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Short Communication

Evaluation of botanicals against colocasia blight caused by *Phytophthora colocasiae* Raciborski

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Abstract

Colocasia leaf blight is the most devastating disease of colocasia incited by oomycetous pathogen *Phytophthora colocasiae* Raciborski. Use of chemicals in the management of this disease may lead to health and ecological hazards thus in the present investigation, the efficacy of botanicals were evaluated both under *in-vitro* and *in-vivo* conditions against colocasia blight. Four different botanicals which included leaf extracts of *Azadirachta indica* (neem), *Melia azedarach* (drek) and botanical formulations of lantana ark and dashparni were evaluated against *P. colocasiae* at four concentrations (5, 10, 12 and 15 per cent). Lantana ark was found to be most effective under *in-vitro* as well as *in-vivo* conditions at 15 per cent concentration resulting in 72.3 per cent mycelial growth inhibition and 65 per cent disease control, respectively followed by *Azadirachta indica*.

Key words: Colocasia, botanicals, colocasia blight, *Phytophthora colocasiae.*

Colocasia (*Colocasia esculenta* (L.) Schott) also commonly known as taro is a tuber crop grown for its edible starchy corms, leaves and petioles. Colocasia is attacked by several diseases like Cladosporium leaf spot (*Cladosporium colocasiae*), Phyllosticta leaf spot (*Phyllosticta colocasiophila*), Pythium rot (*Pythium aphanidermatum*), Fusarium dry rot (*Fusarium solani*), bacterial soft rot (*Erwinia carotovora; E. chrysanthemi*) and dasheen mosaic (Dasheen mosaic virus) but colocasia blight incited by *Phytophthora colocasiae* Raciborski is known to be the most serious disease causing huge yield losses. The pathogen is known to infect all parts of plant *viz*., leaves, petioles, corms and cormels leading to heavy yield losses to the tune of about 60 per cent in severe cases (Maheshwari *et al*. 2007). In Himachal Pradesh, the crop coincides with rainy season thus making the condition even worse. Constant use of chemical fungicide can lead to evolution of more virulent races of the pathogen and development of pesticide residue. Thus, use of botanicals for reducing disease severity can be an alternative which has been investigated in the present investigation.

Isolation of the pathogen

For isolation of *P. colocasiae*, leaves were cut into bits of 2-3 mm size having half portion of diseased and healthy tissue with the help of a sterilized surgical blade. Under laminar air flow these bits were surface sterilized by dipping in 1 per cent sodium hypochlorite solution for 10-15 seconds and were subsequently washed 3-4 times with sterilized distilled water. The bits were then placed between two folds of sterilized blotting sheets to remove excess moisture and transferred to PDA Petri plates under aseptic conditions and incubated at 24±1°C in BOD Incubator. The culture was purified by hyphal tip method and after proving the pathogenicity maintained on PDA medium for further use.

Inoculum preparation

Ten days old culture of the pathogen *P. colocasiae* was used for preparing spore suspension. Mycelial bits of 10 mm diameter were cut and transferred into a Petri plate containing 1 per cent KNO_3 (potassium nitrate) solution using inoculation needle under aseptic conditions in laminar air flow and incubated at 24±1°C for 4 days. The sporangia formed were observed under microscope (10X). The suspension containing at least ten sporangia per microscopic field was used. This suspension was then placed at 10°C for 45-90 minutes to induce zoospore release. After cold treatment plates

were again incubated at 24±1°C for 30 min and checked for zoospore release. When large number of zoospores were found to be released the agar plugs were washed through double layered muslin cloth. The spore concentration was adjusted to 6×10^4 spore per ml with the help of haemocytometer. This concentration was used as standard inoculum for carrying out various experiments.

Pathogenicity test

Pathogenicity test was conducted under net house conditions. Prepared inoculum was sprayed with the help of hand atomizer at 3-5 leaf stage of healthy colocasia plant after which the pots were covered with polyethylene bags. The inoculated plants were sprayed three times a day with sterilized water for three consecutive days to maintain high relative humidity and were observed for symptoms development.

Collection and preparation of leaf extracts and botanical formulations

Aqueous leaf extracts of neem (*Azadirachta indica*), drek (*Melia azedarach*) and botanical formulations of lantana ark and dashparni were evaluated *in-vitro* by poisoned food technique (Grover & Moore, 1962). Leaves of neem and drek were collected and shade dried by spreading them on blotting sheet. After drying, leaves were ground in a blender to obtain fine dry powder and were stored for further use in paper bags at room temperature. Fifty grams of fine powder of each botanical soaked overnight in 100 ml of sterilized distilled water (1:2 w/v) was filtered next day through double layered muslin cloth and the filtrate thus obtained was used as a stock solution. Lantana ark and dashparni were obtained as formulations from the Department of Organic Agriculture and Natural Farming, CSKHPKV, Palampur.

