Himachal Journal of Agricultural Research 47(1): 104-109 (2021)

Short Communication

Evaluation of antagonistic potential of bio-control agents and organic inputs for the management of bean anthracnose

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Manuscript received: 02.11.2020; Accepted: 22.01.2021

Abstract

Management of bean anthracnose remains a great challenge for the farmers owing to variable nature of the pathogen. Generally, disease control involves the use of chemical fungicides but keeping in view their harmful effects on environment, alternative approaches like use of natural antagonists and organic formulations are being explored to mitigate the pollution problem. In the present study, bio-control agents *viz*., *T. harzianum* (TH-11), *T. harzianum* (TH-5), *T. koningii* (DMA-8), *T. koningii* (JMA-11), *T. viride*, *Pseudomonas fluorescens* and bioformulations namely, Jeevamrit, Bijamrit, Tamerlassi, Vermiwash, Cow urine and *Eupatorium* extract were evaluated against *C. lindemuthianum* under *in vitro* and *in vivo* conditions. *In vitro* assessment of the biocontrol agents revealed maximum mycelial growth inhibition by *T. koningii* (DMA-8) (53.69%) whereas among bioformulations, Jeevamrit was observed to be the most effective, resulting in complete mycelial growth inhibition of the pathogen at 4, 6, 8 and 10 per cent concentrations. Similarly, under pot culture conditions in greenhouse, Jeevamrit showed maximum control of seed borne infection (75.60 %) followed by cow urine (68.25%), while artificially inoculated seeds treated with Jeevamrit and cow urine also showed up to 75.34 and 67.13 % disease control, respectively.

Key words: common bean, anthracnose, bio-control agents, bioformulations, Jeevamrit, *Trichoderma*

The common bean (*Phaseolus vulgaris* L.) cultivated as snap bean (green vegetable) and dry bean (pulse) is one of the major grain legume crops with high nutritive value. The crop is cultivated in every part of the world by the farmers for earning their livelihood from agriculture (Sharma *et al*. 2008). Globally it covers an area of about 34.50Mha with 30.43 Mt production (under dry bean) whereas, in India it is grown over 13.54 Mha area with 6.22Mt production (under dry bean) (FAO 2019). Being a Kharif season crop, conditions favouring the plant growth also support various fungal, bacterial and viral pathogens to attack the crop (Sharma *et al*. 1999). Amongst predominantly associated pathogens, *Colletotrichum lindemuthianum* (Sacc. and Magnus) Lams-Scrib. is of regular occurrence in the tropical and sub-tropical areas especially under cool and humid conditions (Sharma *et al*. 1999). Pathogen is known to infect the crop at every stage of the plant growth from seedling to the maturity, depending upon the availability of favorable environmental conditions as required by the pathogen for its growth, spread and disease development. Seed borne nature and cosmopolitan distribution of the pathogen makes its management difficult especially when farmers use their own seed for cultivation year after year. The disease has been reported to infect majority of the widely grown bean cultivars leading up to 100 per cent yield loss in the susceptible ones (Sharma *et al*. 2008) making it one of the major limiting factors for its profitable cultivation. The use of chemicals for the seed treatment has ill effects on the soil health as well as in the environment. Among all the available management practices, highly efficient, simple and economic approach is the use of genetically improved cultivars.

Naturally occurring antagonistic organisms, however, are safer, more eco-friendly and less costly to develop in contrast to fungicides, which are harmful for environment as well as soil health (Amin *et al*. 2014). Further, eco- friendly approaches like use of beneficial microbes and organic inputs provide safer and practicable methods for the management of the plant diseases. Therefore, present investigation was carried out to evaluate the efficacy of different bio-control agents and organic inputs for the management of anthracnose.

Pathogen culture and multiplication of bioagents

Cultures of pathogen and antagonists (*T. harzianum* (TH-11), *T. harzianum* (TH-5), *T. koningii* (DMA-8), *T. koningii* (JMA-11), *T. viride* and *Pseudomonas fluorescens*) were procured from the Department of Plant Pathology, COA, CSK HPKV Palampur. The Pathogen culture was grown and maintained on Mathur's medium for enhanced sporulation at temperature of 22±2°C in walk-in-incubator facility.

