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Biological Management of Colocasia Blight Incited by *Phytophthora colocasiae* using Native Strains of Antagonists in North Western Himalayas

Divya Bhandhari, Amar Singh, J.V. Patel, D.K. Banyal

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ABSTRACT

Background: Colocasia is cultivated globally for its edible corm and leaves. Leaf blight incited by *Phytophthora colocasiae* is the most destructive disease of colocasia. The current study aims at biological management of the disease.

Methods: Nine *Trichoderma* isolates from the colocasia rhizosphere soil along with five designated isolates of *Trichoderma* spp. already available in the Department of Plant Pathology, CSK HPKV, Palampur were tested *in vitro* for antagonistic activity against *P. colocasiae*. Similarly, six unidentified bacterial strains isolated from colocasia phylloplane and available *Pseudomonas fluorescens* were evaluated for antagonistic activity against *P. colocasiae* under *in vitro* conditions. The bioagents found best under *in vitro* conditions were evaluated *in vivo*.

Result: *Trichoderma* isolate Ti-6 was found significantly superior bioagent as it resulted in 72.9 per cent mycelial growth inhibition of *P. colocasiae* followed by Ti-5 (63.2%), Ti-4 (60.1%) and Ti-1 (54.5%). Amongst bacterial antagonists, *Pseudomonas fluorescens* gave maximum mycelial growth inhibition of 50.5 per cent followed by Pb-3 (31.4%) and Pb-6 (30.5%). The efficacy of five *Trichoderma* spp isolates *viz*., Ti-6, Ti-5, Ti-4, Ti-1, *T. viride* and one bacterial isolate of *P. fluorescens* found effective under *in vitro* were also evaluated *in vivo* using three delivery systems under net house condition. Corm treatment with bioagents was found superior for management of colocasia blight. Corm treatment with Ti-6 was found to be significantly superior to other treatments as 93.74 per cent of disease control was observed. For drenching, bioagent Ti-6 was proved best in managing blight disease (88.91%) followed by Ti-5 (88.90%). However, Ti-5 isolate of *Trichoderma* sp. as soil application was found superior with 90.02 per cent disease control.

Key words: Colocasia, *Phytophthora colocasiae*, Biological control agent.

INTRODUCTION

Colocasia [*Colocasia esculenta* (L.) Schott.] is a nutritional tuber crop widely grown for its corms and leaves are being used. Colocasia is attacked by several pathogens that belong to oomycetes; *Phytophthora colocasiae* and *Pythium aphanidermatum* causing colocasia blight and pythium rot, fungi; *Phyllosticta colocasiophila*, *Cladosporium colocasiae* and *Fusarium solani* causing Phyllosticta leaf spot, Cladosporium leaf spot and Fusarium dry rot, bacterium; *Erwinia carotovora* (soft rot) and virus; Dasheen mosaic virus (Ooka, 1990) however, colocasia blight is known to be the most serious disease resulting in considerable losses in terms of quality and quantity. The disease was first reported from Java in 1900 (Raciborski, 1900) and from India, the disease was reported by Butler and Kulkarni (1913).

The pathogen is known to persist through unfavorable conditions either as sporangia, mycelium, or resting structure like oospore and chlamydospore on crop debris and infected corm (Rana, 2006). Secondary spread of the disease is either directly through sporangia or zoospore carried by water splash (Misra *et al*., 2011). Available methods of disease management such as cultural practices and chemical control measures are often limited, as some are ineffective and hazardous to both human health and environment. In contrast, biological control is a safer management alternative enhancing soil and plant health as well as proving sustainable approach (Chakraborty *et al*. 2016).

Biological management is ecofriendly strategy for the effective control of colocasia blight by reducing primary inoculum and also avoids non target effects of chemical control. Such an ecologically safe method can be used as an alternative approach for colocasia blight management. Thus, in the present study, palliative measures were envisaged by the isolation and evaluation of potential phylloplane and rhizosphere antagonist microorganisms along with the available biological control agent.

Department of Plant Pathology, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur-176 062, Himachal Pradesh, India.

