.73
7	15.10	10.78	14.69	5.39
8	17.69	14.47	20.34	13.01
9	22.64	17.57	34.12	20.14
10	34.53	19.21	49.54	20.65
11	50.11	25.69	62.61	33.80
12	58.62	42.23	63.03	46.74
13	60.38	51.23	65.12	54.16
14	57.72	44.93	61.35	49.27
15	50.24	43.52	55.12	45.17

SMW- Standard meteorological week

pupal population of 65.12 and 54.16 were recorded during 13th SMW, 2023 followed by 12th SMW, 2023 in case of former location and 14th SMW, 2023 in case of latter location.

It was found that in Kullu, the pest appeared in fields during 2nd SMW, where as at Karsog, the pest appeared during 8th SMW, 2023 (Table 2). The mean leaf infestation varied from 1.47 to 60.13 per cent in Kullu and1.10 to 46.01 per cent at Karsog. The mean larval & pupal population varied from 0.19 to 65.37 in Kullu and 0.14 to 51.68 at Karsog during entire crop period. The highest mean leaf infestation of 60.13 per cent and mean larval and pupal population of 65.37 were recorded during 14th SMW followed by 16th SMW of 2023 in Kullu, whereas at Karsog the highest mean leaf infestation of 46.01 per cent and mean larval & pupal population of 51.68 were recorded during 16th SMW followed by 18th SMW of 2023.

The present findings regarding the mean leaf infestation and mean larval and pupal population are in consonance with the results obtained by Sood (1992) who reported that the population of leaf miner reached at its peak in April and maximum number of larvae and pupae per plant were recorded on 5th April and 21st March in 1991 and 1992, respectively. Also, the present findings lend support from the results of Guleria (2022) who reported that pea leaf miner incidence ranged from 0.00 to 60.86 per cent during 2021-22 and peak infestation was recorded in the 4th week of March (60.86%) followed by 1st week of April (56.42%). The per cent infestation was highest on April 26, 1991 and April 11, 1992, respectively.

Correlation between pea leaf miner infestation/ larval & pupal population and weather parameters

Correlation coefficients between pea leaf miner infestation and weather parameters (Table 3) revealed that at Kangra, significant positive correlation was observed with maximum temperature (r= 0.792), minimum temperature (r= 0.860) and relative humidity (r= 0.731) while rainfall (r= 0.258) showed non-significant positive correlation. Same trend was followed with correlation between mean larval & pupal

Table 2. Population build-up of *Chromatomyiahorticola* on pea in Kullu and Karsog (*Rabi* 2022-23)

SMW	Mean leaf infestation (%)		Mean population (larvae & pupae/ plant)	
	Kullu	Karsog	Kullu	Karsog
2	1.47	-	0.19	-
4	3.52	-	0.81	-
6	6.05	-	2.34	-
8	10.02	1.10	7.31	0.14
10	18.60	3.71	23.94	0.91
12	40.91	8.50	52.23	4.30
14	60.13	27.96	65.37	35.94
16	55.89	46.01	60.24	51.68
18	46.97	44.28	51.33	49.70
20	-	38.80	-	44.11

SMW- Standard meteorological week

Table 3. Correlation coefficients between pea leaf miner infestation and weather parameters at different locations

Factor	Pea leaf miner infestation			
	Kangra(n= 16)	**Palampur**(n= 16)	**Kullu**(n= 9)	**Karsog**(n= 7)
T_{max}(°C)	0.792^{**}	0.590^{*}	0.770^{*}	0.639^{NS}
T_{min}(°C)	0.860^{**}	0.718^{**}	0.894^{**}	0.781^{*}
Rainfall (mm)	0.258^{NS}	0.364^{NS}	0.802^{**}	0.579^{NS}
Relative humidity(%)	0.731^{**}	0.190^{NS}	0.127^{NS}	0.315^{NS}

**Significant at 1% level; *Significant at 5% level; NS- Non-significant; n- number of observations; T_{max}-Maximumtemperature; T_{min}-Minimum temperature

population and weather parameters (Table 4) i.e., significant positive correlation was observed with maximum (r= 0.844), minimum temperature (r= 0.905) and relative humidity (r= 0.716). However, rainfall showed non- significant positive correlation (r= 0.215) with mean larval and pupal population. At Palampur also significant positive correlation was observed with maximum (r= 0.590) and minimum temperature (r= 0.718).The relative humidity (r= 0.190) and rainfall (r= 0.364) showed non- significant but positive correlation. Same trend was followed with correlation between mean larval and pupal population and weather parameters (Table 4) i.e., significant positive correlation was observed with maximum (r= 0.596) and minimum temperature (r= 0.724). The relative humidity (r= 0.230) and rainfall (r= 0.382) showed non- significant but positive correlation.

At Kullu significant positive correlation was observed between leaf miner infestation andmaximum temperature (r= 0.770), minimum temperature (r= 0.894) and rainfall (r= 0.802) while relative humidity showed non- significant positive (r= 0.127) correlation. Same trend was followed with correlation between mean larval and pupal population (Table 4) and weather parameters i.e., significant positive correlation was observed with maximum (r= 0.777), minimum temperature (r= 0.910) and rainfall (r= 0.745) while relative humidity (r= 0.077) showed non-significant but positive correlation with mean larval and pupal population. At Karsog, significant positive correlation was noticed between leaf miner infestation with minimum temperature (r= 0.781) while maximum temperature (r= 0.639), rainfall (r= 0.579), relative humidity (r= 0.315) was non- significant but positively correlated. Same trend was followed with correlation between mean larval and pupal population (Table 4) and weather parameters i.e., significant positive correlation was observed with minimum temperature (r= 0.722) while maximum temperature (r= 0.631), rainfall (r= 0.552) and relative humidity (r= 0.270) showed non- significant but positive correlation.

These results pertaining to correlation studies between weather parameters and abundance of *C. horticola,* are in close conformity to those of Guleria (2022) who reported that per cent incidence of pea leaf miner was significantly and positively correlated with average maximum temperature (r= 0.905) and average minimum temperature (r= 0.878). Average rainfall was negatively and non-significantly correlated (r= -0.208) with leaf infestation, whereas average relative humidity was negatively and significantly correlated with infestation (r = -0.536). Also, Pathania (2020), reported that leaf miner infestation on pea under different farming practices was having significant positive correlation with maximum and minimum temperature during the two seasons. Earlier, Sood (1992) had reported significant positive correlation between average number of larvae per plant and average maximum temperature, minimum temperature. Relative humidity was negatively non-significant during 1990-91 and positively non-significant during 1991-92. However, it showed non-significant negative correlation with total rainfall during both the years.

Conclusion

The results revealed that the highest pooled mean infestation and larval and pupal populationwas recorded during 13th SMW of 2023 at Kangra and Palampur whereas at Kullu and Karsog it was recorded

Table 4. Correlation coefficients between pea leaf miner population and weather parameters at different locations

Factor	Pea leaf miner (larval & pupal) population			
	Kangra	**Palampur**	**Kullu**	**Karsog**
	(n= 16)	(n= 16)	(n= 9)	(n= 7)
T_{max} (°C)	0.844^{**}	0.596^{*}	0.777^{*}	0.631^{NS}
T_{min} (°C)	0.905^{**}	0.724^{**}	0.910^{**}	0.722^{*}
Rainfall (mm)	0.215^{NS}	0.382^{NS}	0.745^{*}	0.552^{NS}
Relative humidity (%)	0.716^{**}	0.230^{NS}	0.077^{NS}	0.270^{NS}

**Significant at 1% level; *Significant at 5% level; NS- Non-significant; n- number of observations; T_{max}-Maximumtemperature; T_{min}-Minimum temperature

during 14th and 16th SMW of 2023, respectively. The influence of weather factors on pea leaf miner infestation and larval and pupal population revealed that temperature (max. and min.) had significant positive correlation in all the surveyed locations except Karsog, where maximum temperature exerted non- significant positive influence. Relative humidity showed non- significant positive correlation in all surveyed locations except Kangra. Rainfall resulted in non- significant positive correlation in all surveyed locations except Kullu.

Conflict of interest: The authors declare that there is no conflict of interest in this research paper.

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Short Communication

Population buildup of red spider mite, *Oligonychus coffeae* N. in tea

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Abstract

The present investigations entitled "Population buildup of red spider mite, *Oligonychus coffeae* N. in tea" were undertaken from May, 2022 to April, 2023 at Palampur, Himachal Pradesh in conventionally managed tea plantations and organic tea plantations. The mite population was maximum in conventional farming system (5.22 to 19.26/ per leaf) as compared to organic farming system (2.57 to 6.72). The peaks of activity were observed during May and October. Leaf infestation was more in conventional (61 - 99 %) as compared to organic farming system (39 - 82%). Infestation index was also observed highest in conventional tea plantations as compared to organic tea plantations. Population was at its minimum during December - February. Correlation studies revealed that the mite population was positively correlated with temperature (max. & min.) and sun - shine hours, but negatively correlated with relative humidity and rainfall.