Likewise, bio-formulations (Jeewamrit, Bijamrit, Tamerlassi, Vermiwash, Cow urine) were obtained from Department of Organic Agriculture, CSK HPKV, Palampur. These were tested for their efficacy against the pathogen under in *vitro* conditions.

In vitro* evaluation of bio-control agents against *C. lindemuthianum

The fresh cultures of the test pathogen and the bioagent were evaluated on potato dextrose agar medium by using dual culture technique (Huang and Hoes 1976). The per cent mycelial growth inhibition over control was calculated by using the formula given by Vincent (1947).

$$\text{Percent inhibition (I)} = \left(\frac{C-T}{C}\right) \times 100$$

Where C = Growth of mycelium in control and T = Growth of mycelium in treatment

Evaluation of organic formulations and botanicals

Five organic formulations *viz*., Tamerlassi, Jeevamrit, Vermiwash, Bijamrit and Cow urine were procured from Department of Organic Farming, were tested at different concentrations (2%, 4%, 6%, 8% and 10%) by using poisoned food technique (Grover and Moore 1962). The aqueous extract of *Eupatorium* was prepared by soaking 50 g of fine powder in 100 ml of sterilized distilled water (1:2 w/v) overnight in 500 ml conical flask, filtered through a double layer of muslin cloth, twice with Whatman No.1 filter paper to obtain clear filtrate and evaluated by supplementing the medium at similar concentrations as that of bioformulations. The radial growth of the fungus was recorded on the 14^{th} day of incubation till the control plates were fully covered with the mycelium. Mycelial inhibition (%) of the pathogen was calculated by using Vincent's formula given in the above section.

Evaluation under green house conditions

Bio-control agents and organic formulations which were found effective under in *vitro* conditions were further evaluated under pot culture conditions in the greenhouse maintained at 22+1°C. To study the effect test formulations on seed borne infection of *C. lindemuthianum* and effect of seed bio-priming with bio-formulations on anthracnose severity in seedlings developed from artificially inoculated seeds. Among bio-control agents, only *Trichoderma koningii* (DMA-8) was evaluated, whereas, all of the organic formulations (Jeevamrit, Beejamrit, Tamerlassi, Vermiwash, Cow urine and Eupatorium extract) were evaluated using the respective most effective concentration as assessed under in *vitro* conditions. Completely randomized block design was used for statistical analysis.

Naturally infected seeds of *C. lindemuthianum* were dipped for five minutes in the most effective concentrations of each bio-formulation or dressed with the spore mass slurry of the bio-control agent to study the effect of these on seed borne infection. The treated seeds were shade dried for 15 minutes and then sown in the trays having moist sterilized sand. The trays were incubated inside the growth chamber (Bhanu Biotech Pvt Ltd New Delhi) at 22±1ºC and more than 90 per cent humidity was maintained for five days using humidifier. Seed treated with carbendazim (Bavistin) @0.1% were kept as check. In case of seed bio-priming, the apparently healthy seeds of cultivar Jawala were treated with the bio-formulations and bio-control agents first and then shade dried for 15 minutes. After drying, the seeds were dipped in the spore suspension of *C. lindemuthianum* and shade dried for 15-20 minutes. The treated seeds were then sown in the trays filled with sterilized moist sand and placed inside the growth chamber and similar aforesaid conditions were maintained. Similarly, Carbendazim (Bavistin)

(@0.1%) treated seeds were kept as control.