Corresponding Author: Divya Bhandhari, Department of Plant Pathology, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur-176 062, Himachal Pradesh, India.
Email: divyabhandhari112@gmail.com

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MATERIALS AND METHODS

Isolation and identification of pathogen associated with colocasia blight

Colocasia blight samples were collected during cropping season 2019-2020 from various locations of Himachal Pradesh. The samples were sterilized using 1 per cent sodium hypochlorite and inoculated on PDA slants. The slants were incubated at 24±1°C in BOD incubator. The culture was purified by hyphal tip method. Pathogenicity test for the isolated pathogen was conducted on Green Stalked variety of colocasia under net house condition. The identity of the pathogen associated with colocasia blight was established by studying the morpho-cultural traits for the pathogen culture raised on PDA slants by following standard keys (Waterhouse, 1963). Pure culture of the isolate was maintained on PDA medium by periodical sub-culturing and after third sub-culture each isolate was inoculated on healthy host and then re-isolated to avoid loss of virulence.

Isolation of antagonists from rhizosphere soil

Antagonist microorganisms were isolated from the representative soil samples collected during survey from rhizosphere of healthy colocasia plant by serial dilution plate technique (Khang *et al*., 2013). In this method, 10 gm soil was transferred aseptically into conical flask (250 ml) containing 100 ml of sterilized distilled water and mixed thoroughly by shaking for 5 minutes. 10 ml of aliquot was drawn and transferred to 90 ml of sterile distilled water. The suspension was shaken for one minute before it was further diluted till 10^{-4} to 10^{-6} were obtained and used for isolation of microorganisms. 20 ml of sterilized molten (40°C) PDA was poured in Petri plates and allowed to solidify. After solidification of the medium, 1 ml of suspension from respective dilutions were transferred aseptically into Petri plates containing PDA medium and spread over uniformly with the help of a plastic spreader on the medium and incubated at 24±1°C for development of colonies. Four replications were maintained for each dilution. Colonies with characteristics growth of *Trichoderma* were observed under stereo-microscope (Kubicek and Harman, 1998) and mycelial growth from such colonies was sub-cultured on agar slants. The fungal isolates were further purified by hyphal tip method and maintained on *Trichoderma* Specific Medium (TSM) for further studies.

Isolation of antagonists from phylloplane

A survey was conducted during July-August and fresh leaves from healthy plants were randomly collected and brought to the laboratory in a polythene bag. Microflora was isolated by modified leaf washing technique (Chandrakala *et al*., 2018). Fifty leaf discs were cut from fresh leaves with the help of 5 mm sterile cork borer and transferred to conical flask (250 ml) containing 100 ml sterile water. The leaves were then agitated thoroughly for 20 minutes using orbital shaker. Solution obtained was filtered through Whatman filter paper and serial diluted and dilutions (10^{-3} and 10^{-4}) were plated on Petri plates containing PDA medium and incubated at 24±1°C. Four replications were maintained for each dilution. Petri plates were observed daily for any growth and were transferred to PDA slants, purified and maintained on Nutrient agar (NA) for further use.

In-vitro screening of antagonists

Evaluation of antagonists from rhizosphere soil

Trichoderma isolates obtained from rhizosphere along with the available five designated *Trichoderma* spp. were evaluated against *P. colocasiae* by dual culture technique (Ambuse and Bhale, 2015). Sterilized PDA (40°C) was poured into 90 mm diameter sterilized Petri plates (20 ml each) under aseptic condition and allowed to solidify. Mycelial disc of 5 mm diameter was cut from actively growing culture of the test pathogen and *Trichoderma* spp. was inoculated at 6 cm apart. Three replications were maintained for each treatment and were incubated at 24±1°C for 7 days. Monoculture plates of test pathogen served as control. Seven days after incubation, mycelial growth of *P. colocasiae* in dual culture plate was measured and compared with control. Per cent growth inhibition was also calculated by using formula:

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

Where

I= Per cent growth inhibition (%).

C= Mycelial growth in control (mm).

