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Original Research Article

Biological Control of Rots of Betelvine (*Piper betle* L.) by Using *Trichoderma* spp. Grown on Organic Amendments

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Abstract

A field trial was conducted at plant Virus Research Farm, Kalyani, Nadia using biocontrol agents to control foot rot and leaf rot of betelvine (*Piper betle* L.) caused by *Phytophthora* sp. cv. Ghanagette (Bidhan Pan-1). Several isolates of *Trichoderm*a spp. were isolated and through *in vitro* screening, T_7 (*Trichoderma viride*) was found as superior isolate and it was applied in field after multiplying in several organic substrates viz. wheat grain, rice husk, wheat husk, saw dust, cow dung manure, mustard oil cake. Bordeaux mixture was used to compare the treatments in preventing the intensity of rots. The results revealed that treatment T_9 (Application Bordeaux Mixture at pre-monsoon, monsoon and post–monsoon) recorded lowest disease incidence which was statistically at par with the treatments T_1 (Application of mass multiplied *Trichoderma* sp. inoculated wheat grain at pre-monsoon, monsoon and post–monsoon), T_4 (Application of mass multiplied *Trichoderma* sp. inoculated cow dung manure at pre-monsoon, monsoon and post–monsoon),T_5 (Application of mass multiplied *Trichoderma* sp. inoculated mustard oil cake at pre-monsoon, monsoon and post–monsoon) and T_7 (Application of mass multiplied bio-formulated product of *T. harzianum* (Kesana) pre- monsoon, monsoon and post–monsoon) in case of foot rot and T_1,T_2 (Application of mass multiplied *Trichoderma* sp. inoculated rice husk at pre-monsoon, monsoon and post–monsoon),T_7 in case of leaf rot. Treatment T_9 recorded highest leaf yield which was statistically at par with T_1 and also increased fresh weight of leaves; whereas, treatment T_4 was found highly remunerative. Although bio control approach was not superior to chemical control, yet, it was at par in all aspects. Therefore, use of biological control agents is recommended to control foot and leaf rot of betelvine looking at the long term prospects and to avoid the possibility of health hazards due to consumption of toxic betel leaf.

Keywords

Biocontrol

Bioformulation

Phytophthora spp.

Piper betle

Trichoderma spp.

Introduction

Betelvine suffers from many root and aerial diseases. Among them, *Phytophthora* spp. perhaps ranks first in its destructiveness under both field and storage conditions. The extent of losses may vary from 30-100% in case of foot rot and 20-40% in case of leaf rot leading to almost total crop failure (Dasgupta et al., 2000).

The disease development on the stem, vine, leaf, and root render less economic value. These features make management mandatory. A tiny amount of pesticide carried by the leaf would be hazardous to human health due to the residual toxicity of the pesticide as they are non-biodegradable. There is no doubt that biological control of plant disease is gaining importance in the light of hazard caused by chemicals. For massive application of antagonist (biological agent), it is needed to biomass production. Some common waste materials act very well as the substrate of mass multiplication of *Trichoderma* spp. The organic substrates help *Trichoderma* spp. for mycelial growth and sporulation. Various substrates viz. mustard oil cake, wheat bran, cow dung, poultry manure, farm yard manure (FYM), gobar gas slurry, press mud are used for the mass production of antagonist in laboratory condition and that substrates induce the sporulation.

Elad et al., (1984) used wheat bran, saw dust and water at a ratio 3:1:3 for mass multiplication of *T. harzianum*. Upadhyay and Mukhopadhyay (1986) used sorghum grain for mass multiplication of *Trichoderma* (Panicker and Jayarajan, 1993). Prakash et al. (1999) showed tea waste was the best media for biomass production of *Trichoderma* spp. Dutta and Das (1999) screened that FYM and banana peeled skin (BPS) were found to be the best substrate. Coffee, cherry husk, fruit skin, berry mucilage, poultry manure and mushroom grown waste having the population 20-30 million c.f.u / g used for large scale fungal multiplication (Sawant et al., 1995). Liquid culture media such as molasses, brewer's yeast are used to produce viable inoculums of antagonist in a deep tank fermentation system for large scale industrial production (Papavizas et al., 1984). The production of mass amount of culture of antagonist facilitates the large scale application of bioagent in the field.