***In-vitro* evaluation of botanicals**

Different botanicals were evaluated against *P. colocasiae* at four different concentrations namely 5, 10, 12 and 15 per cent prepared from stock solution. Double strength PDA was prepared and equal quantity of different double strength concentration of botanical formulation was mixed under aseptic conditions. Medium mixed with equal quantity of sterilized distilled water served as control. Mycelial bits of 5 mm diameter were cut with the help of sterilized cork borer from the margins of 7 days old actively growing culture of *P. colocasiae* and were placed in the center of the plates containing amended medium. The inoculated plates were then incubated at 24±1°C with each treatment replicated four times. Colony diameter of the pathogen in each treatment was measured when control plates were fully covered by the test pathogen. Per cent growth inhibition was calculated by using formula (Vincent, 1947):

$$I = C\text{-}T/C \times 100$$

Where

I= Per cent Growth Inhibition (%)

C= Colony diameter in control (mm)

T= Colony diameter in treatment (mm)

***In-vivo* evaluation of botanicals**

The minimum concentration of leaf extracts and botanical formulations at which maximum per cent growth inhibition was observed *in-vitro* were used for leaf bioassay. Disease free healthy leaves of same age were taken from the plants of Green Stalked variety of colocasia grown under net-house conditions. These were placed on moist blotting sheet kept in plastic Petri plates (150 mm diameter). Each leaf disc was inoculated with 10 µl of spore suspension (6×10^4 spores per ml). After 30 minutes of inoculation, botanicals were sprayed on the inoculated leaves while the leaves sprayed only with pathogen were kept as control. One set of leaves sprayed with only water was also kept as check. The Petri plates were kept moist in order to maintain high relative humidity and incubated at 24±1°C with each treatment repeated four times. The inoculated leaves were observed daily for disease development.

Identification of the pathogen

The pathogen isolated was purified and identified as *P. colocasiae* Raciborski on the basis of morphological characteristics (Waterhouse 1963). Colony produced by *P. colocasiae* was white with cottony growth pattern. Mycelium was hyaline, coenocytic with less than 1 µm diameter. Sporangia were formed terminally on aseptate sporangiophore and were semi-papillate, caducous, ovoid in shape with mean diameter ranging from 77×43.2 µm with short pedicel (3.7-5.9 µm). Pathogenicity was proved on Green Stalked variety of colocasia using pure culture of the pathogen. Characteristic symptoms of the disease were produced after four days of inoculation under net-house conditions.

***In-vitro* efficacy of botanicals**

Data on mycelial growth of *P. colocasiae* were represented in table 1. The perusal of data revealed that all the botanicals under study resulted in significant reduction in the growth of pathogen at all the concentrations tested. Among all the botanicals

tested, Lantana ark proved to be the most effective resulting in the maximum mycelial growth inhibition of 50.3, 56.3, 65.9 and 72.3 per cent at 5, 10, 12 and 15 per cent concentration, respectively. *Azadirachta indica* was the next in order of efficacy with mycelial growth inhibition of 27.4 per cent followed by *Melia azedarach* while dashparni was the least effective (13.6%). It was also apparent from the data that with increase in concentarion the efficacy of the botanicals also increased being maximum at 15 per cent concentarion. Shakywar *et al*. (2012) reported that at 10 per cent concentration, *Azadirachta indica* showed maximum growth inhibition of 50.13 per cent. Similarly, Saykar *et al*. (2018) found sapindus (soapnut rind) best under *in-vitro* conditions with maximum growth inhibition of 94.07 per cent followed by nanma (neem and cassava leaf extract) with 90.4, neemraj (neem leaf extract) with 72. 6 and *Lantana camera* with 51 per cent mycelial growth inhibition. Recently, Khan *et al*. (2019) while working with *P. infestans* concluded that among all the botanicals tested, *Azadirachta indica* showed maximum growth inhibition (59.8%). In the present studies also neem and lantana have shown the inihibitory properties against *P. colocasiae* which is in conformity with available literature.

Leaf bioassay of botanicals for disease management

Different botanicals tested *in-vitro* were evaluated *in-vivo* by using leaf bioassay technique on detached leaves of colocasia at 15 per cent concentration. Lantana ark was found to be superior to others with disease control of 65 per cent followed by *Azadirachta indica, Melia azedarach* and dashparni with 57.8, 43.1, 36.8 per cent disease control, respectively as represented in table 2. Shakywar *et al*. (2014) reported that two foliar spray of *Azadirachta indica* leaf extract (10%) were most effective with disease incidence of 72.18 and 72.73 per cent during 2006 and 2007, respectively as compared to 96.79 and 95.51 per cent in control during two cropping years. Saykar *et al*. (2018) concluded that under *in-vivo* conditions, minimum disease incidence was recorded in plot sprayed with *Sapindus* (33.67%), followed by nanma (37%) and neemraj (39.67%), *Lantana camera* (45.33%) with 64.85 per cent in control. In the present study also, lantana ark and *Azadirachta indica* extract were found most effective in reducing the severity of colocasia blight with correspondingly higher disease control.

Conflicts of interest: The authors declare that there is no conflict of interest involved with this manuscript.

Table 1. In-vitro efficacy of botanicals against *Phytophthora colocasiae*

Botanical	Mycelial growth (mm) Concentration (%)				Mycelial inhibition (%) Concentration (%)			
	5	10	12	15	5	10	12	15
Azadirachta indica	65.3	63.3	53.6	48.8	27.4	29.6	40.4	45.7
Melia azedarach	76.3	68.5	64.9	57.3	15.2	23.8	27.8	36.3
Lantana ark	44.7	39.3	30.6	24.8	50.3	56.3	65.9	72.3
Dashparni	77.7	73.5	72.0	67.7	13.6	18.2	20.0	24.7
Control	90.0	90.0	90.0	90.0	-	-	-	-
CD (P=0.05)	0.31	0.67	0.37	0.49				

Table 2. Efficacy of botanicals for the management of colocasia blight by leaf bioassay

Botanical	Disease Severity (%)	Per cent Disease Control
Azadirachta indica	13.5	57.8
Melia azedarach	18.2	43.1
Lantana ark	11.2	65.0
Dashparni	20.2	36.8
Control	32.0	
CD (P=0.05)	3.02	

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