T= Mycelial growth in treated plates (mm).

Evaluation of antagonists from phylloplane

Phylloplane bacteria *viz*., Pb-1, Pb-2, Pb-3, Pb-4, Pb-5, Pb-6 and *Pseudomonas fluorescens* maintained on Nutrient Agar (NA) was streaked on four sides of *P. colocasiae* culture disc placed at the centre of the Petri plate on PDA media. Three replications were maintained for each antagonist. Plates were then incubated in an incubator at 24±1°C in inverted position. Seven days after incubation, mycelial growth of the pathogen in dual culture plate was measured and compared with control. Per cent growth inhibition was also calculated as per formula mentioned in above section.

In-vivo evaluation of potential bioagents

Antagonists which were found most effective *in-vitro* were evaluated *in-vivo* as corm treatment, soil application and drenching. Prior to application of any treatment, corms and soil were treated with standard inoculum of the test pathogen. For treatment with phylloplane bacteria 10^9 cfu/ml bacterial suspension was used and in case of *Trichoderma*, spore suspension was prepared from 14 days old culture broth which was then homogenized. After homogenizing solution was filtered and diluted 2.5 times to adjust it to 2-5 $\times$ 10^6 cfu/ml concentration. For corm treatment, corms were treated with bioagent suspension for 30 minutes in case of bacteria and for 1 hr in case of *Trichoderma*. Treated corms were then shade dried and sown in pots. For soil application, potential bacterial antagonists and *Trichoderma* were mixed

uniformly with sterilized soil used for pot filling. In case of drenching, prepared suspension of each bioagent was drenched after sowing at the rate of 100 ml per pot. Low temperature and high humidity was maintained by covering them with polythene bags. Each treatment was replicated thrice. Data was recorded on disease severity by using disease rating scale (0-6) given by Little and Hills (1978) and per cent disease control was also calculated by using formula:

$$\text{Per cent disease control} = \text{C-T/C} \times 100$$

Where,

C = Per cent disease severity in control.

T= Per cent disease severity in treatment.

RESULTS AND DISCUSSION

Isolation and Identification of the test pathogen

Pathogen was established as pure culture and identified as *Phytophthora colocasiae* Raci. on the basis of morphological characteristics. 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 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 with pure culture of the isolate and maintained for further studies. Symptoms of the disease began as small light brown water-soaked lesions which enlarged rapidly to form large dark brown lesions. Characteristic symptoms of the disease were produced four days of inoculation, under net house conditions.

Isolation of antagonistic microorganisms

Antagonistic microorganisms were isolated from colocasia phylloplane by using modified leaf washing technique and rhizospheric soil samples by using serial dilution technique on potato dextrose agar medium (PDA) from different locations. The account of fifteen isolates comprising 9 Trichoderma isolates obtained from colocasia rhizospheric soil and 6 phylloplane bacteria is given in Tables 1 and 2. These isolates along with five designated biocontrol agents *viz*., *Trichoderma koningii* (DMA-8), *T. harzianum* (SMA-5), *T. koningii* (JMA-11), *T. viride, T. harzianum* (TH-11) and *Pseudomonas fluorescens* obtained from the Department of Plant Pathology were evaluated for their antagonistic potential against *P. colocasiae*.

Carnot *et al*. (2017) isolated fourteen antagonistic microorganisms from phylloplane and rhizosphere of colocasia identified as *Penicillium* sp, *Trichoderma* sp, *Aspergillus* sp, *Pythium* sp and bacterial isolates identified were *Bacillus* sp, *Rhizobium*, *Streptomyces* and other 7 unidentified isolates.

In-vitro screening of antagonists

In-vitro analysis of antagonistic activity of different isolates *viz., Trichoderma* spp. isolated from rhizosphere of colocasia plant (Ti-1, Ti-2, Ti-3, Ti-4, Ti-5, Ti-6, Ti -7, Ti-8, Ti-9) along with those from Department of Plant Pathology (*Trichoderma koningii* (DMA-8), *T. harzianum* (SMA-5), *T. koningii* (JMA-11)), *T. viride* and *T. harzianum* (TH-11)) were evaluated against *P. colocasiae* by dual culture method and results on mycelial growth inhibition as presented in Table 3 and graphically represented in Fig 2a.

