ISSN 0971-930X
Journal of Biological Control
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Journal of Biological Control, 26 (1): 84-87, 2012

Research Note

Studies on efficacy of different delivery systems of *Pseudomonas fluorescens* for the biosuppression of damping-off disease in tobacco seed beds

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ABSTRACT: Aexperiment in tobacco nursery was conducted to evaluate effect of different delivery systems for *Pseudomonas fluorescens* in the management of damping-off in tobacco seed beds. When talc enriched with *P. fluorescens* was applied, significantly highest population of *P. fluorescens* was recorded in seed treatment and soil application combination (32.3 x 10^7 CFUs/g soil) at 60 days after sowing and the same treatment recorded the lowest population of *Pythium aphanidermatum* (0.52 x 10^3 CFUs / g soil) of soil. As a result, 33 percent incidence of damping-off was recorded. Application of mancozeb + metalaxyl at 0.2 % recorded 25 percent of disease incidence and the untreated control recorded the maximum disease incidence of 83%. The study suggests that talc based delivery supports better survival of *P. fluorescens* in rhizosphere when compared to FYM and filter press cake and have potential for controlling *Pythium aphanidermatum*.

KEY WORDS: *Pseudomonas fluorescens, Pythium aphanidermatum,* talc, tobacco.

(Article chronicle : Received: 11.11.2011 Revised: 20.01.2012 Accepted: 01.02.2012)

Damping-off is the most common and widespread nursery disease of tobacco (*Nicotiana tabacum* L.) caused by the soil-borne, 1hydrophilic fungus *Pythium aphanidermatum* (Edson) Fitzp. The pathogen is responsible for poor seedling stand. Conspicuous symptom of this disease is the sudden collapse of young seedlings in patches leading to uneven stand. Brown watery soft rot of young seedlings, girdling of hypocotyls, toppling, wet rot and finally leading to death of seedlings are the characteristic symptoms (Shah-Smith and Burns, 1996). The pathogen spreads quickly and affects the entire seed bed causing enormous loss of seedlings in tobacco (Subashini and Padmaja, 2010). The use of chemical fungicides at present is unavoidable.

However, chemical control of the disease is expensive and disturbs soil ecology (Gnanamanickam, 2002). Biological control of plant pathogens is a promising approach in combating soil borne pathogens. Certain strains of *Pseudomonas fluorescens* when applied to seed and soil were found to provide biological control of root pathogens (De Boer, 2003). Farm Yard Manure (FYM) was the best substrate for multiplication of *P. fluorescens* in poly bags (Najam and Singh, 2004). Delivery system of the antagonists is critical for effective implementation of biocontrol of various crop diseases. The objective of this study was to evaluate the efficacy of various carriers of powder based formulations of *P. fluorescens* for the management of damping-off disease in tobacco seed beds.

Isolation of the pathogen

Tobacco seeds @ 0.5g /m^2 of seed bed were sown in pots filled with soil from nursery and kept under glasshouse conditions. The pots were watered daily and a fortnight later seedlings showing damping-off symptoms were collected and the pathogen, *P. aphanidermatum* was isolated by tissue segment method (Rangaswami, 1958) on potato dextrose agar medium (PDA). It was purified by single hyphal tip method and maintained on PDA.

Isolation and screening of bacterial antagonists

Rhizobacteria were isolated from the rhizosphere soil of tobacco crop as described by Vidhyasekaran and Muthamilan (1995). Five native bacterial antagonists of CTRI were coded as *Pf* -1, *Pf* -2, *Pf*-3, *Bacillus* sp.-1 and *Bacillus* sp.-2 These five isolates were used under *in vitro* conditions against damping–off disease and the strain Pf-1 (48.3% inhibition over control) was identified as a potential strain after comparing with reference strain, Pf-TNAU which showed 42.8% inhibition (Subhashini and Padmaja

2009).

Exploiting the biocontrol potential of the selected bacterial antagonist under field conditions

A field trial was conducted during 2008 at Central Tobacco Research Institute, Rajahmundry to study the biocontrol potential of the antagonistic bacteria (*P. fluorescens*). King's B broth was prepared and distributed in 50ml quantities in 250ml Erlenmeyer flasks and autoclaved. After sterilization the flasks were inoculated with cell suspension of *P. fluorescens* prepared from 48h culture @ 0.5ml. The flasks were continuously shaken in rotatory shaker (l20rpm) for four days. The talc-based formulation of *Pf*-1 was prepared following the method of Vidhyasekaran and Muthamilan, (1995). FYM was prepared at CTRI Farm, Katheru and well decomposed material was brought and used in the experiment. Filter press cake was obtained from the sugar factory. The *P. aphanidermatum* was mass multiplied on potato dextrose agar medium and was inoculated in the seed beds at a concentration of 5 per cent (w/w). The experiment consisted of micro plots of 1 m^2 beds in a randomized block design.

Red sandy loam soil was used for the nursery experiment. The soil analysis before starting the experiment for its showed chemical characteristics as pH 7.6, electrical conductivity 1.77 dsM^{-1}, available N 0.25%, available P 0.23% and available K 0.18%.

Tobacco seeds @ 0.5g were mixed with 250g of carrier materials *viz*., talc, FYM, and filter press cake containing 25 ml of *P. fluorescens* @ 1 x 10^7 CFUs ml^{-1} for each m^2 seed bed. The treated seeds were sown on seed beds containing unsterilized soil. Soil application of various formulations was carried out. Combination of seed treatment and soil application was also carried out. Soil treatment with Mancozeb + Metalaxyl at 0.2 % and Bordeaux mixture at 0.4% were kept for comparison. The incidence of damping-off in tobacco seed beds was assessed and it was expressed as per cent disease incidence. Soil samples were collected from each treatment at 20, 40 and 60 days after sowing (DAS) from rhizosphere. The survival of *P. fluorescens* and *P. aphanidermatum* was assessed by serial dilution plating method.