Key words: *Oligonychus coffeae*, tea, seasonal incidence

Tea, *Camellia sinensis* (L.) is a perennial plantation crop which is cultivated in more than 50 countries all over the world (Idris *et al.* 2020). India is the second largest tea producer and tea is grown on an area of about 5.79 lakh ha in India (Babu 2021) with production of 1.35 million metric tonne (PIB, Ministry of Commerce and Industry 2023). Tea production has been hampered by numerous constraints, among which insect and mite pests are considered the most damaging factors (Yang *et al.* 2022). Globally, over 1031 insect and mite pest's species have been recorded to be associated with tea (Hazarika 2009). *Oligonychus coffeae* is an economically important mite pest of agricultural and ornamental crops (Haque *et al.* 2007). The severe infestation of *O. coffeae* could cause 17 - 46 per cent of crop loss because of its year - round presence (Bharathi *et al.* 2022).

The observations on build-up of phytophagous tea mite complex population were recorded at Palampur in University tea garden Lat: 32.1067^{0}N; Long: 76.5517^{0}E (organic tea plantation) and Rajpur Lat: 32.086^{0}N; Long: 76.5344^{0}E (conventionally managed tea plantation) during second week of every month, throughout the year starting from May 2022. One hundred leaves from upper, middle and lower canopy of ten randomly selected tea bushes were collected in a polyethylene bag and brought to laboratory for microscopic examination. The motile stages of the mites were counted under stereo - zoom microscope. Based on the total number of leaves observed (100 leaves), per cent leaf infestation was worked out as follow:

$$\text{Leaf infestation \%} = \frac{\text{Number of leaves with mites}}{\text{Total number of leaves observed}} \times 100$$

Based on the mite population count and leaf infestation, infestation index was determined as suggested by Bhalla and Verma (1991) with slight modifications.

$$\text{Infestation index} = \frac{\text{Mean mite population} \times \text{per cent leaf infestation}}{100}$$

Also, a relationship between mite population and abiotic weather factors namely, mean of monthly temperature (^{0}C), relative humidity (%), sun shine (hrs) and cumulative monthly rainfall (mm) was worked out. For this, daily meteorological data were procured from the Agro - meteorology section of the

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Population buildup of *Oligonychus coffeae* on tea at Palampur in conventional and organic tea plantation was recorded from May 2022 to April 2023 and findings have been presented in Table 1 and the effect of abiotic factors on its buildup was assessed. Observations recorded on population build-up of *O coffee* revealed them to differ significantly in conventional and organic tea plantations, with the mite population being higher in conventional farming system (Table 1). Observations initiated during May 2022 revealed the corresponding population levels of 19.26 and 6.72 mites / leaf in conventional and organic tea plantations. A declining trend in population under both the farming systems was observed up to August 2022 with the population levels of 7.29 and 2.57 mites / leaf in respective farming systems. During September 2022 rise in population took place and second peak of population was evident during October 2022 with the corresponding population levels of 13.42 and 5.49 mites / leaf. Thereafter, the population declined and reached the minimum levels in February 2023 with the population count of 5.22 and 2.63 mites / leaf in conventional and organic tea plantation systems, which increased to 14.45 and 6.26 mites / leaf during April 2023.

Leaf infestation in tea by red spider mite varied from 61 - 99 per cent in conventional and 39 - 82 per cent in organic farming systems with the peak of leaf infestation occurring during June and May, 2022 in respective farming systems. Based on the mite population and leaf infestation, infestation index was worked out and is presented in Fig 1. It was observed that the value of the infestation index was maximum in June 2022 in conventional (18.71) and during May 2022 in organic (5.51) farming systems. Thereafter, a decline was set in and a peak was evident during October 2022 with the corresponding values of 11.68 and 3.07.

Correlation of *O. coffeae* with abiotic factors

The relationship deduced between mite population and different environmental factors presented in Table 2 revealed that a positive correlation with the maximum and minimum temperature and sun - shine hrs was evident. However, it was significant with maximum temperature (r = 0.6131, P = 0.05) in conventional tea plantations only. Whereas, it showed negative relationship with rainfall and relative humidity, being significant in organic tea plantations with relative humidity (r=-0.6012).There exists a positive relationship between mite population and temperature and sunshine hrs. Whereas, the population was affected negatively by rainfall and relative humidity. These findings are in conformity to the findings of Dantanarayana and Ranaweera (1972), Choudhary *et al.* (2006), Ahmad *et al.* (2012),

Table 1. Population buildup of *Oligonychus coffeae* in tea at Palampur in conventional and organic farming systems

Month of observation	Conventional tea plantation		Organic tea plantation	
	Mite population ± SE / leaf	Leaf infestation (%)	Mite population ± SE / leaf	Leaf infestation (%)
May 2022	19.26 ± 1.73	95	6.72 ± 0.86	82
June 2022	18.90 ± 1.50	99	6.37 ± 0.99	70
July 2022	8.05 ± 1.14	78	2.68 ± 0.68	41
August 2022	7.29 ± 1.23	68	2.57 ± 0.67	41
September 2022	12.26 ± 1.33	86	3.03 ± 0.82	39
October 2022	13.42 ± 1.51	87	5.49 ± 1.08	56
November 2022	7.00 ± 0.96	76	4.09 ± 0.84	54
December 2022	5.22 ± 1.05	67	2.45 ± 0.60	41
January 2023	5.73 ± 1.03	63	2.98 ± 0.70	46
February 2023	5.22 ± 0.10	61	2.63 ± 0.73	45
March 2023	11.37 ± 1.54	83	4.61 ± 0.89	58
April 2023	14.45 ± 1.83	84	6.26 ± 0.83	57
P = (0.05), Independent t-test		Significant		

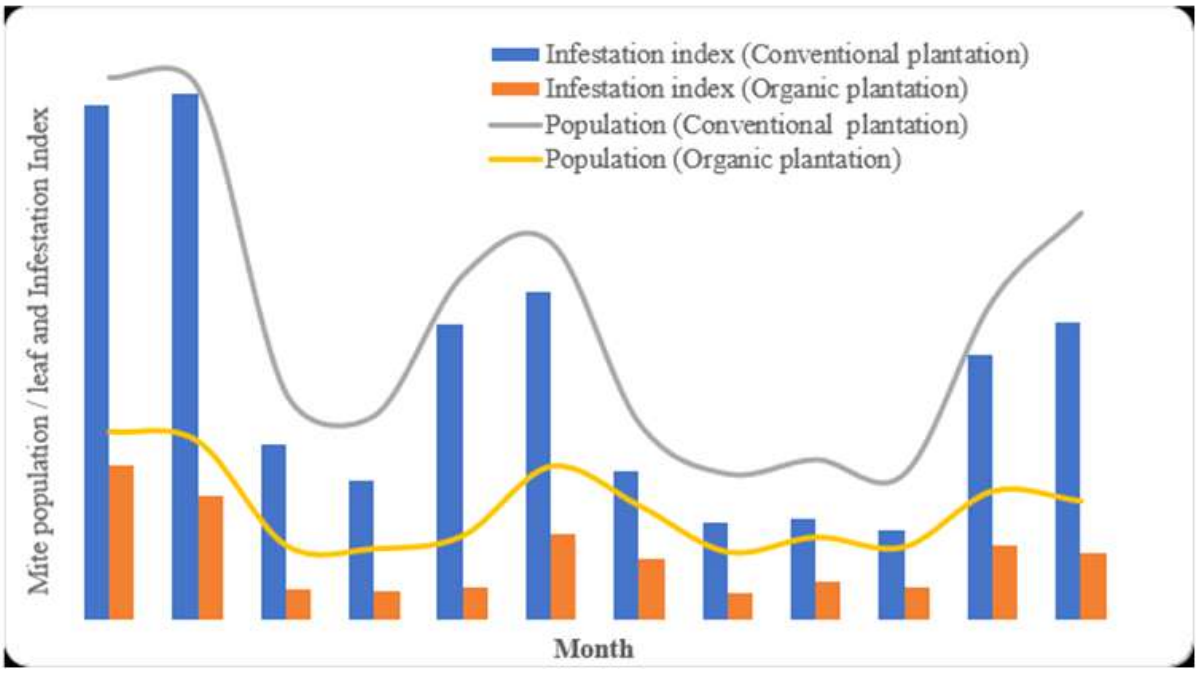

Figure 1: Population buildup of *O. coffeae* in conventional and organic farming systems at Palampur

Table 2. Correlation coefficient (r) between abiotic factors and mean mite population in conventional and organic farming systems

Abiotic factor		Correlation Coefficient (r)	
		Conventional tea plantation	Organic tea plantation
Temperature (°C)	Maximum	0.6131*	0.5072
	Minimum	0.4725	0.2931
Relative humidity (%)	Morning	-0.4647	-0.6012*
	Evening	-0.4564	-0.2837
Rainfall (mm)		-0.0001	-0.3191
Sunshine (hrs)		0.0455	0.3157

* Significant at P = 0.05

Kachhawa and Rahman (2013).