All the isolates were found to inhibit the growth of *P. colocasiae* Fig 1a. However, maximum per cent mycelial growth inhibition was shown by Ti-6 (72.9%) followed by Ti-5 (63.2%), Ti-4 (60.1%), Ti-1 (54.5%) and Ti-8 (50.6%). Ti-3 showed minimum inhibition of 19.6 per cent. The *Trichoderma* spp. obtained from the Department of Plant Pathology showed less mycelial growth inhibition than those isolated from *colocasia rhizosphere* Fig 1c. Amongst *Trichoderma* spp. obtained from department maximum per cent mycelial inhibition was shown by *T. harzianum* (SMA-5) with 49.6 per cent inhibition which is less than Ti-6, Ti-5, Ti-4, Ti-1 and Ti-8. Singh and Islam (2010) concluded that *T. harzianum* (0034H) showed highest inhibitory effect *in-vitro* against *P. nicotianae* with per cent growth inhibition (61%) while *T. viride* (0034S) showed minimum per cent mycelial growth inhibition of 32 per cent. Later, Ambuse and Bhale (2015) studied efficacy of *T. viride*, *T. koningii*, *T. harzianum*, *T. virens* and *T. pseudokoningii* and found *T. viride* and *T. harzianum* were most effective. Recently, Moise *et al*. (2018) reported that 34.77 per cent and 41.77 per cent inhibition of mycelia growth of *P. colocasiae* with *T. harzianum* (Edtm) and *T. aureoviridae* (T4), respectively.

The mode of action of *Trichoderma* spp. was observed as mycoparasitism in which hyphae of *Trichoderma* spp. coils around and interacts with the *P. colocasiae*, eventually leading to lysis or degradation of pathogen mycelium Fig 1b. In 2016, Jiang *et al*. found that *T. asperellum* surrounded

Table 1: *Trichoderma* spp. isolated from colocasia rhizosphere.

Isolate	District	Location
Trichoderma isolate-1 (Ti-1)	Kangra	Palampur
Trichoderma isolate-2 (Ti-2)	Kangra	Bawarna
Trichoderma isolate-3 (Ti-3)	Kangra	Upper Bhattu
Trichoderma isolate-4 (Ti-4)	Kangra	Upper Bhattu
Trichoderma isolate-5 (Ti-5)	Kangra	Machkher
Trichoderma isolate-6 (Ti-6)	Shimla	Thiog
Trichoderma isolate-7 (Ti-7)	Kangra	Madhar
Trichoderma isolate-8 (Ti-8)	Kangra	Madhar
Trichoderma isolate-9 (Ti-9)	Sirmour	Maryog

Table 2: Bacteria isolated from colocasia phylloplane.

Isolate	District	Location
Phylloplane bacteria-1 (Pb-1)	Kangra	Palampur
Phylloplane bacteria-2 (Pb-2)	Kangra	Palampur
Phylloplane bacteria-3 (Pb-3)	Kangra	Palampur
Phylloplane bacteria-4 (Pb-4)	Kangra	Palampur
Phylloplane bacteria-5 (Pb-5)	Mandi	Bharol
Phylloplane bacteria-6 (Pb-6)	Kullu	Ramshila

and penetrated pathogen hyphae thus, resulting in the collapse of colony morphology of *P. capsici* by breaking its hyphae into fragments.