***In vitro* evaluation of bio-control agents and bioformulations**

The observations recorded for the mycelial growth inhibition of *C. lindemuthianum* by various bio-control agents under *in vitro* conditions are presented as figure 1. The antagonistic potential of all the six bio-control agents tested ranged between 35.63 to 53.69 per cent. Among the tested bio-control agents the highest mycelial growth inhibition of 53.69 per cent was observed with *T. koningii* (DMA-8) which was statistically at par with *T. harzianum* (TH-11) with 49.14 per cent, *P. fluorescens* (47.99 %) and *T. viride* (45.45 %). However, *T. harzianum* (TH-5) and *T. koningii* (JMA-11) were less effective with minimum inhibition of 35.63 and 39.43 per cent, respectively.

Our results are in accordance with the earlier findings by Jebessa and Ranamukhaarachchi (2006) who tested three *Trichoderma* isolates (*T. harzianum, T. koningii and T. pseudokoningii*) against *C. gloeosporioides* and found that *T. koningii* was most effective in controlling the growth of the anthracnose pathogen. Similarly, Krishan (2014) reported *T. koningii* (DMA-8) to be most effective as compared to the other *Trichoderma* isolates {*T. koningii* (JMA-11), *T. harzianum* (SMA-5) and *T. harzianum* (JMA-4)} evaluated against *Phytophthora nicotianae var parasitica* showing 51.93% inhibition of mycelial growth of the pathogen.

However, contrary to the present results Padder *et al*. (2010) who evaluated three bioagents (*Trichoderma viride, T. harzianum* and *Gliocladium virens*) against *C. lindemuthianum* under *in vitro* conditions and found highest mycelial growth inhibition with *T. viride* (69.21%) and *T. harzianum* (64.20%). Further, Rajesha *et al*. (2010) and Pandey *et al*. (2019) also found *Trichoderma harzianum* to be the best in inhibiting the mycelial growth of the *C. lindemuthianum* upto the extent of 73.54 & 73.87% respectively. The inconsistency in the results of such studies may be recognized due to variation in the isolates used by various workers as well as environmental conditions and experimental setup. The efficacy of different bioformulations (Jeevamrit, Bijamrit, Tamerlassi, Vermiwash, Cow urine and *Eupatorium* extract) against the pathogen *C. lindemuthianum* under *in vitro* conditions is presented in figure 2. Among all the tested bioformulations, Jeevamrit was observed to be most effective, as it resulted in 100 per cent mycelial growth inhibition at 4 per cent and above concentrations followed by Cow urine and Tamerlassi where complete mycelial inhibition was recorded at 6 per cent and above concentrations. However, *Eupatorium* extract and Bijamrit applied at 10 per cent concentration showed 80.81 per cent and 77.78 per cent mycelial growth inhibition, respectively.

