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Plant Pathology

Management of Damping off (*Pythium aphanidermatum*) in chilli (*Capsicum annum cv* VNS-4) by *Pseudomonas fluorescens*

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Abstract

Pseudomonas fluorescens 0.5% W.P. formulation applied as seed and furrow (soil application) in Chilli significantly reduced the damping off disease of chilli caused by *P. aphanidermatum.* The yield of chilli was also significantly enhanced. The formulation did not have any phyto-toxic effect on chilli plants at all the dosage levels tested for bioefficacy. The *Pseudomonas fluorescens* 0.5% W.P. application had no adverse effect on the beneficial rhizospheric microbes, like Arbuscular Mycorrhizae *(Glomus* spp.) in chilli rhizosphere at all dosages which were confirmed by microscopic observations. Based on the above findings, the *Pseudomonas fluorescens* 0.5% W.P. formulation is found safe and effective and may be used as an efficient & eco-safe alternative of synthetic fungicides for the management of damping off disease of chilli and for obtaining higher yields.

Highlights

- Talc based formulation of *Psedomonas fluorescens* significantly controlled damping off disease of chilli caused by *Pythium aphanidermatum*
- The formulation had no adverse effect on the beneficial microbes in the rhizosphere of chilli

Keywords: Damping off, *Pythium aphanidermatum*, Chilli, *Psedomonas fluorescens*, Biological control

Introduction

Chilli (*Capsicum annum* L.) commonly known as pepper, belonging to solanaceae family, forms an indispensible culinary spice of almost every cuisines of the world. Valued principally for its pungency and anti oxidant content it is considered an economically important tropical and sub tropical crop owing to its high consumption as well as nutritional significance. India has been the second largest exporter of chilli in the international market, exporting products ranging from dried form of chilli to chilli powder and oleoresins across 90 countries (Muthukumar *et al*., 2010; Peter, 1999). However, there is still lower production of chilli in the country when compared to other countries. This can be attributed to the various bacterial as well as fungal diseases incidences affecting the production at seedling to post and pre harvest stages of the crop.

Among the fungal disease about 60 % losses at seedling stage in both nursery and field level have been reported due to damping off disease in chilli caused by *Pythium aphanidermatum* (Jadhav and Ambadkar, 2007). Pythium is a soil borne plant pathogen causing seed rot and damping

off diseases in many crops especially affecting tomato and chilli (Shah Smith and Burns, 1996). Being generalistic and unspecific in its host range, it is capable of causing serious loss under field and greenhouse condition affecting newly emerging seedlings. The ability of the pathogen to persist in the soil for longer times and infect a wide variety of plant hosts by rapidly infesting germinating seeds prove as a barrier for bio-effective controls (Ellis *et al*., 1999).

Though fungicides have proved to be an effective control measure for damping off in chilli, phytotoxicity and fungicides residues have been a serious concern leading to environmental pollution and human health hazards. Also, fungicide resistance developed by *Pythium* further discourages its use for its management (Whipps and Lumsden, 1991). Another strategy of using resistant cultivar of chilli against *Pythium* is also in vain as till date no successful resistant cultivar of chilli has been developed. These constraints have provoked the use of alternate control strategies, particularly the use biological control agents in past two decades. The use of *Psuedomonas fluorescence* (Trevisan) Migula and *Trichoderma viride* Pers. has been developed commercially as a talc based formulation and tested against several crop diseases previously showing promising results in managing disease incidence under both field and greenhouse conditions (Sarma *et al*., 2009,Roberts *et al*., 2005).

However, not much study has been conducted to analyze the effect of such talc based formulations on the rhizospheric microbial population of the soil as well as its phytotoxicity on the plants. In pursuit of proving talc based formulation of *Pseudomonas fluorescens* 0.5% W.P. formulation (TNAU Strain; CFU count: 2 x 10^8 CFU/gm minimum) an eco-friendly alternative for management of damping off in chilli under field conditions, the present study was carried out with following objectives of (1) assessing its field bioefficacy against *P. aphaniderrnatum* along with (2) evaluating its effect on population of beneficial microorganisms in chilli rhizosphere and (3) its phytotoxic effect on chilli plants if any.

Materials and Methods

A field trial was conducted during December, 2008 at an experimental field of Banaras Hindu University, Varanasi district of U.P. and the crop was harvested in May 2009. The experiments were conducted in a randomized block design with six treatments and three replications as mentioned in Table 1. A susceptible variety of chilli VNS-4 was selected for the field trials and all agronomical practices for the crop were followed according to packages of practices.

The disease severity was rated after 25 days of sowing in nursery on the basis of plant mortality caused by pathogens as described by Hartman *et al.* (2000). Disease incidence was recorded (Average disease severity) & growth promotion parameters (plant height and no. of branches/ plant) from different treatment plots and control plot were recorded.

Qualitative phyto-toxicity symptoms i.e. injury on leaf tips & leaf surface, wilting, vein clearing, necrosis, epinasty & hyponasty from ten randomly selected chilli plants from each treatment were recorded as per the Manual for testing the phytotoxicity of pesticides (1989). The effect on the beneficial rhizospheric microbes like Arbuscular Mycorrhiza *(Glomus* spp.) in chilli rhizosphere at all dosage levels in treated plots with *Pseudomonas fluomscens* 0.5% W.P. and control plot was also recorded.