It can be concluded that population buildup of *Oligonychus coffeae* studied under conventional and organic farming system revealed the population to vary from 5.22 to 19.26 and 2.45 to 6.72 in respective farming system at Palampur. The peaks of activity were observed during May and October. Population was at its minimum during December - February. Leaf infestation was more in conventional as compared to organic farming system.Infestation index also revealed its value to be more in conventional farming (3.18 - 18.71%) with the value of 1.00 to 5.51 in organic farming system. The peaks of index occurred during June - October in both the farming systems. The mite population was positively correlated with temperature (maximum & minimum) and sun - shine hrs, but it was related negatively with relative humidity (morning & evening) and rainfall.

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Short Communication

Comparative biology of *Spodoptera frugiperda* (JE Smith) on maize and sorghum

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Abstract

Biology of fall armyworm, *Spodoptera frugiperda* (JE Smith) was studied under laboratory conditions on maize and sorghum. The studies revealed that the larval period was shorter when the larvae were fed on maize as compared to sorghum. The adult longevity was longer in maize-fed larvae. Fecundity was also higher when the larvae were reared on maize plants.

Key words: *Spodoptera frugiperda*, maize, sorghum

The fall armyworm, *Spodoptera frugiperda* belonging to order Lepidoptera and family Noctuidae, is one of the most invasive pests of maize (Assefa and Ayalew 2019; Kenis *et al*. 2022). It has a wide host range of up to 353 plant species belonging to 76 families (Montezano *et al.* 2018). Its high fecundity and strong ability to migrate is probably attributed to its infestation in newer areas replacing other stem borers like *Busseola fusca* Fuller and *Chilo partellus* Swinhoe infesting maize (Wan *et al*. 2021). Yield losses to the tune of 8 to 20 million tonnes by this pest have been reported in Africa alone (Anon. 2020), where its first incidence was reported in 2016 only (Georgen *et al.* 2016). *S. frugiperda* invaded into India in 2018 when it was first reported from Karnataka (Sharanabasappa *et al.* 2018). Subsequently, the major outbreak of this invasive pest was reported in Una district of Himachal Pradesh during *Kharif* 2020 when its incidence ranging from 37.1 to 64.7% was observed on maize (Sharma 2021). In the present investigations, an attempt was made to study the biology of this pest under laboratory conditions on maize and sorghum to find out its preference for the host plants.

Seedlings of maize and sorghum were raised in plastic pots (dia: 100 mm). These pots were filled with a mixture of soil and farm yard manure (FYM) in equal proportions. Twenty pots were maintained for each crop. The mass culture of *S. frugiperda* in laboratory was started from field-collected larvae. The larvae, identified by a characteristic inverted 'Y' mark on head and four dots arranged in a square pattern on the 8th abdominal segment, were collected from the field and transferred to plastic vials (100 mL) provisioned with tender maize leaves. The larvae were fed till pupation and then left undisturbed until adult emergence. The freshly emerged moths from mass culture were used in further laboratory studies. These male and female moths in the ratio of 1:1 were released in the ovipositional jar (20 x 15 cm^2). Cotton swabs soaked in 10% honey solution were given as food for adult moths. Folded blotting papers were provided for resting and egg-laying by female moths. The open end of ovipositional jar was covered with muslin cloth held tightly in position by a rubber band. The jar was regularly inspected for egg laying. When the egg-laying commenced, blotting papers having egg masses of *S. frugiperda* were removed and replaced with new ones. The collected egg masses were placed in plastic boxes (19 x 12 cm^2) and were kept in an insectary under controlled conditions (Temperature: 25+2°C; RH: 60-65%, Photoperiod: 14L:10D).

For studying growth and developmental biology of *S. frugiperda*, 30 neonates were gently picked up with camel hair brush and placed singly in plastic Petri plates (diameter: 9 cm) having Whatman blotting

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paper at the base. These larvae were fed with tender and succulent leaves of these host crops. Only 1st and 2nd instars of *S. frugiperda* were reared in the Petri plates. Whereas, 3rd to 6th instar larvae were shifted to plastic vials having perforated lids. These larvae were also daily provided with fresh leaves. The pupae forming after completion of larval stage were left undisturbed in the same vials till the emergence of adults.

Observations were recorded on durations of immature stages *viz*., egg, larva and pupa to work out incubation period, larval period and pupal period, respectively. Similarly, data on pre-oviposition, oviposition and post-oviposition periods were noted to get longevity of adult female. Likewise, male moths were also observed to know their longevity. In addition, fecundity of female was also observed.

The data presented in Table 1 revealed that the mean incubation period of *S. frugiperda* eggs obtained from females whose larvae were reared on maize and sorghum was statistically similar and lasted for 2.55 and 2.57 days, respectively. However, the total duration of larva reared on maize was significantly shorter (16.89 days) than that on sorghum (17.84 days). Wijerathna *et al.* (2021) also reported that larval period of *S. frugiperda* on maize was 16 days. Nandhini *et al.* (2023) reported larval duration of 14.40 days on maize. Similarly, Sharanabasappa *et al.* (2018) found the larval period of *S. frugiperda* to last for 14 – 19 days. Also, Ashok *et al.* (2020) observed that the larval stage of *S. frugiperda* lasted for 14.48 days on maize under controlled conditions (Temperature- 27°C, RH- 70%, Photoperiod- 14L:10D). Thus, these earlier studies corroborated the present findings. On the other hand, pupal period of *S. frugiperda* was statistically at par with each other when larvae were reared on maize (10.27 days) as well as sorghum (10.30 days). Rashed (2023) reported pupal period of *S. frugiperda* as 9.93 days on maize. Similarly, Salem *et al*. (2021) stated that the pupal duration of this pest on maize was 10.75 days. Krishnarao *et al.* (2022) also found its pupal duration to last for 10.13 days on maize. Therefore, the present results agree with results of these authors. Generally, the males of *S. frugiperda* comparatively lived longer than females. At the same time, longevity of adult male and female where larval stages were fed on maize and sorghum was 8.28, 8.00 and 11.32, 10.15 days, respectively (Table 1). Longevity of male and female adult on maize was 8.30 and 10.33 days, respectively (Krishnarao *et al*. 2022). The adult males survived for 11.1 days (Ashok *et al.* 2020) and for 6 – 7 days (Kranthi *et al*. 2021). These earlier results are also in close proximity to durations observed in present studies.

The pre-oviposition period of *S. frugiperda* females derived from larvae fed on maize and sorghum was statistically at par with each other and was recorded to be 3.50 and 3.45 days, respectively (Table 2). However, the female from maize-fed females oviposited for statistically longer duration (4.50 days) than those where larvae were fed on sorghum (3.40

Table 1. Durations of immature stages of *Spodoptera frugiperda*

Host plant	Biological parameter				
	Incubation period (Days)	Larval period (Days)*	Pupal period (Days)*	Adult longevity (Days)*	
				Male	Female
Maize	2.55 (1.88)	16.89 (4.23)	10.27 (3.36)	8.28 (3.05)	11.32 (3.51)
Sorghum	2.57 (1.89)	17.84 (4.34)	10.30 (3.36)	8.00 (3.00)	10.15 (3.34)
CD (P=0.05)	NS	0.07	NS	0.02	0.04

*Figures within parentheses are square root transformed means

Table 2. Pre-oviposition, oviposition, post-oviposition periods and fecundity of *Spodoptera frugiperda*

Host plant	Pre-oviposition period (Days)*	Oviposition period (Days)*	Post-oviposition period (Days)*	Fecundity (No. of eggs/female)*
Maize	3.50 (2.12)	4.50 (2.35)	3.35 (2.08)	827.5 (28.7)
Sorghum	3.45 (2.11)	3.40 (2.10)	3.30 (2.07)	538.2 (23.2)
CD (P=0.05)	NS	0.04	NS	(1.0)

*Figures within parentheses are square root transformed means

days). On the other hand, the post-oviposition period was statistically similar for females whose larvae were fed on maize and sorghum. But, the females from larvae reared on maize laid significantly a greater number of eggs than those on sorghum. The respective fecundity of females for maize and sorghum-reared larvae was 827.5 and 538.2 eggs per female. According to Praveen and Mallapur (2019), the fecundity of *S. frugiperda* female was 680 and 650 eggs per female on maize and sorghum, respectively. Likewise, Nandhini *et al.* (2023) reported the fecundity of *S. frugiperda* as 720 and 725 – 850 eggs per female on maize and sorghum, respectively. The fecundity of female observed in current studies is in consonance with these earlier reports.