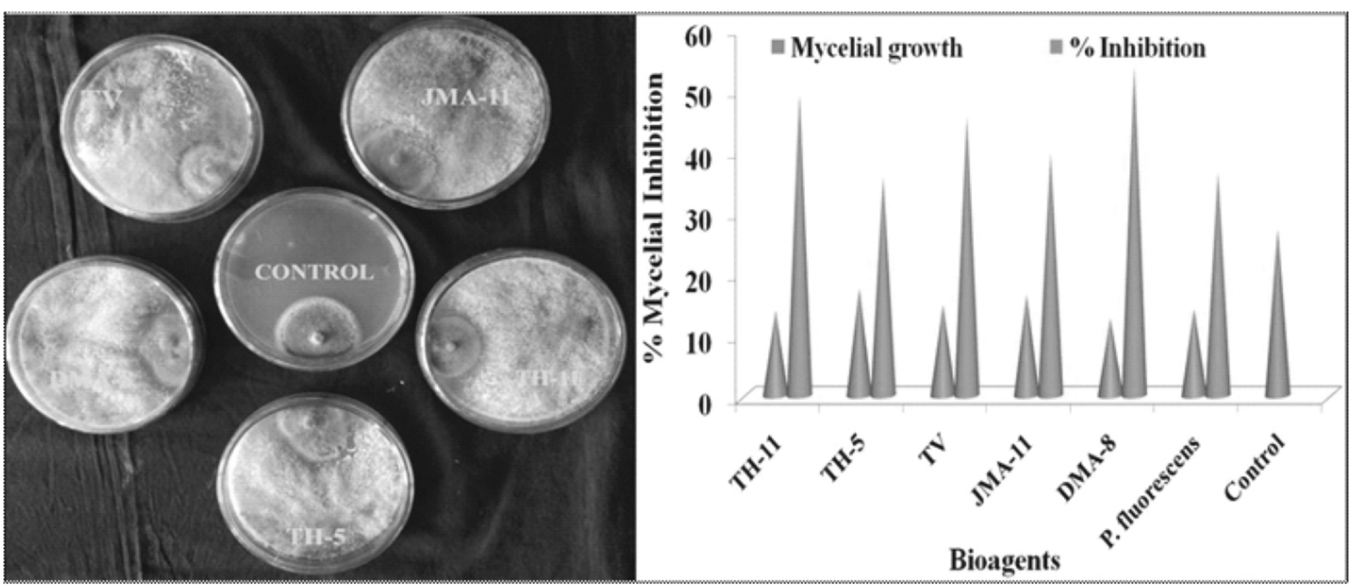

Fig.1 *In vitro* evaluation of bio-control agents against *C. lindemuthianum*

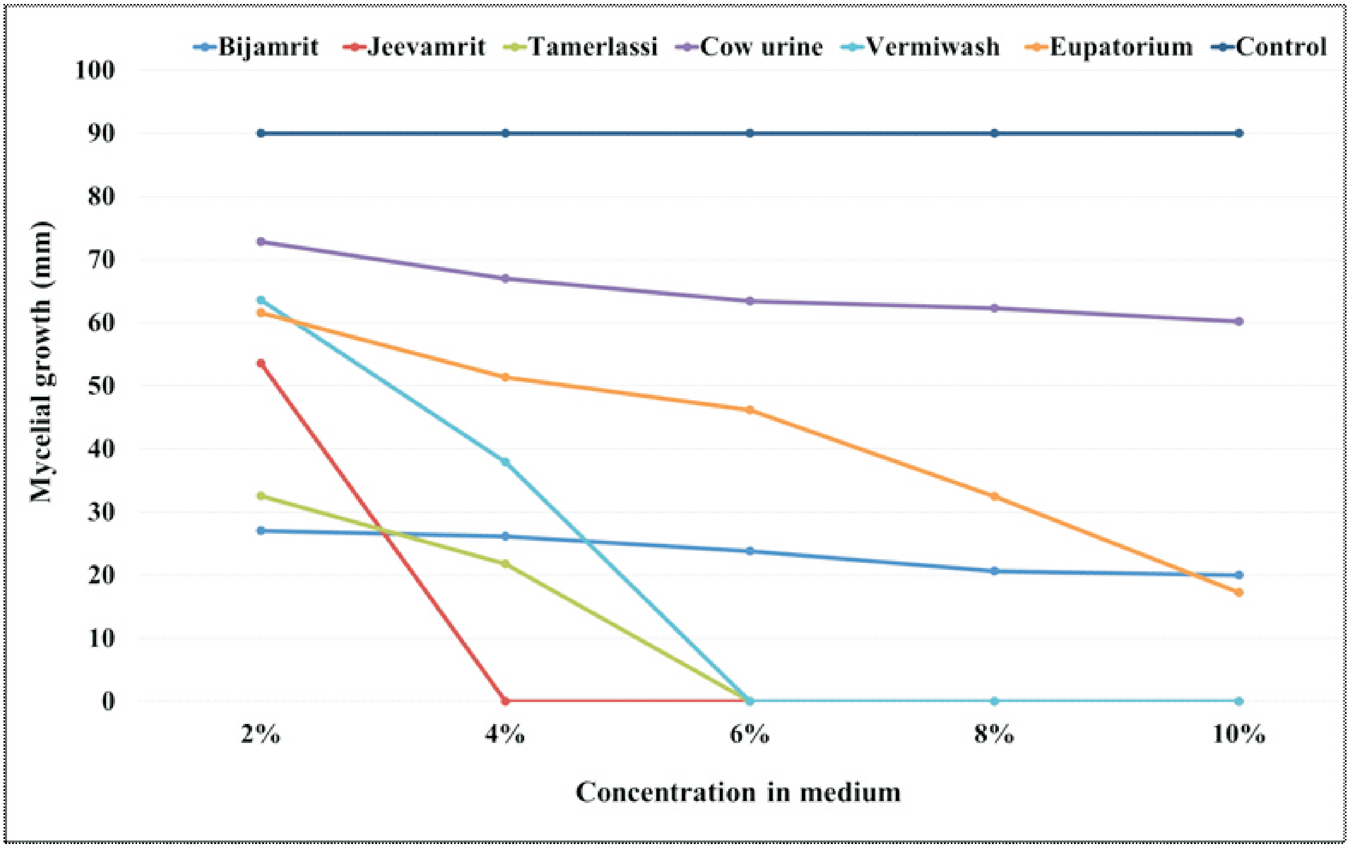

Fig. 2 ***In vitro* assessment of different bioformulations for the management of *C. lindemuthianum***

However, at 2 per cent concentration, Bijamrit revealed 70 per cent inhibition of the pathogen mycelia followed by Cow urine which showed 63.81 per cent inhibition. The authors did not find any reports regarding the effectiveness of different bioformulations against *C. lindemuthianum*, hence compared the present results in the context studies conducted the test bioformulations on other pathogens of different beneficial crops. The present observations support the findings by Pandia *et al*. (2019) who evaluated the effectiveness of Jeevamrit and its various components against *Alternaria alternata* causing leaf spots in mung bean. They observed that Jeevamrit-1, Jeevamrit-2 and Jeevmrit-3 (different solutions of Jeevamrit) resulted in 90.99, 93.34 and 77.23 per cent inhibition of mycelial growth, whereas among the components of Jeevamrit, Cow urine itself was recorded to show 92.23 per cent mycelial growth inhibition at 7.5 per cent concentration.

Present results are in conformity with those of Rana (2013) who studied the efficacy of various organic inputs and botanicals against paddy blast fungus (*Pyricularia oryzae*) and found that fermented buttermilk (Tamerlassi) and Cow urine in 1:1 mixture were most effective, showing 100 per cent inhibition at 10 per cent concentration, whereas at 2, 4, 6 and 8 per cent concentrations resulted in 72.97, 78.15, 81.53 and 83.11 per cent inhibition, respectively.

Our results showed that *Eupatorium aqueous* extract applied at 10% concentration caused 80.81% mycelial growth inhibition, which is in contrary to the report of Rana (2013) where the author recorded complete mycelial inhibition of the rice blast fungus at and above 0.5% concentration of *Eupatorium* extract in the medium.

***In vivo* assessment of bioformulations and bio-control agent**

The bioformulations and bioagent found effective under *in vitro* evaluation were further tested in pot culture conditions under green house conditions using naturally infected and artificially inoculated common bean seeds. The data recorded on seed borne infection and artificial inoculation of *C. lindemuthianum* has been given in Table 1.

In naturally infected seeds treated with bioformulations, overall germination percentage was

Table 1. Effect of bioformulations on anthracnose severity in naturally infected and artificially inoculated seeds under greenhouse conditions