The present investigation was under taken for biocontrol of rots of betelvine (*Piper betle* L.) using *Trichoderma* spp. grown on different organic amendments.

Materials and methods

The field trial was conducted for the management of rots of betelvine caused by *Phytophthora parasitica* using biocontrol agent *Trichoderma* spp. grown on organic amendments. The "Boroj" or "Bareja" was taken having 10m length and 8m width was selected at Gene Bank or Plant Virus Research Farm, Kalyani, Nadia. The variety used was Ghanagette (Bidhan Pan-I), a moderately susceptible host variety.

Several organic substrate viz. wheat grain, rice husk, wheat husk, saw dust, cow dung manure, mustard oil cake were used for inoculation and multiplication of bioagent 3 days old disc of *Trichoderma* sp. (T_7) was inoculated on that organic substrate and incubated at 28°±1°C for 28 days. The isolate of *Trichoderma* sp. (T_7) was selected on the basis of the superior antagonistic effect of it (T_7) in dual plate culture against *Phytophthora* sp. (Bell, et al., 1982). After having the growth that substrates were added in moistened Mustard oil cake (MOC) and covered with polyethylene bag for 7 – 10 days. Before starting the experiment all the rotten leaves and the dead plants were removed. Five hundred grams of each mass multiplied substrate was applied in every line as per treatment. Each treatment had 3 replicas. The application started in June and continued till the end of monsoon.

The trial was formulated as follows:

T_1 - Application of mass multiplied *Trichoderma* sp. inoculated wheat grain at pre-monsoon, monsoon and post–monsoon.

T_2 - Application of mass multiplied *Trichoderma* sp. inoculated rice husk at pre-monsoon, monsoon and post–monsoon.

T_3 - Application of mass multiplied *Trichoderma* sp. inoculated wheat husk at pre-monsoon, monsoon and post–monsoon.

T_4 - Application of mass multiplied *Trichoderma* sp. inoculated cow dung manure at pre-monsoon, monsoon and post–monsoon.

T_5 - Application of mass multiplied *Trichoderma* sp. inoculated mustard oil cake at pre-monsoon, monsoon and post–monsoon.

T_6 - Application of mass multiplied *Trichoderma* sp. inoculated saw dust at pre-monsoon, monsoon and post–monsoon.

T_7 - Application of mass multiplied bio-formulated product of *T. harzianum* (Kesana) at pre-monsoon, monsoon and post-monsoon.

T_8 - Application of mass multiplied bio-formulated product of *Pseudomonas fluorescens* (Biomonas) at pre-monsoon, monsoon and post–monsoon.
T_9 - Application Bordeaux mixture at pre-monsoon, monsoon and post–monsoon.
T_{10} - Control treatment.

The following data's were recorded 30 days after last treatment: Leaf yield (lakh/ha); Fresh wt. of 100 leaves (g); PDI (foot rot and leaf rot); Cost benefit ratio.

Statistical analysis

Randomized Block Design (RBD) Analysis.

Percent Disease Incidence (PDI) was calculated with the formula of Vernell and Hecloud (1975).

$$PDI = \frac{\text{Number of infected leaf/stem}}{\text{Total number of leaves/stem}} \times 100$$

Results and discussion

PDI of foot rot

The result (Table 1) revealed that, in year 2010, the highest disease incidence (41.15%) was found in treatment T_{10} (control) which was statically at par with treatment T_2, T_3, T_6 and T_8 at 5% level of significances and T_2, T_3, T_6 and T_8 at 1% level of significances. The lowest disease incidence (21.14%) was observed in treatment T_9 (Application of Bordeaux Mixture at pre-monsoon, monsoon and post-monsoon) which was statistically at par with T_1(*Trichoderma* sp. inoculated wheat grain at pre-monsoon, monsoon, post monsoon),T_4(*Trichoderma* sp. inoculated cow dung manure at pre-monsoon, monsoon, post-monsoon),T_5(*Trichoderma* sp. inoculated mustard oil cake at pre-monsoon, monsoon, post-monsoon) andT_7 [Bio-formulated product (Kesana) at pre- monsoon, monsoon and post–monsoon] at both 5% and 1% level of significances. Among the treatments, following hierarchy was observed: $T_{10} \geq T_2, T_3, T_6, T_8 \geq T_1, T_4, T_5, T_7 \geq T_9$.