The studies concluded that host plants influenced the overall biological parameters of *S. frugiperda.* The adults lived longer on maize plants and the fecundity was also higher on maize plants as compared to sorghum.

Conflict of interest: There is no conflict of interest among the authors in this research paper.

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Assessment of Level of Emotional Intelligence and Gender Differences among college going youth of Chandigarh

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Abstract

Emotion is an essential part of a person's life as it is involved in every activity, action and reaction of human being. Many students come to college, prepared academically but not emotionally. During higher education, the need of emotional intelligence increases as it helps students to reduce their academic pressure. Therefore, the present study was undertaken with the objective to determine the level of emotional intelligence as well as to find out the gender difference between emotional intelligence of college students. A total sample of 200 students in the age group of 18-24 years were selected using stratified random sampling. The data was analyzed with the help of standardized tool, percentage and frequency distribution, t-test and SPSS software. The results indicated that majority of boys and girls scored higher in domain motivating oneself and overall has average level of emotional intelligence. Significant gender differences were revealed where boys scored higher than girls in domain managing emotions and empathy.

Key words: Emotions, emotional intelligence, gender difference, relationships

India has been regarded as the youngest country in the world by the United Nations as it has the world's largest youth population where more than 365 million people are between the age group 10 and 24 years. The young person in India constitutes one of the precious resources of India characterized by growth and development and is a phase of vulnerability often influenced by several intrinsic and extrinsic factors that affect their health and safety. As parents are responsible for promoting and supporting the physical, emotional, social and intellectual development of a child from infancy to young adulthood (Thakur *et al.* 2012). They may differ in how they try to control or socialize their children and the extent to which they do so, it is assumed that the primary role of all parents is to influence, teach, and control their young children (Thakur *et al.* 2012). Young people undergo maximum emotional fluctuations in the process of transition from school to college. College students face many challenges that may affect their mental health including relationship dynamics, academic pressures, and financial struggles. On a deeper level, at college, there are new and often unexpected challenges to their identity, sense of efficacy, mental health, personality, satisfaction etc. due to which understanding and honing the ability to identify, express, and manage emotions is just as important to them as studying for an exam. In today's time, the aim of education is not only to have individuals who have knowledge but also to have people with emotional intelligence (EI) with an effective use of it. Wherever there is a human component involved, there is also emotional intelligence in action.

Emotional Intelligence offers tremendous practical applications in the major part of our lives. It refers to the ability of an individual to recognize his own emotions and those of others, distinguish and differentiate between them and classify them appropriately. Thus, emotional intelligence is viewed as the ability of an individual to appropriately and successfully respond to a vast variety of emotional

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stimuli being elicited from the inner self and immediate environment (Singh 2003). People who show high levels of emotional intelligence are individuals who know themselves and their needs, strengths, weaknesses and manage to control themselves and forms sound relationships. The value of emotional intelligence is immense; developing emotional intelligence encourages many positive traits, from resilience to communication, motivation to stress management, all of which can be seen as conducive to effectively achieving personal, physical and occupational health, and success. Parentscan empathizes their child by using soothing words of affection which helps the child label the emotion he/she is feeling and offers guidance on regulating emotions.Therefore, the relationship between a parent and child is of utmost importance - the nature of interaction, discipline and dealing with the child's behaviour and emotions have an impact on the developing child. These examples set by parents is extremely important developing for interpersonal relations and social behavior (Rani and Singh 2014).

Therefore, once the nature and extent of their emotional intelligence is identified, it would enable teachers, parents, social workers and policymakers to plan and develop intervention strategies for improving their emotional intelligence. Improving emotional intelligence in college students can help them improve self-awareness, self-regulation, decision making, empathy, critical thinking, communication skills, boost self-motivation, social skills etc. and can reduce their risky behaviors. Therefore, the present study was conducted with objectives to determine the level of emotional intelligence and to find out the gender differences in emotional intelligence among college going youth of Chandigarh.

Materials and Methods

The present study was conducted in selected educational institutions of Chandigarh. For the selection of colleges, a list of all co-education and girl's colleges in Chandigarh was obtained from the website of Director of Higher Education. Two co-education colleges and two girl's colleges were randomly selected from the obtained list. A total sample of 200, consisting of 100 boys and 100 girls were selected for the purpose of study. Using stratified random sampling and questionnaire survey method, 50 undergraduate students within age range of 18-24 years from each college were selected. After data collection, the results were collected, arranged, tabulated, coded and analyzed using statistical tools such as percentage, frequency distribution, t- test. The analysis of data was done by using SPSS software.

For determining the emotional intelligence of the respondents, a standardized Emotional Intelligence Test developed by Dr. Ekta Sharma (2011) was used. It is one of the most popular instruments to measure emotional intelligence. The EIT constitutes of 60 items from five domains of Emotional Intelligence *i.e.*, Self-Awareness, Managing Emotions, Motivating Oneself, Empathy and Handling Relationships. All the 60 items are socially acceptable, though positively and negatively stated. The response pattern in the scale is of 5-point Likert-type ranging from strongly Always (5) to Never (1) and reverse for negative items. Thus total score was obtained from all items. In this way, the expected score may range from 60-300 with high score showing high degree of emotional intelligence and low score showing low score degree of emotional intelligence.

Results and Discussion

The percentage distribution of college going students with regard to their socio-demographic attributes, as reported by them, has been presented in Table 1. Results in Table 1 indicates that the majority (45% and 53%, respectively) of sample was in the age group of 20-22 years and belonged to Hindu religion (69% and 78% respectively). Majority of boys (45%) were studying in 1st year while most of the girls (61%) in 2nd year. Most of the boys (67%) as well as girls (77%) belonged to nuclear families and were first born (51% and 48%, respectively). It was also found that majority of mothers (71% and 76%, respectively) of both the samples were not working while the fathers (52% and 57%, respectively) were employed in private sector. A large number of boys (54%) defined themselves as serious and determined while the girls (63%) see themselves as easy going. But both the samples scored higher (39% and 44%, respectively) on over thinking. Maximum number of boys (36%) reported to join civil services as their aim of life unlike most of the girls who fall under miscellaneous category

Table 1. Socio-demographic information among sample of 200

Variables	Categories	Boys (%)	Girls (%)	Total (%)
• Age	18-20years	37.0	32.0	34.5
	20-22years	45.0	53.0	49.0
	22-24years	18.0	15.0	16.5
• Standard	1st year	45.0	28.0	36.5
	2nd year	36.0	61.0	48.5
	3rd year	19.0	11.0	15.0
• Religion	Hindu	69.0	78.0	73.5
	Sikh	23.0	16.0	19.5
	Muslim	3.0	2.0	2.5
	Others	5.0	4.0	4.5
• Type of	Nuclear	67.0	77.0	72.0
Family	Joint	33.0	23.0	28.0
• Birth Order	1st born	51.0	48.0	49.5
	2nd born	30.0	36.0	33.0
	3rd born	19.0	16.0	17.5
• Occupation(Father)	Govt.	48.0	43.0	45.5
	Private	52.0	57.0	54.5
(Mother)	Working	29.0	24.0	26.5
	Housewife	71.0	76.0	73.5
• Challenges	Very Emotional	26.0	27.0	26.5
	Over Thinks	39.0	44.0	41.5
	Self-Criticism and Lack of Confidence	22.0	20.0	21.0
	Others	13.0	9.0	11.0
• Define Themselves	Serious and determined	54.0	37.0	45.5
As	Easy Going	46.0	63.0	54.5
• Aim	Medical Profession	11.0	9.0	10.0
	Bank Professional	10.0	8.0	9.0
	Civil Services	36.0	22.0	29.0
	Education	13.0	26.0	19.5
	Miscellaneous	30.0	35.0	32.5

which included fashion designing, dietician, psychologist, artist, singer etc.

The percent distribution of emotional intelligence among college going young adults revealed that more than half of boys 58% and girls 54% scored high only in the category of motivating oneself followed by 39% boys and 46% girls in average level respectively (Table 2). In general, more than half of sample also showed high level under this category. Majority of participants including boys and girls have scored in average level for remaining four categories. Most of the boys 89% have achieved highest average scored in managing emotions and about 83% girls scored under average level for same category. Whereas almost all girl's 91% have highest average scored for empathy while most of boys 77% scored average under this category. In the category of self-awareness only 67% boys and 78% girls showed average level. Half of the boys 56% and girls 66% obtained average score with regard to handling relationships category.