The results revealed that combined application (seed treatment + soil application) of *P. fluorescens* significantly increased the plant growth and decreased the disease incidence (Table 1). Among the carriers tested, talc based formulation effectively increased the per cent germination (78.6), shoot length (10.80cm), seedling dry weight (6.35 g), chlorophyll content (1.51 mg/g leaf fresh weight) and number of healthy transplants (412/ m^2 seed bed) and minimum per cent disease incidence (33) was recorded followed by FYM (34) without significant difference between the two. Filter press cake recorded 47% disease incidence though superior to untreated control, was inferior to the rest of the treatments. Fungicides Mancozeb + Metalaxyl @ 0.2% recorded 25 per cent while Bordeaux mixture @ 0.4% recorded 36 per cent disease incidence and it is on par with the talc based formulation of *P. fluorescens*

Table 1. Effect of seed treatment and soil application of *Pseudomonas fluorescens* on seedling growth and damping-off incidence in tobacco seed beds (60 DAS).

Formulation @ 250 gm/ m^2	Per cent Germination	Shoot length (cm)	Seedling Dry weight (gm)	Chlorophyll content	Per cent disease incidence/ m^2	No. of healthy transplants/ m^2
Talc	78.6	10.80	6.35	1.51	33	412
FYM	77.0	10.36	6.15	1.49	34	410
Filter press cake	73.6	9.98	5.20	1.38	47	397
Mancozeb+Metalaxyl (0.2%)	81.0	10.53	6.52	1.50	25	423
Bordeaux mixture (0.4%)	74.3	10.03	5.41	1.34	36	395
Control	32.6	8.70	2.13	0.63	83	271
SEM ±	0.98	0.11	0.13	0.01	1.86	5.37
CD (*P*=0.05%)	3.09	0.36	0.42	0.03	5.85	16.91
CV%	2.44	1.95	4.36	1.46	7.48	2.42

(33). Untreated control recorded the maximum disease incidence (83%).

The methods of application of formulated products of biocontrol agents reported by earlier workers include seed treatment (Rosales and Mew, 1997), root dip (Maurhofer *et al.,* 1994), soil application (Vidhyasekaran *et al.,* 1997) and foliar application (Jayalakshmi *et al*., 2005; Singh and Sinha 2005). Combination of different methods of application could be more effective in disease management than a single method of application (Nandakumar *et al*.,

Table 2. Effect of different treatments on the rhizosphere survival of the antagonist and the pathogen in talc colonized *Pseudomonas fluorescens*

Treatment	*P. fluorescens* population (x 107 CFUs g^{-1} of soil)			*P. aphanidermatum* population (x 10^{-3}CFUs g^{-1} of soil)		
	20 DAS	40 DAS	60 DAS	20 DAS	40DAS	60DAS
Seed Treatment	15.6	21.0	26.0	1.22	1.33	0.91
Soil Application	18.0	23.0	28.7	1.05	1.17	0.83
Seed Treatment+ Soil Application	20.7	25.0	32.3	0.97	1.14	0.52
Mancozeb + Metalaxyl (0.2%)	5.3	7.3	11.0	1.12	1.25	0.92
Bordeaux mixture (0.4%)	6.3	9.0	12..0	1.17	1.38	1.06
Control	1.6	2.7	4.3	4.91	5.95	8.80
SEM ±	0.50	0.52	0.80	0.03	0.02	0.34
CD (*P*=0.05%)	1.58	1.63	2.52	0.09	0.07	1.07
CV%	7.71	6.10	7.28	2.88	1.93	27.09

2001). These reports support the present findings. Seed treatment followed by soil application of talc based powder formulation has effectively checked wilt of chickpea, wilt of pigeon pea and cotton under field condition and increased the yield as reported by Srinivasan and Mathivanan (2006). In FYM and Filter Press Cake there may be other competing microorganisms which may have reduced the potential of *Pseudomonas flourescens*. Since, talc is an inert material the full potential of the isolate was realized.

The rhizosphere survival of *P. fluorescens* with different methods of application was estimated (Table 2). Among the treatments tested, the survival of *P. fluorescens* was significantly higher in seed treatment + soil application at 60 DAS (32.3 x 10^7 CFUs g^{-1}) followed by soil application (28.7 x 10^7 CFUs g^{-1}). With regard to survival of pathogen in the rhizosphere region, the treatment with talc colonized *P. fluorescens* as seed treatment + soil application significantly reduced population of the pathogen in rhizosphere soil when compared to control.

P. fluorescens possesses the capacity to adhere to plant root (Subhashini and Padmaja, 2009). Subhashini and Padmaja (2009) observed high population of *P. fluorescens* in rhizosphere soil of tobacco due to seed treatment and soil application of *P. fluorescens* in integration with talc.

The treatment of talc enriched with *P. fluorescens* applied as seed treatment and soil application significantly reduced the population of *P. aphanidermatum* with 0.52 x 10^3 CFUs g^{-1} of soil followed by soil application 0.83 x 10^3 CFUs g^{-1}. Usharani *et al.,* (2009) FYM enriched with *P. fluorescens* as seed and soil application was very effective in minimizing leaf incidence in tomato and significantly highest population of *P. fluorescens* was recorded with FYM enriched with *P. fluorescens* was used for seed treatment and soil application and the same treatment recorded the lowest population of pathogen *Fusarium oxysporum*.

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