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Biological control of foot rot of betelvine (*Piper betle* L.) caused by *Phytophthora parasitica* Dastur

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A B S T R A C T

The experiment was carried out over two consecutive years to study the impact of incidence of foot rot of betelvine caused by *Phytophthora parasitica* and growth, yield, and keeping quality by applying two bioagents, viz., P. *fluorescens* and *Trichoderma harzianum. P. fluorescens* inoculated in 500 kg oil cake ha^{-1} was applied once at pre-monsoon, twice during pre- and post- monsoon and four times at quarterly intervals. *T. harzianum* inoculated in 500 kg oil cake ha^{-1} was applied at quarterly intervals. Bordeaux mixture (BM) was used to compare the treatments in preventing the intensity of foot rot. The results revealed that minimum foot rot disease occurred where four drenching and eight sprayings of BM at monthly and fortnightly intervals respectively were applied in the 1st year. In the 2nd year, the minimum foot rot disease was recorded in the treatment where four applications of the *Trichoderma* preparation ha^{-1} at quarterly intervals were given. The maximum foot rot disease was recorded in control treatment. The yield parameters like fresh weight of 100 leaves and leaf yield were good in treatments where BM was applied. *Trichoderma* applications resulted in better c : b ratio during 1st year, where as applications of *P. fluorescens* over two year analysis of pooled data gave the best c : b ratio.

***Keywords**:* Biocontrol, *Phytophthora parasitica, Piper betle, Trichoderma, Pseudomonas,* bioformulations

Introduction

Phytophthora spp. (*P. parasitica*, *P. nicotianae* var. *parasitica*, *P. palmivora, P. capsici*), are perpetual menace to the crop of betelvine, causing foot rot and leaf rot. The extents of losses vary from 5 to 90 percent (Dasgupta & Sen 1999; Dasgupta *et al*. 2008). Low temperature, high humidity and diffused light that prevail inside the baroj favours vine growth and are also congenial for the growth of the pathogen. The disease appears at the onset of monsoon and remains in high intensity throughout the rainy season. It wanes during the winter and may also occur in summer months when sudden hail storms occur.

The foot rot caused by phytophthoras were claimed to be ameliorated by soil application of BM (Dastur 1935; Dasgupta & Sen 1999; Dasgupta & Maiti 2008 and others). It was completely checked when cuttings were dipped in streptomycin solution and the plants were sprayed with BM (1%) twice a month (Saksena 1977). Dasgupta *et al*. (1988) and Mohanty and Dasgupta (2008) showed that fosetyl-Al and BM mixture were effective in controlling this disease. Sengupta *et al*. (2011) recorded lower return where biological control agents (BCAs) were used for its management. However, to reduce toxic hazards to human beings and to get maximum return, attempts were made by many workers to replace application of fungicides with BCAs (Tiwari & Mehrotra 1968; D'Souza *et al*. 2001; Mohanty *et al.* 2000). The present investigations were carried out to ~~to~~ device a strategy for effective management of this scourge of betelvine.

Materials and Methods

The experiments were carried out in RBD for two consecutive years using six treatments and four replications for each. Before the start of the experiment all infected plants in treatment rows were removed. Two rows containing 200-250 vines were considered as a treatment plot. Each treatment was separated by a buffer row.

For field testing, the selected BCAs were grown in oil cake medium for mass production and incubated at 28±1^{0}C for 30 days to allow production of chlamydospores. These were mixed with mustard oilcake previously soaked in water for 7 days in the ratio of 1:10 and kept for another seven days covering it with polyethylene sheet. The antagonists were then placed within the rows of vines and lightly covered with soil at prescribed ratios.

The treatments were:

T_1 = One application of *P. fluorescens* inoculated in 500 kg oil cake ha^{-1} (form-1) at pre-monsoon + three applications of uninoculated oilcake at 500 kg ha^{-1} per application at quarterly intervals.

T_2 = Two applications of *P. fluorescens* (form-1) at pre- and post- monsoon + two applications of uninoculated oil-cake at 500 kg ha^{-1} per application at quarterly intervals.

T_3 = Four applications of *P. fluorescens* (form-1) at quarterly intervals.

T_4 = Four applications of *Trichoderma* inoculated in 500 kg oil cake ha^{-1} (form-2) at quarterly intervals.