The sample of both boys and girls (63% and 77%, respectively) have an average level of emotional intelligence (Figure 1). As a whole, more than half of participants, 70%, were showing average level of emotional intelligence. Similar results are found in studies of (Aleena and Vigraanth 2021; Kumar 2020;

Table 2. Percentage distribution of emotional intelligence among the sample

Category	Sub Category	Boys (%)[n-100]	Girls (%)[n-100]	Total (%)[N=200]
Self-Awareness	High	30.0	22.0	26.0
	Average	67.0	78.0	72.5
	Below Average	3.0	0.0	1.5
Managing Emotions	High	9.0	7.0	8.0
	Average	89.0	83.0	86.0
	Below Average	2.0	10.0	6.0
Motivating Oneself	High	58.0	54.0	56.0
	Average	39.0	46.0	42.5
	Below Average	3.0	0.0	1.5
Empathy	High	19.0	9.0	14.0
	Average	77.0	91.0	84.0
	Below Average	4.0	0.0	2.0
Handling Relationships	High	41.0	34.0	37.5
	Average	56.0	66.0	61.0
	Below Average	3.0	0.0	1.5

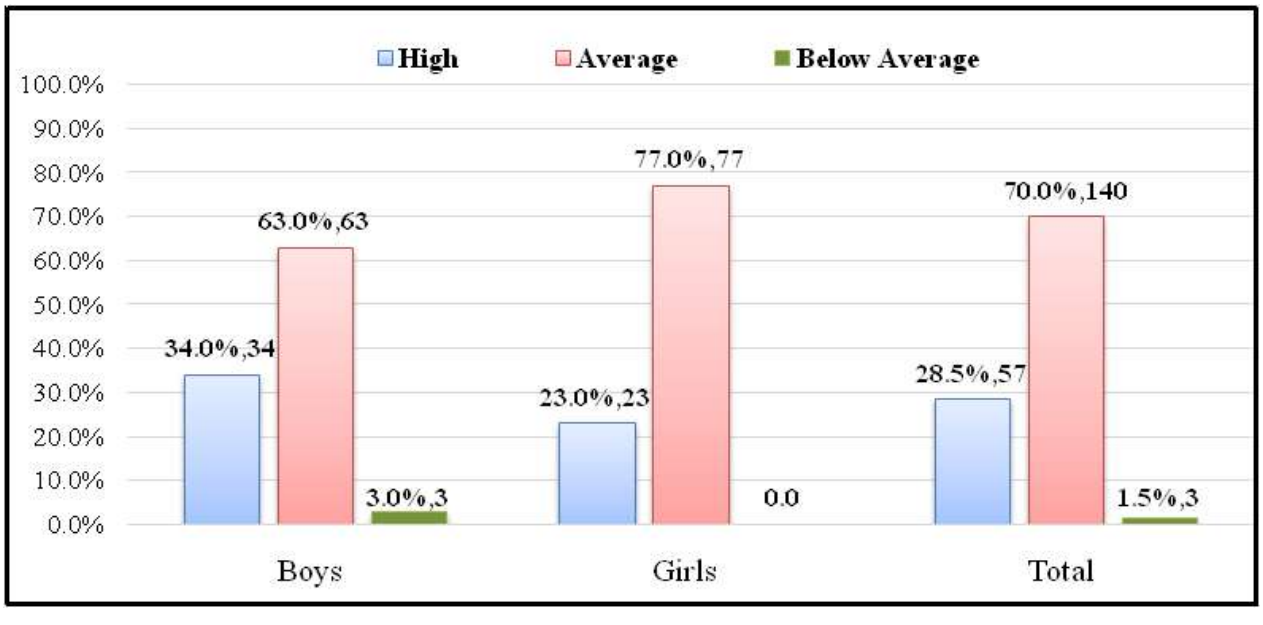

Figure 1. Level of emotional intelligence of boys, girls and total sample

Llego *et al*. 2019; Sowmyashree and Sreenivas 2019; Thamizhselvan and Vembu 2019; Sinha *et al.* 2017; Thomas and Arjunan 2016; Anitha and Jebaseelan 2014; Joshi *et al.* 2012; Katyal and Awasthi 2005) who have reported that more than half of the students had an average level of emotional intelligence.

Based on t-test, the gender differences in emotional intelligence of college going young adults (Table 3) revealed that there existed a statistically significant difference with regard to managing emotions ($t=-2.456$, $p<0.05$) and empathy ($t=-1.87$, $p<0.10$) referring that boys are more empathetic and manage their emotions better than girls. Similar results are found in studies of (Chu 2002; Dunn 2002; Singh 2002; Mishra and Ranjan 2008; Ahmad *et al.* 2009; Joibari and Mohammadtaheri 2011; Lenka and Kant 2012; Khan and Bhat 2013; Mokhlesi and Patil 2018; Usha and Solomon 2022) and revealed in their studies that boys were found to be having significantly higher score on emotional intelligence and are more

Table 3. Difference between emotional intelligence based on gender

Dimensions	Gender	Mean	SD	SEM	t-value	p-value
Self- Awareness	Girls	39.87	5.15743	0.51574	-0.678	0.499
	Boys	40.45	6.83185	0.68319		
Managing Emotions	Girls	20.25	3.16667	0.31667	-2.456	**0.015****
	Boys	21.32	2.99117	0.29912		
Motivating Oneself	Girls	52.42	7.38984	0.73898	0.036	0.971
	Boys	52.38	8.34336	0.83434		
Empathy	Girls	30.06	3.80011	0.38001	-1.87	**0.063***
	Boys	31.31	5.49875	0.54988		
Handling Relationships	Girls	67.6	6.27485	0.62748	0.73	0.467
	Boys	66.68	10.93588	1.09359		
Overall Emotional	Girls	210.57	15.01801	1.5018	-0.51	0.611
Intelligence	Boys	212.16	27.31733	2.73173		

*Significance Levels: *** 99% ** 95% * 90%*

empathetic than the girls. In contrary, Alam (2018) found significant difference of emotional intelligence among adolescents with reference to gender and socio-economic status.

Conclusion

Summarizing the results, it was concluded that majority of boys and girls showed average level of emotional intelligence and scored higher on the category of motivating oneself. Significant gender differences were revealed with regard to managing emotions, empathy where boys scored higher than girls. This may be due to the reason that boys show more assertiveness, self-recognition about themselves and show more independence and management according to the situations than the girls.

Conflict of Interests: Authors have declared that no competing interests exist.

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Six-limb-lead electrocardiogram in common dog breeds: reference values

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Abstract

The objective of this study was to establish the standard electrocardiographic (ECG) values for six dog breeds and to evaluate the influence of gender and age on various ECG parameters. Labrador Retrievers (22), Pomeranians (11), mongrels (13), German Shepherds (10), Golden Retrievers (13) and Gaddi dogs (9) were included in the study. Dogs were positioned in right lateral recumbency without the use of any chemical restraint and six-lead ECGs were recorded, comprising three bipolar standard limb leads (I, II and III) and three augmented unipolar limb leads (aVR, aVL and aVF). Parameters like amplitude, duration of P-wave, QRS complex, PR interval, QT interval, mean electrical axis and heart rate were measured for each recording. Amplitude and durations of ECG waves across all six leads were not statistically affected by gender or age. P(mV) was significantly greater in Pomeranians as compared to mongrels whereas, Gaddi breed showed significantly greater Q(mV) as compared to Golden Retrievers.

Key words: Augmented baseline data, unipolar lead, ECG, Gaddi dog

Electrocardiography (ECG) is a valuable tool for assessing parameters such as heart rate (HR), cardiac rhythm, conduction integrity and the theoretical axis of the heart (De Caterina *et al*. 2012). Traditionally, ECGs of healthy dogs have been analysed using standard bipolar limb lead I, which provides information in only one direction. However, clinical situations involve three-dimensional aspects that are more complex. Therefore, multiple electro cardiographic leads are necessary to capture the variations in heart distribution within the thorax (Hsieh and Hsu, 2012).

Studies have shown significant breed differences in ECG parameters among healthy dogs, which could be attributed to variations in shape of thoracic cavities or genetic differences (Avizeh *et al*. 2010). This study aimed to establish a comprehensive set of ECG reference values and assess the influence of breed, sex, and age on the electrocardiogram of six breeds of dogs. It is widely employed in diagnosing various cardiac conditions in humans and animals, including cattle and small ruminants (Pavan *et al*. 2015) and horses (Scheffer *et al*. 1995) as well as in assessing non-cardiac illnesses. In dogs, cardiac arrhythmias and intra-cardiac conduction disturbances are common issues that can be effectively analysed using ECG (Gugjoo *et al*. 2014).