Sr No	Treatment	Naturally infected seeds			Artificially inoculated seeds		
		Germination	Disease severity	% Disease control	Germination	Disease severity	% Disease control
1.	Bijamrit	93.00(75.02)*	88.89(71.14)*	2.44	95.33(77.51)*	72.25(58.33)	19.83
2.	Jeevamrit	96.00(78.69)	22.23(23.21)	75.60	97.67(81.41)	22.22(28.06)	75.34
3.	Tamerlassi	80.00(63.41)	62.22(56.11)	31.71	91.00(72.53)	50.61(45.33)	43.84
4.	Cow Urine	89.00(70.75)	28.89(31.85)	68.29	84.00(66.48)	29.62(32.93)	67.13
5.	Vermiwash	92.00(73.83)	73.33(59.62)	19.51	91.67(73.39)	82.71(65.50)	8.22
6.	Eupatorium	92.67(74.97)	68.89(52.45)	24.39	93.67(75.44)	70.37(57.11)	21.92
7.	Trichoderma (DMA-8)	86.67(68.61)	80.00(63.62)	12.19	83.67(66.29)	72.84(58.62)	19.17
8.	Bavistin (Check)	99.00(85.36)	0.00	100	98.00(82.02)	0.00	100
9.	Control	84.33(66.86)	91.11(72.86)		94.67(76.70)	90.12(74.89)	
	CD (P=0.05)	6.35	19.09		4.11	9.86	

* The figures within the parentheses are arc sign transformed values

in the range of 80 to 96 per cent, it was found to be maximum in seeds treated with Jeevamrit (96%) which was observed to be statistically at par with Bijamrit (93%), *Eupatorium* extract (92.67%) and Vermiwash (92%) whereas minimum germination was observed to be 80 per cent in Tamerlassi treated seeds. Disease severity ranged from 22.23-88.89 per cent in different seed treatments, where it was minimum in the Jeevamrit (22.23%) followed by cow urine (28.89%) and both were observed to be statistically at par with each other. However, maximum disease severity was recorded in seed treatment with Bijamrit (88.89%) followed by *Trichoderma* and Vermiwash showing 80 per cent and 73.33 per cent disease severity, respectively.

Maximum control of seed borne infection was observed in the seed treatment with Jeevamrit which was 75.60 per cent followed by cow urine (68.29%) whereas, 31.17 per cent disease control was noticed in Tamerlassi treated seeds. In the remaining treatments *i.e., Eupatorium*, Vermiwash, *Trichoderma* and Bijamrit, disease control was observed to be 24.39, 19.51, 12.19 and 2.44 per cent, respectively. Similar observations were recorded in artificially inoculated seeds where seed germination was found to vary in the range 83.67-97.67 per cent, maximum being in Jeewamrit (97.67%), statistically at par with Bijamrit (95.33%) whereas, minimum germination of 83.67 per cent was revealed in *Trichoderma* (DMA-8). Reduced disease severity was observed again in Jeevamrit treated seeds (22.22%) with higher per cent of disease control (75.34%) followed by Cow urine with 29.62 per cent disease severity and 67.13 per cent disease control which was statistically at par with Jeevamrit. The treatment of inoculated seeds with Tamerlassi, *Eupatorium* extract, Bijamrit and *Trichoderma* showed 43.84, 21.92, 19.83 and 19.17 per cent control of disease respectively. Bavistin as a check treatment revealed 100 per cent disease control in case of both naturally infected as well as artificially inoculated seed treatments.

The present findings are in accordance with the observations by Pandia *et al*. (2019) who studied *in vivo* efficacy of Jeevamrit and its components against *Alternaria alternata* causing leaf spot in mung bean. They reported lowest disease severity {Percent disease index (PDI) 17.34}and maximum disease control (75.22 per cent) in plants with foliar spray of Jeevamrit-2 solution, which was found significantly superior to all the other treatments followed by Jeevamrit-1 (PDI 22.0 and PDC 68.57) and Jeevamrit-3 (PDI 24.67 and PDC 64.75). Similarly, cow urine was also found to be highly effective in controlling the infection caused by *Alternaria alternata* revealing PDI of 28.34 and PDC of 59.51 at 5% concentration which varied with the increasing concentration (7.5%) depicting PDI of 26.67 and PDC of 61.90. However, the potential bioagent and the bioformulation found effective to minimize the seed borne infection under in *vitro* and *in vivo* conditions needs to be tested under field conditions to validate their efficacy against the target pathogen.

Acknowledgements

The authors are thankful to Head, Department of Plant Pathology for providing necessary facilities and Head, Department of Organic Agriculture, CSK HPKV Palampur for bioformulations.

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

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