T_5 = BM : 4 drenches + 8 sprays at monthly and fortnightly intervals respectively + four split doses of uninoculated oilcake at 500 kg split^{-1} ha^{-1} at quarterly intervals.

T_6 = Control : 4 split doses of oilcake at 500 kg split^{-1} ha^{-1} at quarterly intervals.

The mortality of vines, fresh weight of 100 leaves and yields per ha in each treatment was recorded 30 days after the last treatment application. The disease incidence and mortality of vines were calculated using McKinney's (1923) formula. The c : b ratios were also calculated using standard approach. The results obtained were subjected to analysis of variance of annual and two year pooled data.

Results and Discussion

Percent disease incidence

The results (Table 1) showed that minimum foot rot disease occurred under T_5 treatment (5.80, 9.34 %) that was statistically superior to all other treatments. In the 2nd year, the minimum foot rot disease was recorded in T_4 treatment (12.55 %) where Trchoderma was applied, it being statistically at par with treatments T_3 (12.93 %) and T_5 (12.88 %). The maximum foot rot disease was recorded in T_6 (control) treatment (19.49, 24.74, 22.11). The disease incidence in all the treated plots was in the descending order, T_6 T_1 T_2 T_3, T_4 T_5 (Pooled).

The different treatment combinations of pseudomonads with MOC and single treatment of *T. harzianum* on MOC showed different disease reducing ability in two different years and also in the pooled mean. All the treatment combinations reduced foot rot of betelvine significantly when compared to untreated control. Minimum disease incidence was observed in T_5 treatment (9.34 %) and maximum disease in T_6 (22.11 %). Treatments T_3 and T_4 showed no significant difference in disease reduction in both the years and in the pooled mean. The results therefore indicated that BM application gave better result in disease reduction when compared to application of bioagents. Similar results were noticed earlier (Dutta *et al.* 1996; Dasgupta *et al.* 2003; Sengupta *et al.* 2011).

Fresh weight of 100 leaves (g)

The results (Table 1) showed that the highest fresh weight of 100 leaves was recorded in T_5 treatment (339.25, 342.50, 340.87 g) where BM + MOC were applied and it was statistically superior to all other treatments in 1st year, 2nd year and pooled analysis of two years data. Minimum fresh weight of 100 leaves was recorded in T_6 (control) treatment (241.25 and 266.87 g). This again was statistically lower than in all other treatments in 1st year and pooled analysis of two years data. In the 2nd year the fresh weight of 100 leaves recorded in the control (292.50 g) was statistically at par with the treatments T_1 (292.75 g), T_2 (296.25 g) and T_3 (295.00 g) The fresh weight of 100 leaves (g) as a result of treatments was in the order; T_5 T_4 T_3 T_2, T_1 T_6 (Pooled).

These results revealed that there is a sharp increase in fresh weight of 100 leaves in every treatments in comparison to control treatment. The highest fresh weight was observed in T_5 treatment. This led to the conclusion that bio-agents and BM had significant effects in increasing the fresh weight of 100 leaves of betelvine. These results are in consonance with the findings of Sengupta *et al.* (2011).

Leaf Yield (Lakh/ha)

Highest leaf yield in 1st year was recorded in T_5 treatment (35.86 lakh ha^{-1} $year^{-1}$) being statistically at par with T_4 (34.98 lakh ha^{-1} $year^{-1}$). In the 2nd year and in pooled analysis of two years, highest leaf yield was recorded in the treatment T_5 (39.52 and 37.69 lakh ha^{-1} $year^{-1}$), being statistically superior to all other treatments. Minimum leaf yield was recorded in T_6 (control) treatment in 1st year, 2nd year and pooled analysis of two years data (22.48, 26.45 and 24.46 lakh ha^{-1} $year^{-1}$) (Table 1).

The results of leaf yield (lakh ha^{-1} $year^{-1}$) in different treatments by application of BCAs may be represented as T_5 T_4 T_2, T_3 T_1, T_6 (Pooled).

The different treatments showed different results in two different years and also in pooled mean and such differences were statistically significant. The results presented here showed that every treatment increased the leaf yield significantly as compared to control where only MOC were used. This experiment suggested that the BCAs and BM had significant effects on leaf yield of betelvine in comparison to control as they reduced the different harmful diseases which was ultimately reflected as increase of leaf yield.