However, there is a lack of comparative studies examining variations in ECG parameters among different dog breeds. Therefore, this study aims to assess the variation in ECG parameters among different breeds of dogs, namely Labradors, Pomeranians, Mongrels, German Shepherds, Golden Retrievers and Gaddi. Additionally, there is a lack of data regarding the basic cardiac parameters of these dogs in specific geo-climatic regions. It is anticipated that the data generated from this study will be valuable for future assessments in this field.

Materials and Methods

The study involved 78 clinically healthy dogs of either gender from locations in and around Palampur, H.P., India. The dogs were categorized into six different breeds: Labradors (n=22), Pomeranians (n=11), mongrels (n=13), German Shepherds (n=10), Golden Retrievers (n=13) and Gaddis (n=9). Age-based classification was carried out into three subgroups: (i) <2 years, (ii) 2–6 years and (iii) >6

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years.

A 3-channel RMS Vesta 301i ECG machine was used to capture the electrographic recordings, with calibration and gain set to 10 mm=1 mV and paper speeds of 50 and 25 mm/s. On thermosensitive ECG paper, recordings were made from all of the standard bipolar limb leads (Lead-I, II and III) as well as the unipolar augmented limb leads (Lead - aVR, aVL and aVF). The small square on the horizontal axis indicates 0.02 seconds. Wave amplitude or voltage is shown on the vertical axis, where 0.1 mV is represented by a 1 mm height. All of the dogs were kept in right lateral recumbency and manually restrained without the use of anaesthesia at the time the recording was made.

Measurement of Heart rate, complexes, intervals, and mean electrical axis (MEA)

Waves of ECG recorded by Lead II are considered to be the typical waves as the depolarization vector is directed toward the electrode of Lead II (Becker *et al*. 2006). Amplitudes of P, Q, R, S and T-waves were measured together with PR interval, QRS interval and QT interval. HR was calculated by successive R-R interval.

The procedures outlined by Edwards (2000) were followed to calculate the mean electrical axis-

1) Mean Electrical Axis was identified by locating the isoelectric lead on the ECG.
2) The six-axis reference chart revealed a lead that was perpendicular to the isoelectric lead.
3) The MEA value is determined by the electrocardiogram's positive or negative perpendicular lead deflection.

Statistical analysis

Using computer software Instat from Graphpad software, the collected data was statistically analysed. The mean values of different parameters between control and diseased group, pre and post treatment were compared at 5% and 1% level of significance using "t" test and "ANOVA".

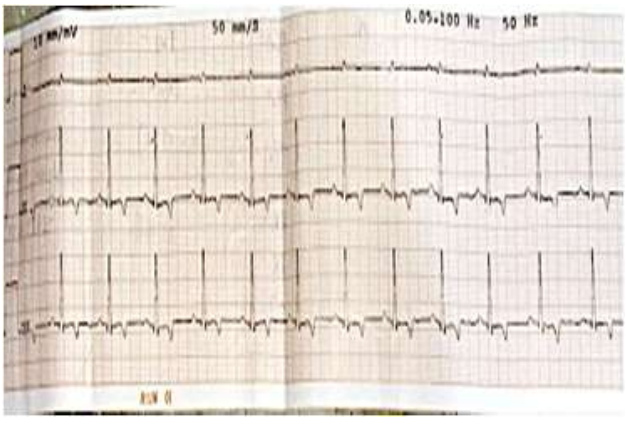
Results and Discussion

Mean ± SEM of different ECG parameters (Lead II) in different breeds of dogs has been presented in Table1. The ECG parameter reference values for the various dog breeds in this investigation were found to be consistent with previous reports in the German Shepherd (Rezakhani *et al*. 1990), Labrador (Gugjoo *et al*. 2014), and Beagle (Detweiler *et al*. 1997). Fig. 1-6 represents the normal electrocardiograph of healthy dogs in different breeds.

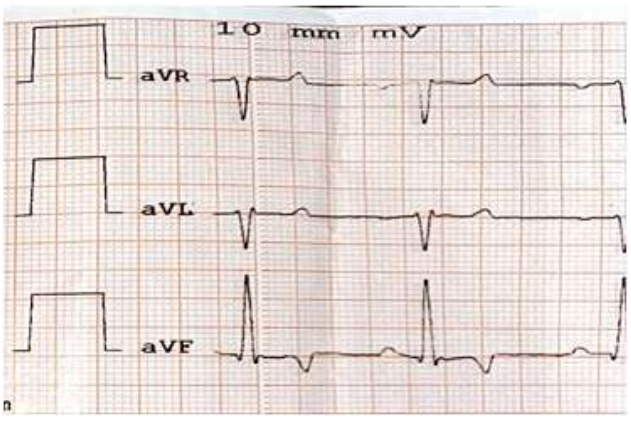
There were no significant variations in HR among different breeds. Though HR was found to be highest in Pomeranians (134 ±15.49 bpm) followed by German Shepherd (125.3±11.25 bpm), Gaddi (123.33±6.67 bpm), Golden Retriever (116.67 ± 6.67 bpm), Labrador (111.67± 9.46) and Mongrel (108.75 ± 5.26 bpm).Heart rate in the current investigation was found to be comparable to the reference values given by Becker (2006).

P-amplitude was maximum in Pomeranians (0.18 ± 0.03 mV) followed by Gaddi (0.16 ± 0.03 mV), Labradors (0.16 ± 0.02 mV), Golden retriever (0.15 ± 0.04 mV), German Shepherd (0.13 ± 0.03mV) and mongrels (0.10 ± 0.02 mV). Pomeranians showed statistically ($p<0.05$) greater P(mV) as compared to mongrels, but within normal range. The P-wave amplitude travels from the Sinoatrial node to the Atrioventricular node and represents the degree of atrial depolarization. Pomeranian breeds showed a higher HR (134 ± 15.49 bpm) than the other five breeds studied, which could account for the breeds' higher P-wave amplitude. According to Avizeh *et al*. (2010) and Ferasin *et al*. (2012), stress displayed during ECG recording may account for variation in P-wave amplitude. Additionally, Mukherjee *et al*. (2020) noted that stress and heart rate may be related to variations in P wave amplitude. In all six limb leads, the gender influence on the P wave was found to be non-significant. These findings were consistent with the results of several earlier studies (Hanton and Rabemampianina 2006 and Changkija 2007).

Q-amplitude was maximum in Gaddi dogs (0.25±0.03 mV) followed by Pomeranians (0.21±0.02 mV), mongrels (0.18±0.03 mV), Labradors (0.18±0.02 mV), German Shepherds (0.17±0.03 mV) and Golden Retrievers (0.15±0.03mV). Gaddi breed showed statistically ($p<0.05$) greater Q(mV) in comparison to Golden retrievers but within normal range. Rest all ECG parameters varied non-significantly within normal ranges among different breed under study (Table 1). Q-wave of QRS complex produced due to ventricular depolarization and the electrical transmission through interventricular septum after P-

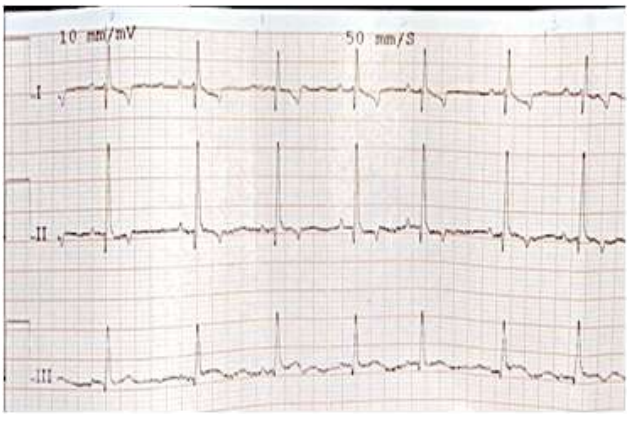


(a) Standard bipolar limb lead I, II and III

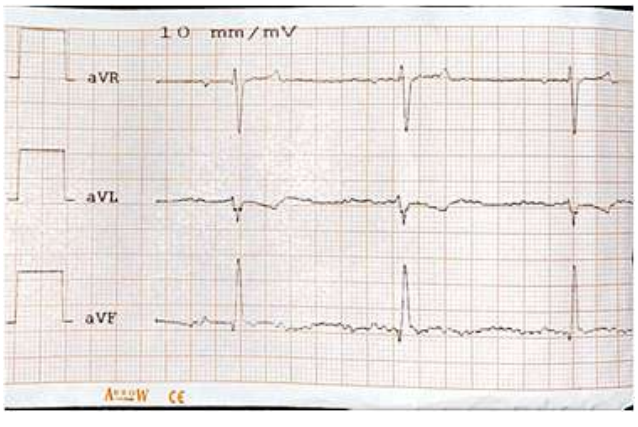


(b) Augmented unipolar lead avR, avL and aVF

Fig 1 Electrocardiograph in healthy Labrador dog (Speed: 50mm/sec)