The antagonistic activity of isolates obtained from phylloplane of *P. colocasiae* was evaluated along with *Pseudomonas fluorescens* obtained from the Department of Plant pathology *viz*., Pb-1, Pb-2, Pb-3, Pb-4, Pb-5, Pb-6 and *P. fluorescens* by dual culture method against *P. colocasiae* Fig 1d. All isolates showed mycelial growth inhibition with inhibition ranging from 22.5 to 50.5 per cent (Table 4) and graphically represented in Fig 2b. Data revealed that *P. fluorescens* showed maximum per cent

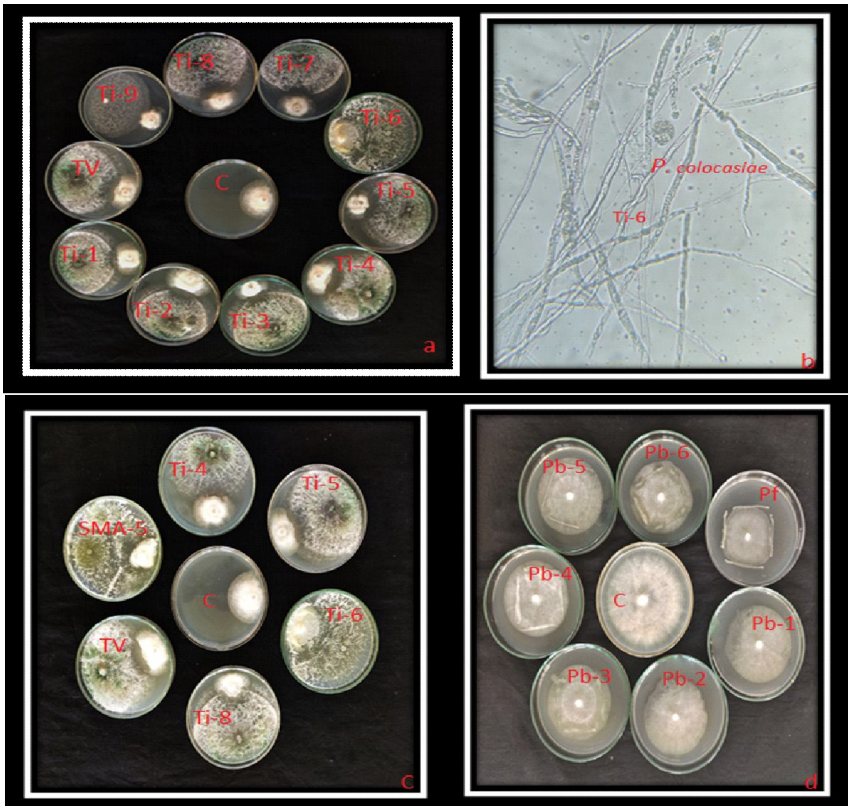


Fig 1: Antagonistic activity of bioagent against *Phytophthora colocasiae* a) *Trichoderma* spp. b) Mycoparasitic interaction (coiling) between *Trichoderma* spp. and *Phytophthora colocasiae* at 40X c) *Trichoderma* isolates comparison with the *Trichoderma* spp. from the Department of Plant Pathology d) Phylloplane bacteria and *Pseudomonas fluorescens*.

Table 3: *In-vitro* evaluation of *Trichoderma* spp. against *Phytophthora colocasiae* for antagonism.

Bioagent isolates	*Phytophthora colocasiae* mycelium growth (mm)	Per cent inhibition (%)
T. harzianum (TH-11)	33.3	21.2
T. koningii (JMA-11)	32.5	23.1
T. koningii (DMA-8)	33.3	21.2
T. viride	30.6	27.6
T. harzianum (SMA-5)	19.2	49.6
Trichoderma isolate-1 (Ti-1)	31	54.5
Trichoderma isolate-2 (Ti-2)	34	26.7
Trichoderma isolate-3 (Ti-3)	16.8	19.6
Trichoderma isolate-4 (Ti-4)	15.5	60.1
Trichoderma isolate-5 (Ti-5)	11.4	63.2
Trichoderma isolate-6 (Ti-6)	32.3	72.9
Trichoderma isolate-7 (Ti-7)	20.9	23.6
Trichoderma isolate-8 (Ti-8)	31.7	50.6
Trichoderma isolate-9 (Ti-9)	30	25.1
Control	42.3	0
CD (p=0.05)	1.89	

mycelial growth inhibition with 50.5 per cent followed by Pb-3(31.4%), Pb-6 (30.5%), Pb-4 (29.7%) and Pb-5 (27.6%). Minimum per cent mycelial growth inhibition was recorded in Pb-2 (22.5%).