Cost : benifit Ratio (CBR)

CBR in 1st year revealed that the treatment T_4 containing Trichoderma was most remunerative (1:33.76). In the 2nd year and pooled data of two years, the most remunerative treatment was T_2 (1:15.71 and 1:15.02 C:B ratio) that contained pseudomonas. The least remunerative in the 1st year, 2nd year and pooled mean (1:15.71 and 1:15.02) of two years was treatment T_5 (1:4.32, 1:4.22 and 1:4.27) where BM was applied (Table 1)

These results are in consonance with earlier findings (Mohanty *et al.* 2000; Dasgupta *et al.* 2003. They revealed that although biological control approach was not superior to chemical control in terms of yield, PDI and fresh weight of 100 leaves, when we consider the CBR, biological control with *P. fluorescens* at pre- and post- monsoon and quarterly application of *Trchoderma* was significantly more promising among all treatments. Therefore, these biological control agents may be recommended to the growers for the present to achieve higher economic returns and provide environmentally safer leaves for the consumers who chew it almost immediately after harvest. In the meanwhile researches need to continue to device more efficient biocontrol strategies for optimizing the yield while retaining the safety considerations.

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Table 1.
Effect of bio-agent on growth, yield and disease incidence of betelvine

Treatment	Foot rot *Phytophthora* spp.			Fresh weight of 100 leaves (g)			Leaf yield in (Lakh $ha^{-1} year^{-1}$)			Cost: benefit ratio		
	1st year	2nd year	Pooled	1st year	2nd year	Pooled	1st year	2nd year	Pooled	1st year	2nd year	Pooled
T_1	15.00(22.77)	16.89(24.26)	15.94(23.52)	292.50	292.75	292.62	24.89	27.86	26.37	1:10.01	1:5.86	1:7.94
T_2	10.92(19.24)	13.78(21.77)	12.35(20.56)	304.00	296.25	300.12	29.38	34.01	31.69	1:14.44	1:15.71	1:15.02
T_3	9.87(18.29)	12.93(21.07)	11.40(19.72)	308.75	295.00	301.87	28.94	30.47	29.70	1:6.71	1:4.17	1:5.44
T_4	9.85(18.25)	12.55(20.74)	11.19(19.54)	319.00	304.25	311.62	34.98	34.68	34.83	1:33.76	1:8.55	1:10.77
T_5	5.80(13.86)	12.88(21.02)	9.34(17.78)	339.25	342.50	340.87	35.86	39.52	37.69	1:4.32	1:4.22	1:4.27
T_6	19.49(26.19)	24.74(29.82)	22.11(28.05)	241.25	292.50	266.87	22.48	28.45	25.46	1:1	1:1	1:1
SEm(±)	0.691	0.289	0.225	3.750	3.269	2.903	1.009	0.911	0.815	1:10.01	1:5.86	1:7.94
CD (P=0.05)	2.082	0.870	0.677	11.299	9.850	8.747	3.040	2.745	2.455	1:14.44	1:15.71	1:15.02

[1]Average of four replications
Figure in parentheses are the angular transformed values percent disease incidence.
Treatment: T_1= One application of *Pseudomonas fluorescens* inoculated in 500 kg oil cake ha^{-1} at pre-monsoon + three application of uninoculated oilcake at 500 kg ha^{-1} per application at quarterly intervals. T_2 = Two applications of *Pseudomonas fluorescens* inoculated in 500 kg oil cake ha^{-1} at pre and post monsoon + two application of uninoculated oil-cake at 500 kg ha^{-1} per application at quarterly intervals. T_3 = Four applications of *Pseudomonas fluorescens* inoculated in 500 kg oil cake ha^{-1} at quarterly intervals. T_4 = Four applications of *Trichoderma* inoculated in 500 kg oil cake ha^{-1} at quarterly intervals. T_5 = Bordeaux mixture 4 drenches +8 sprays at monthly and fortnightly intervals (respectively)+ four split doses of uninoculated oilcake at 500 kg $split^{-1} ha^{-1}$ at quarterly intervals. T_6 = Control (4 split doses of oilcake at 500kg $split^{-1} ha^{-1}$ at two quarterly intervals).