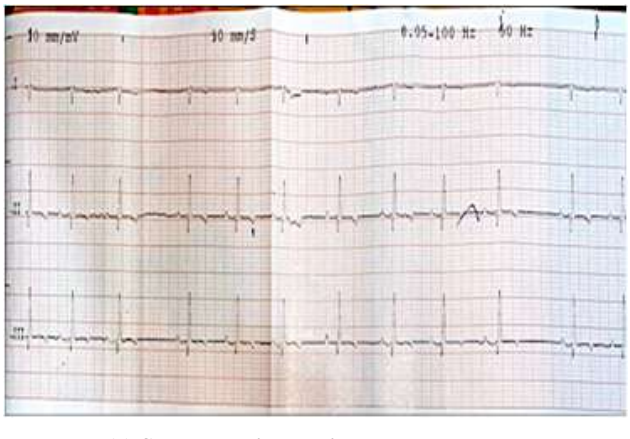


(a) Standard bipolar limb lead I, II and III

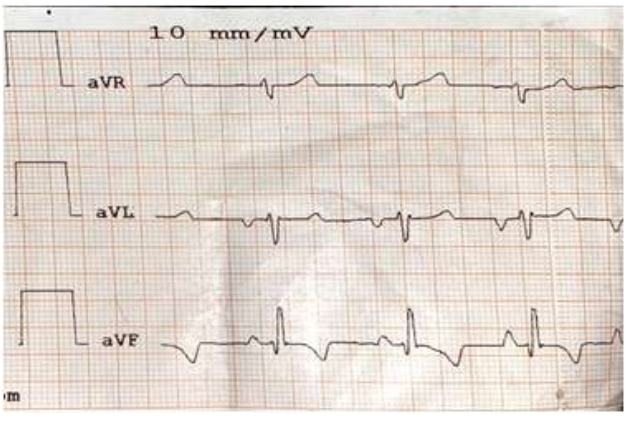


(b) Augmented unipolar lead avR, avL and aVF

Fig 2 Electrocardiograph in healthy Pomeranian dog (Speed: 50mm/sec)

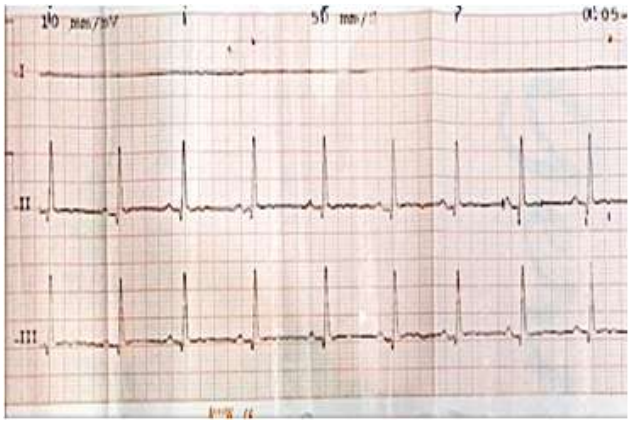


(a) Standard bipolar limb lead I, II and III

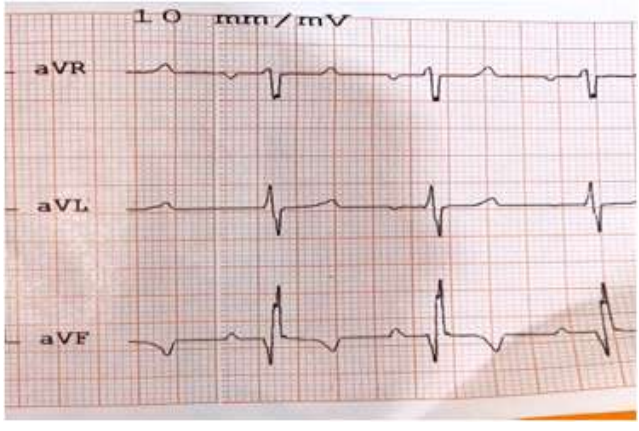


(b) Augmented unipolar lead avR, avL and aVF

Fig 3 Electrocardiograph in healthy mongrel dog (Speed: 50mm/sec)

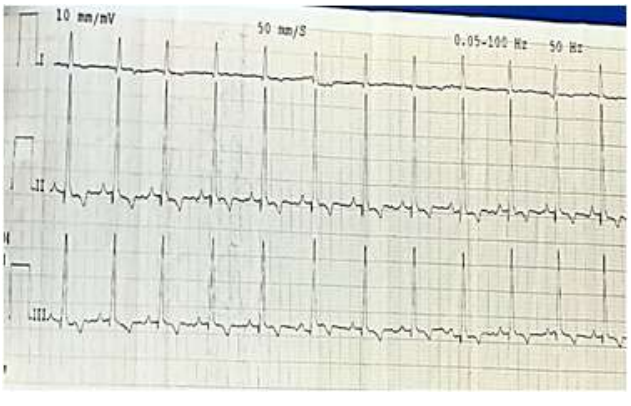


(a) Standard bipolar limb lead I, II and III

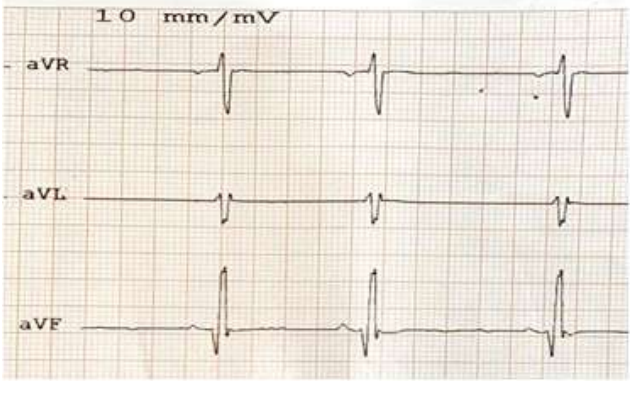


(b) Augmented unipolar lead avR, avL and aVF

Fig 4 Electrocardiograph in healthy German Shepherd dog (Speed: 50mm/sec)

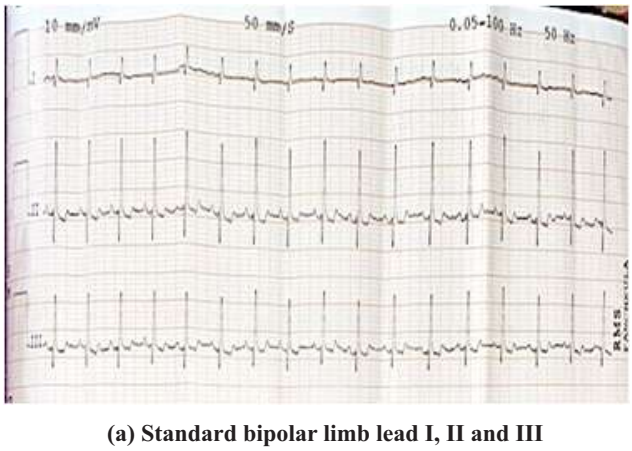


(a) Standard bipolar limb lead I, II and III

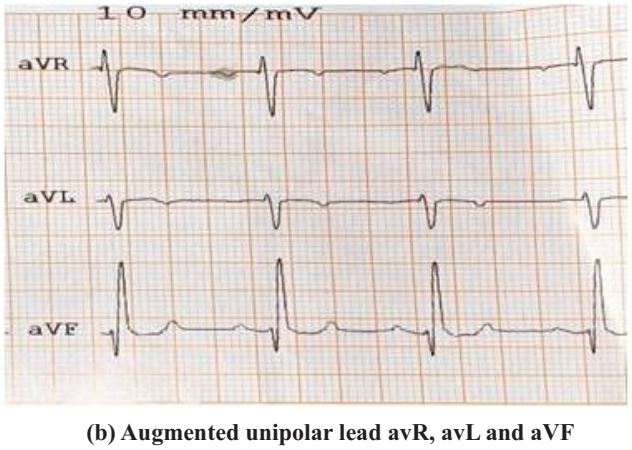


(b) Augmented unipolar lead avR, avL and aVF

Fig 5 Electrocardiograph in healthy Golden Retriever dog (Speed: 50mm/sec)



(a) Standard bipolar limb lead I, II and III



(b) Augmented unipolar lead avR, avL and aVF

Fig 6 Electrocardiograph in healthy Gaddi dog (Speed: 50mm/sec)