Table 1: Details of the treatments used for field trial

Treatment Code	Dose used
T_1	Seed treatment @ 5g/kg of seed
T_2	Seed treatment @ 10g/kg of seed
T_3	Seed treatment @ 20g/kg of seed
T_4	Furrow (soil application) treatment of *Pseudomonas fluorescens* 0.5% W.P. @ 2.5kg/ hectare
T_5	Bavistin @ 0.2% (seed treatment)
T_6 - Control	No application of bioagent or fungicide
Replications	Three
Variety	VNS-4

Results

The data from the present investigation as represented in Table 2 indicates that the per cent disease incidence was significantly lower in treated plants than in control in both methods of treatment of chilli crop by the *P. fluorescens* 0.5% W.P. formulation. The per cent reduction in disease incidence following seed treatment was (40.4, 68.1 & 69.3%) at 5, 10 and 20 gm/kg of seeds, respectively and following furrows (soil application) (63.6%). Plant height due to seed treatment of chilli by *P. fluorescens* 0.5% was increased by 17.2, 35.3 and 35.4% at similar doses whereas increase due to furrow (soil application) treatment was 17.8% (Fig 1). Similarly, the number of branches also increased by 17.4, 52.9 and 56.4% in seed treatment with *P. fluorescens* 0.5% W.P.; whereas the increase in furrow

Table 2: Field Bio-efficacy of *Pseudomonas fluorescens* 0.5% W.P. against damping off disease of Chilli caused by *P. aphanidermatum*

S.No.	Treatments	Per cent disease incidence	Disease reduction (%)	Plant height (cm)	No. ofbranches/ plant	Yield (q/ha)
1.	T_1	38.52±1.92	40.38±1.36	57.84±3.44	4.37±0.61	20.83t2.21
2.	T_2	20.61±1.11	68.10±1.51	66.28±5.62	5.69±0.71	25.18±2.29
3.	T_3	19.82±1.05	69.32±1.82	66.81±4.97	5.82±0.60	25.69±2.22
4.	T_4	23.49±1.51	63.64±1.82	58.14±4.29	5.14±0.52	20.89±2.24
5.	T_5	27.72±2.44	57.10±1.92	54.11±3.64	4.01±0.63	20.16±2.27
6.	T_6 (Control)	64.62±3.19	-	49.35±3.22	3.72±0.44	15.74±2.24
	CD at 5 % ± S.E.	1.92	1.91	2.09	1.12	2.42

Fig 1: Effect of *Pseudomonas fluorescens* 0.5% W.P. on growth and yield of chilli (treated right) control (left)

treatment (soil application) was 38.1%. The yield of chilli also showed an increase of 32.3, 59.9 and 64.6% in seed treatment at different doses and 32.7% in furrow (soil application) treatment with *P. fluorescens* W.P.

The *P. fluorescens* 0.5% W.P. formulation had no phyto-toxic affect on the chilli plants following its application at all the dosage levels tested for bio-efficacy. The *Pseudomonas fluorescens* 0.5% W.P. formulation had also no adverse effect.on the beneficial rhizospheric microbes like Arbuscular Mycorrhiza *(Glomus* spp.) in chilli rhizosphere.

Discussion

Damping off in chilli caused by *P. aphanidermatum* is a severe disease resulting in high crop losses in chilli production. Management strategies involving cultural practices and toxic chemicals have both advantages and disadvantages. The use of plant growth promoting

rhizobacteria for biocontrol of plant diseases is currently one of the major areas of research in biology. Various mechanisms are attributed for their effective biocontrol potential which may be antibiosis or mycoparasitism, creating competition for nutrients in soil or by inducing defence responses in host plant against the phytopathogen (Saxena *et al*., 2013). *Pseudomonas* spp. in combination with other biocontrol agents or applied singly have been tested for their biocontrol potential against many diseases as well as for the damping off of chilli (Jain *et al*., 2012; Muthukumar *et al*., 2010).

From the present study, it may be concluded that treatment of chilli seeds with *Pseudomonas fluorescens* 0.5% W.P. formulation (TNAU Strain) [Project sponsored by M/s. Indore Biotech Inputs & Research (P) Ltd.,1] @ 5, 10 and 20 g/kg of seeds resulted in efficient control of the damping off disease and also significant enhancement in the crop yield. There was no phyto-toxic effect on chilli plants and also no adverse effect on beneficial microbes in chilli rhizosphere viz. *Glomus* spp. was observed during the bio-efficacy trial.

In view of the above findings, the formulation of *Pseudomonas fluorescens* 0.5% W.P. formulation (TNAU Strain) @ 10g/kg of seeds as seed treatment may be used as an efficient & ecologically safe alternative of synthetic chemicals for the management of damping off disease of chilli and for obtaining higher yields.

Future research needs to be focused towards identifying the biochemical and molecular mechanisms involved in the growth stimulation of plants and inhibition of *Pythium aphanidermatum* by the beneficial rhizobacteria. Such biocontrol agents, which provide multiple benefits, may provide useful pointers to improving afforestation practices and establishment of plants in diverse inhospitable/barren habitats, besides their promise as multifaceted bioinoculants in organic farming practices popular in present day agriculture.

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