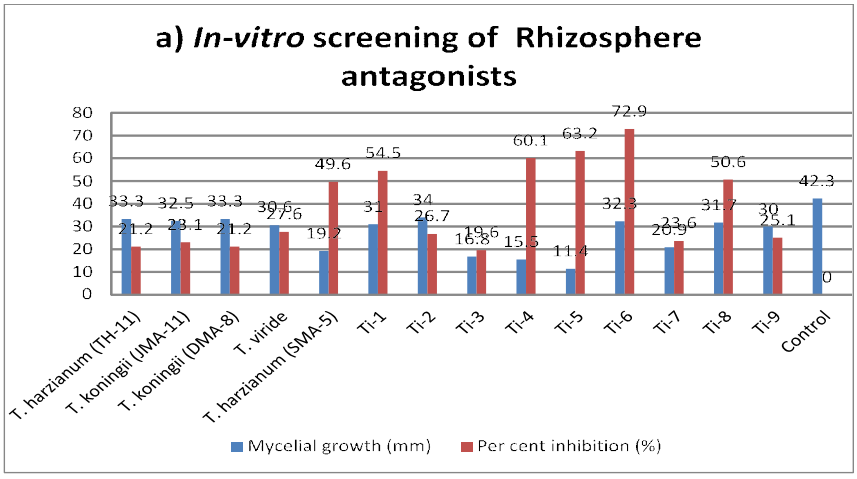
Zegeye *et al*. (2011) studied antagonistic activity of *T. viride* and *P. fluorescens* against *P. infestans* and concluded that *in-vitro* 36.7 per cent growth inhibition and complete overgrowth of *T. viride* later whereas *P. fluorescens* inhibited growth of *P. infestans* by 88 per cent. Padmaja *et al*. (2015) concluded that Phylloplane bacteria 1 showed maximum growth inhibition of 72.7 per cent followed by Phylloplane bacteria 3 (67.8%), Phylloplane bacteria 4 (64.1%) and Phylloplane bacteria 2 (61.6%).

In-vivo evaluation of potential bioagent

The bioagents which were found best under *in-vitro viz*., Ti-1, Ti-4, Ti-5, Ti-6, *T. viride* and *P. fluorescens* were further evaluated under net house in pot culture conditions. The efficacy of bioagents applied as corm treatment, soil drenching and soil application is given in Table 5. Corm treatment with Ti-6 was found superior with 93.74 per cent disease control followed by Ti-4 (74.99%), *P. fluorescens*

Table 4: *In-vitro* evaluation of bacterial isolates against *Phytophthora colocasiae* for antagonism.

Phylloplane antagonists	*Phytophthora colocasiae* mycelium growth (mm)	Per cent inhibition (%)
Pseudomonas flourescens	44.5	50.5
Phylloplane Bacteria-1 (Pb-1)	67.7	24.7
Phylloplane Bacteria- 2 (Pb-2)	69.7	22.5
Phylloplane Bacteria- 3 (Pb-3)	61.7	31.4
Phylloplane Bacteria- 4 (Pb-4)	63.2	29.7
Phylloplane Bacteria- 5 (Pb-5)	65.1	27.6
Phylloplane Bacteria- 6 (Pb-6)	62.5	30.5
Control	90.00	0
CD (p=0.05)	0.463	