Table 1. Biological control by using *Trichoderma* spp. grown on organic amendments.

Treatment	Percent Disease Incidence (PDI)		Leaf yield in lakh / ha / year	Fresh weight of 100 leaves in g	Benefit : cost ratio
	Foot rot	Leaf rot			
T_1 (*Trichoderma* sp. inoculated wheat grain at pre-monsoon, monsoon, post monsoon)	20.00 (26.07)	22.67 (28.25)	36.00	373.33	1 : 2.53
T_2 (*Trichoderma* sp. inoculated rice husk at pre-monsoon, monsoon, post monsoon)	30.00 (33.00)	25.33 (29.99)	33.30	319.33	1 : 2.62
T_3 (*Trichoderma* sp. inoculated wheat husk at pre-monsoon ,monsoon, post monsoon)	33.33 (35.22)	29.67 (32.89)	33.97	318.66	1 : 2.68
T_4 (*Trichoderma* sp. inoculated cow dung manure at pre-monsoon ,monsoon, post monsoon)	26.67 (30.29)	26.00 (30.56)	34.15	329.33	1 : 5.10
T_5 (*Trichoderma* sp. inoculated mustard oil cake at pre-monsoon ,monsoon, post monsoon)	26.67 (31.00)	26.00 (30.56)	34.95	356.00	1 : 1.79
T_6 (*Trichoderma* sp. inoculated saw dust at pre-monsoon ,monsoon, post monsoon)	33.33 (34.93)	32.00 (34.42)	32.00	324.00	1 : 2.99
T_7 (Bio-formulated product (Kesana) at pre-monsoon, monsoon and post–monsoon.)	23.33 (28.28)	20.00 (26.07)	32.85	330.00	1 : 1.24
T_8 (Bio-formulated product (Biomonas) at pre-monsoon ,monsoon, post monsoon)	30.00 (33.00)	26.00 (30.58)	35.05	321.33	1 : 3.25
T_9 (Bordeaux Mixture at pre-monsoon, monsoon and post–monsoon)	13.33 (21.14)	13.33 (21.14)	36.45	392.66	1 : 1.71
T_{10} (Control)	43.33 (41.15)	39.67 (39.02)	30.25	235.00	1 : 1.00
SEM (±)	5.30	3.86	0.71	4.34	0.44
CD at 5%	11.13	8.11	1.49	9.12	0.92
CD at 1%	13.52	9.85	1.81	11.07	1.12

*Average of 3 replications; Figures in parentheses are the angular transformed values of percent disease incidence.

PDI of leaf rot

The result (Table 1) showed that highest disease incidence (39.02%) was found in the control treatment (T_{10}) which was statistically at par with T_3 (Application of *Trichoderma* sp. inoculated in Wheat husk at pre-monsoon, monsoon and post-monsoon) and T_6 (Application of *Trichoderma* sp. inoculated in Saw dust at pre-monsoon, monsoon and post-monsoon) at 5% level of significances and with T_3, T_4, T_5, T_6 and T_8 at 1% level of significances. On the other hand lowest disease incidence (21.14%) was observed in treatment T_9 (Application of Bordeaux Mixture at pre-monsoon, monsoon and post-monsoon) and it was statistically at par with the treatment T_1 (*Trichoderma* sp. inoculated wheat grain at pre-monsoon, monsoon, post- monsoon), T_2 (*Trichoderma* sp. inoculated rice husk at pre-monsoon, monsoon, post-monsoon) and T_7 [Bio-formulated product (Kesana) at pre- monsoon, monsoon and post–monsoon] at both 5% and 1% level of significances. Among the treatments, following hierarchy was observed: $T_{10} \geq T_3$, $T_6 > T_4$, T_5, $T_8 \geq T_1$, T_2, $T_7 \geq T_9$.