Table 1. Reference ECG parameters categorised breed, age, and gender wise

	Heart Rate (bpm)	P-wave amplitude (mV)	P-wave duration (sec)	Q-wave amplitude (mV)	PR-interval (sec)	R-wave amplitude (mV)	QRS duration (sec)	T-wave amplitude (mV)	T-wave duration (sec)	QT segment (sec)	RR-interval (sec)	MEA
Selected Breed												
Labrador (n=22)	111.67 ± 9.46[a]	0.16 ± 0.02[ab]	0.04 ± 0.001[a]	0.18 ± 0.02[ab]	0.10 ± 0.001[a]	1.20 ± 0.16[a]	0.05 ± 0.002[a]	0.17 ± 0.02[a]	0.04 ± 0.00[a]	0.19 ± 0.01[a]	0.55 ± 0.07[a]	67.5 ± 4.09[a]
Pomeranian (n=11)	134 ± 15.49[a]	0.18 ± 0.03[a]	0.03 ± 0.001[a]	0.21 ± 0.02[ab]	0.08 ± 0.01[a]	1.10 ± 0.21[a]	0.04 ± 0.01[a]	0.20 ± 0.03[a]	0.05 ± 0.01[a]	0.17 ± 0.02[a]	0.52 ± 0.09[a]	56.36 ± 6.42[a]
Mongrel (n=13)	108.75 ± 5.26[a]	0.10 ± 0.02[b]	0.03 ± 0.001[a]	0.18 ± 0.03[ab]	0.10 ± 0.01[a]	0.89 ± 0.18[a]	0.04 ± 0.002[a]	0.18 ± 0.05[a]	0.06 ± 0.01[a]	0.18 ± 0.01[a]	0.47 ± 0.09[a]	56.92 ± 5.83[a]
German Shepherd (n=10)	125.3 ± 11.25[a]	0.13 ± 0.03[ab]	0.03 ± 0.01[a]	0.17 ± 0.03[ab]	0.10 ± 0.01[a]	1.18 ± 0.11[a]	0.05 ± 0.01[a]	0.22 ± 0.05[a]	0.06 ± 0.01[a]	0.19 ± 0.01[a]	0.51 ± 0.11[a]	71 ± 6.74[a]
Golden Retriever (n=13)	116.67 ± 6.67[a]	0.15 ± 0.04[ab]	0.04 ± 0.002[a]	0.15 ± 0.03[a]	0.09 ± 0.01[a]	1.18 ± 0.64[a]	0.04 ± 0.002[a]	0.20 ± 0.04[a]	0.05 ± 0.00[a]	0.17 ± 0.03[a]	0.48 ± 0.12[a]	75.38 ± 5.62[a]
Gaddi (n=9)	123.33 ± 6.67[a]	0.16 ± 0.03[ab]	0.04 ± 0.01[a]	0.25 ± 0.03[b]	0.09 ± 0.01[a]	0.95 ± 0.30[a]	0.05 ± 0.001[a]	0.20 ± 0.02[a]	0.06 ± 0.01[a]	0.18 ± 0.01[a]	0.39 ± 0.11[a]	76.67 ± 5.27[a]
Age												
<2 years (n=17)	158.26 ± 5.32[a]	0.13 ± 0.01[a]	0.0315 ± 0.0016[a]	0.22 ± 0.01[a]	0.08 ± 0.005[a]	1.04 ± 0.08[a]	0.04 ± 0.001[a]	0.16 ± 0.03[a]	0.04 ± 0.005[a]	0.17 ± 0.01[a]	0.45 ± 0.04[a]	55.6 ± 5.56[a]
2-6 years (n=31)	111.41 ± 4.07[b]	0.16 ± 0.02[a]	0.0374 ± 0.0024[ab]	0.27 ± 0.02[a]	0.09 ± 0.002[ab]	0.98 ± 0.11[a]	0.04 ± 0.004[a]	0.20 ± 0.01[a]	0.05 ± 0.005[a]	0.19 ± 0.00[a]	0.51 ± 0.05[a]	69.75 ± 5.11[a]
>6 years (n=30)	105.58 ± 4.98[b]	0.17 ± 0.02[a]	0.041 ± 0.0023[b]	0.19 ± 0.07[a]	0.11 ± 0.003[b]	1.12 ± 0.12[a]	0.05 ± 0.003[a]	0.20 ± 0.02[a]	0.05 ± 0.01[a]	0.19 ± 0.01[a]	0.51 ± 0.05[a]	74.65 ± 8.75[a]
Gender												
Male (n=56)	121.11 ± 4.03[a]	0.15 ± 0.07[a]	0.04 ± 0.01[a]	0.24 ± 0.02[a]	0.09 ± 0.02[a]	1.04 ± 0.58[a]	0.04 ± 0.01[a]	0.18 ± 0.17[a]	0.05 ± 0.02[a]	0.19 ± 0.04[a]	0.49 ± 0.26[a]	67.67 ± 4.83[a]
Female (n=22)	126.67 ± 9.40[a]	0.18 ± 0.11[a]	0.03 ± 0.01[a]	0.29 ± 0.04[a]	0.09 ± 0.02[a]	1.20 ± 0.72[a]	0.04 ± 0.02[a]	0.20 ± 0.19[a]	0.04 ± 0.02[a]	0.17 ± 0.02[a]	0.48 ± 0.25[a]	56.29 ± 5.88[a]

Mean ± SE values with different superscript in same column are considered significant ($p<0.05$)

wave. Q (amplitude) was significantly greater in Gaddi dogs when compared to Golden Retrievers. According to Sato *et al*. (2000), variations in Q wave amplitude among breeds may be linked to thoracic characteristics in individual dogs. This observation may be explained by the thoracic idiosyncrasies of various dog breeds as well as the relationship between the depth of the Q wave and the activation processes of the cardiac ventricles, or by variations in the distribution of purkinje fibres within the ventricular walls. According to Bernal *et al*. (1995), statistical study based on age and sex revealed non-significant differences in the Q wave amplitude which was consistent with our results.

The primary ventricular muscles' depolarization is represented by the R wave, which is used to assess left ventricular function. In the current study, we found that while R wave did not differ significantly amongst breeds. Labrador, GSD and Golden Retrievers had non-significantly higher values. Previous studies by Mukherjee *et al*. (2020) also reported that larger dog breeds have higher R wave amplitudes than smaller breeds because of larger ventricular surfaces and thicker walls.

The QT interval is a dynamic physiological characteristic that can be changed by the velocities of both ventricular conduction and repolarization. The QT interval in this investigation was consistent with previous publications (Gugjoo *et al*. 2014 and Mattera *et al*. 2012).

According to previous observations (Gugjoo *et al*. 2014 and Kumar *et al*. 2011), T-wave amplitude and duration, which are directly related to the repolarization of the ventricular myocardial cells, were determined to be within normal range in the current investigation. In our investigation, inverted T-waves were frequently observed. While the altered polarity of the T-wave in Lead II may be induced by elevation of the diaphragm during breathing, the genesis of the T-wave is highly complex (Tilley 1992; Kumar *et al*. 2014), and the factors determining T-wave polarity are still not well known (Potse *et al*. 2007).

As previously reported by Gugjoo *et al*. (2014), MEA values of the heart in several breeds of dogs under investigation were determined to be within the normal range.

Under age wise categorization, dogs below 2 years of age showed highest heart rate with mean ± SEM of 158.26±5.32 bpm which was significantly greater than other age groups probably due to higher metabolic rate in growing pups. Also, the geriatric age group (>6 years) showed longer P(duration) - 0.041 ± 0.0023 sec when compared to other age groups and showed significant variation ($p<0.05$) with younger age group (<2 years). Similarly, PR(sec) in geriatric group (>6 years) was more - 0.11 ± 0.003sec in comparison to other groups and showed statistical variation ($p<0.05$) with the younger age group (<2 years). The rest of the ECG parameters varied non-significantly among different age groups. Gender wise classification revealed no statistical variations among ECG parameters as shown in Table 1.

Conclusion

The present study provides the reference values for different ECG parameters in Labrador, Pomeranian, mongrel, German Shepherd, Golden Retriever and Gaddi dog breeds acclimatized to Indian climate with cardiovascular adjustments. This study found significant differences with respect to the Q (amplitude) between the Gaddi and Golden Retrievers and P (amplitude) among Pomeranians and Mongrels breeds. Heart rate in younger age group (<2 years) dogs was significantly higher when compared to other age groups. Also, P (duration) and PR wave was significantly greater in geriatric dogs when compared to younger dogs.

Conflict of interest: The authors have no conflict of interest in this research paper.

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