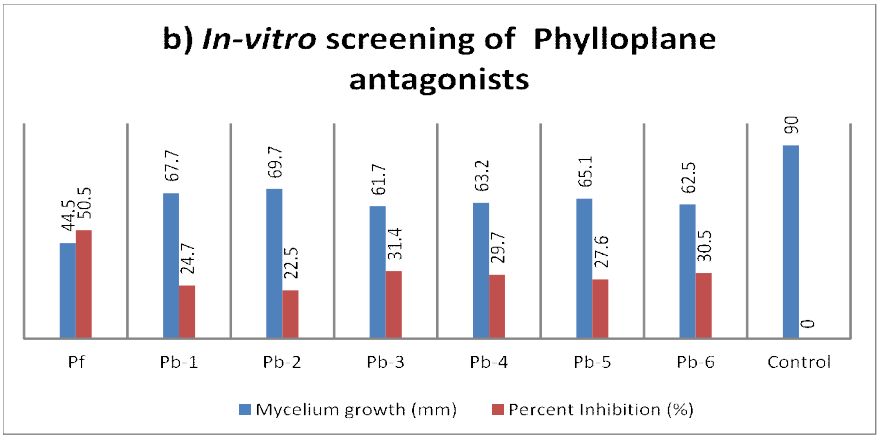


Fig 2: *In-vitro* screening of antagonists against *Phytophthora colocasiae* a) *In-vitro* screening of Rhizosphere antagonists b) *In-vitro* screening of Phylloplane antagonists.

Table 5: *In-vivo* efficacy of potential bioagents for the management of colocasia blight.

Bioagents	Disease severity (%)			Per cent disease control		
	Corm treatment	Drenching	Soil application	Corm treatment	Drenching	Soil application
Trichoderma isolate-1	61.12	11.08	38.88	31.23	77.82	29.99
Trichoderma isolate-4	22.23	27.76	22.23	74.99	44.47	59.98
Trichoderma isolate-5	27.78	5.547	5.54	68.74	88.90	90.02
Trichoderma isolate-6	5.557	5.543	22.23	93.74	88.91	59.98
Pseudomonas fluorescens	22.23	22.23	11.08	74.99	55.54	80.04
Trichoderma viride	38.88	22.26	27.78	56.25	55.47	49.97
Control	88.89	50.00	55.55			
CD(p=0.05)	0.04	0.671	0.058			

(74.99%), Ti-5 (68.74%), *Trichoderma viride* (56.25%) whereas Ti-1 was least effective with 31.23% control. Ti-6 and Ti-5 were found most effective for soil drenching, 88.91 and 88.90 per cent disease control, respectively, and Ti-4 (44.47%) was found least effective. For soil application, Ti-5 was superior with disease control of 90.02 per cent followed by *P. fluorescens* (80.04%), Ti-6 (59.98%) and Ti-4 (59.98%). Ti-1 (29.99%) and *T. viride* (49.97%) were found least effective for soil application.

Narula and Mehrotra (1987) screened phylloplane microorganisms *in–vivo* against *P. colocasiae* and found *Streptomyces albidoflavus* reduced infection by 90-93 per cent and *Streptomyces diasticus* by 76 per cent. Among fungi, *Botrytis cinerea* gave best disease control (33%). Sriram and Misra (2007) reported that under polyhouse condition, when applied as seed tuber treatment, rhizobacterial culture S1B3, S11B4, S13B5 and S23B5 showed no disease incidence as compared to control where disease severity was 2.92 on 0-5 rating scale. In soil application, disease incidence was nil as compared to control where disease severity was 2.83 when rhizobacterial culture S4B5, S13B5 and S23B5 were used. Similarly, foliar application with S1B4 and S11B3 reduced disease severity to 0-0.33 compared to the control of 2.66 per cent disease severity. Similarly, Carnot *et al*. (2017) concluded high inhibitory effect of *Trichoderma* spp. and *Rhizobium* under greenhouse conditions among fourteen antagonistic microorganisms isolated from phylloplane and rhizosphere of colocasia.

CONCLUSION

The rising population has led to an increase in food demand. So, an effective disease management strategy is of immense importance. Colocasia blight is a devastating disease causing substantial crop losses. Keeping in view problems associated with the use of chemical pesticides (like residue and resistance development) the current study was planned for biological management using native antagonists, which is an environment friendly as well as sustainable approach. Available bioagents were evaluated with the native antagonist biogents isolated from phylloplane and rhizosphere of colocasia. It was found that some of the native bioagents were more effective than available bioagents both under *in-vitro* and *in-vivo* conditions. Delivery method found best for management was corm treatment.

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