Leaf yield

The analysis of leaf yield parameters showed (Table 1) that the treatment T_9 (Application of Bordeaux Mixture at pre-monsoon, monsoon and post-monsoon) showed highest leaf yield (36.45 lakh ha^{-1}) and it was statistically at par with treatment T_1 (Application of *Trichoderma* sp. inoculated in Wheat grain at pre-monsoon, monsoon and post-monsoon) at both 5% level and 1% level of significances. The lowest leaf yield (30.25 lakh ha^{-1}) was found in control treatment (T_{10}) and it was most inferior to any other treatments at both 5% and 1% level of significances. Among the treatments, following hierarchy was observed: $T_9 \geq T_1 > T_2$, T_3, T_4, T_5, T_6, T_7, $T_8 \geq T_{10}$.

Fresh weight of leaf

It was revealed (Table 1) that the highest fresh weight of 100 leaves (392.66 gm) was found in treatment T_9 (Application of Bordeaux Mixture at pre-monsoon, monsoon and post-monsoon) which was statistically most superior isolate than another treatments at both 5% and 1% level of significances. On the other hand, the lowest fresh weight of leaf (235.00 gm) was observed in control treatment (T_{10}) and it was most inferior treatment than other treatments at both 5% and 1% level of significances. Among the treatments, following hierarchy was observed: $T_9 > T_1$, T_2, T_3, T_4, T_5, T_6, T_7, $T_8 \geq T_{10}$.

Cost Benefit Ratio (CBR)

The most remunerative cost benefit ratio (1: 5.10) was observed (Table 1) in treatment T_4 (Application of *Trichoderma* sp. inoculated in Cow dung manure at pre-monsoon, monsoon and post-monsoon) which was most superior to all the treatments at both 5% and 1% level of significances. The lowest remunerative (1: 1.0) cost benefit ratio found in treatment T_{10} (control) which was statistically at par with T_5 (Application of *Trichoderma* sp. inoculated in Mustard oil cake at pre-monsoon, monsoon and post-monsoon), T_7 [Application of multiplied bio-formulated product of Kesana (*T. harzianum*) at pre-monsoon, monsoon and post–monsoon] and T_9 (Application of Bordeaux Mixture at pre-monsoon, monsoon and post-monsoon) at both 5% and 1% level of significances. Among the treatments, following hierarchy was observed: $T_4 > T_1$, T_2, T_3, $T_6 > T_8 > T_5$, T_7, $T_9 \geq T_{10}$.

The overall result revealed that in both case of foot rot and leaf rot T_9 (Bordeaux Mixture at pre-monsoon, monsoon and post-monsoon) was the most statistically superior treatment to all other treatments which was statistically at par with T_1,T_4,T_5,T_7 in case of foot rot and T_1,T_2,T_7 in case of leaf rot. It may be concluded that the application of T_9 treatment is much more effective in combating the deadly diseases i.e. foot rot and leaf rot of betelvine than modern day's chemicals. Bordeaux Mixture application in betelvine was found effective against *Phytophthora* sp. infection by a number of workers starting from very early workers like Hector (1930), Dastur (1931 and 1935) to the present day workers like Mahanty et al. (2000, 2011).

Yield of the leaves in lakh / ha and fresh weight of 100 leaves revealed that T_9 (Bordeaux Mixture at pre-monsoon, monsoon and post–monsoon) showed the superiority over other treatments confirmed the findings of Mahanty (2004), and the result was statistically at par with T_1 (Application of *Trichoderma* sp. inoculated in wheat grain at pre monsoon, monsoon and post monsoon) in case of yield of leaf. The economic analysis showed that T_4 (*Trichoderma* sp. inoculated cow dung manure at pre-monsoon, monsoon, post monsoon) treatment was most remunerative and gave most notable additional return.

The calculated CBR is not totally flawless and standardized because the low cost of marketed product of bio-control agent is far more than the cost of small scale bio-agent production at laboratory. Along with that market demand, quality parameter, grading of product needed to be incorporated to make a viable economic analysis. Although, biocontrol approach was not superior to chemical control in terms of PDI, yield and leaf weight, it was at par in all cases. Therefore, use of biological control agents is recommended for to control foot and leaf rot of betelvine looking at the long term prospects and to avoid the possibility of health hazards due to consumption of betel leaf if the chemical pesticides are used to control both the diseases.

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