Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; SP1: 3000-3003

Dr. JN Srivastava
Bihar Agricultural University, Bhagalpur, Bihar, India

Dr. AK Singh
Sher-e-kashmir University of Agricultural Sciences and Technology, Jammu J&K, India

Dr. Mithilesh Kumar
Bihar Agricultural University, Bhagalpur, Bihar, India

Anupam Kumai
Bihar Agricultural University, Bhagalpur, Bihar, India

Dr. Rakesh Sharma
Fruit Science Division, SKUAST-Jammu, Jammu J & K, India

Correspondence
Dr. JN Srivastava
Bihar Agricultural University, Bhagalpur, Bihar, India

Management of damping-off disease of seedling caused in solanaceous and cruciferous vegetable through integrated approach

Dr. JN Srivastava, Dr. AK Singh, Dr. Mithilesh Kumar, Anupam Kumai and Dr. Rakesh Kumar

Abstract
Three species *Trichoderma* namely: *Trichoderma viride* (Tv) *Trichoderma harzianum* (Th), and *Trichoderma koningii* (Tk**)** and *Pseudomonas fluorescence* as seed treatment two neem-based formulation as seed treatment and soil drench were evaluated in greenhouse experiment of damping–off caused by *pythium aphanidermatum* in brinjal, brinjal, chilli, cauliflower and cabbage. Treatment with all the isolates of *Trichoderma* spp. and *Pseudomonas fluorescence* resulted in significantly higher seed germination, less disease and higher dry biomass over the non-treated control in all the treated crops. Two neem-based formulations also significantly suppressed the disease and were on par or superior in effectiveness as compared to the fungicides metalaxyl 8% mancozeb 64% (Ridomil MZ 72) and captan 50% WP (Captra 50 WP).

Keywords: Nursery vegetable, damping-off, integrated management

Introduction
The vegetable crops propagated by seeds and to be sown directly in the field whereas some crops like, tomato, brinjal, chilli etc. are first sown in nurseries for raising seedling and then transplanting. The disease is common in nursery bed and young seedlings by several fungi *Pythium*, *Phytopathora*, *Fusarium*, *Rhizoctonia*, *Sclerotium Colletotrichum*.
Damping off disease of seedlings is widely distributed all over the world. It was first studied by Hens (1874) in Germany. Damping off is a seedling disease common to most of solanaceous vegetable *viz.*, tomato, brinjal and chilli but disease has wide host range and attracts the plants belonging to families Crucifereae, Leguminoceae and Chenopodiaceae (Alexander,1931).
The disease is of common in nursery bed and young seedlings. Several seed and soil borne fungi can kill before the tender radical and plumule established in the nursery bed (Fageria *et al.*, 2003).
The pathogen attracts to the seed and seedling roots during germination either before or after emergence. Within days, more number of seedlings destroyed is by pathogens, and also later several weeks damping-off seedling, may develop root rot or stem canker (Atkinson, 1895). The pathogen attracts underground, soil line or crown roots of seedlings. Depending upon host variety and environmental factor, 25-75 per cent losses are caused due to this disease (Gupta and Paul, 2001).
Pythium aphanidermatum is an essentially soil borne fungus and chemical fungicides are generally used for its control. Frequent and indiscriminate use of chemicals is associated with ill effects such as environment pollution and development of resistance (Whipps and Lumsden, 1991). Hence, bio-control has been advocated as the most promising alternate strategy to overcome these problems (Mukhopadhyay *et al.*, 1992). While, bio-control agents (BCAs) have been successfully used to control soil borne diseases (Copper and Compbell, 1986; Elad and chet, 1987; Tiwari and Mukhopadhya, 2001, Ranjan *et al.*, 2002), the potential of botanicals like neem are yet unexplored. In the present study, Three species namely: *Trichoderma viride* (Tv) *Trichoderma harzianum* (Th), and *Trichoderma koningii (*Tk***)** and *Pseudomonas fluorescence* as seed treatment and two neem-based formulation as seed treatment and soil drench were evaluated for management of damping–off caused by *pythium aphanidermatum* in greenhouse experiment in solanaceous winter vegetables, namely, tomato, brinjal, chilli, cauliflower and cabbage.

Materials and Methods

Isolation and purification of pathogens and bio-control agents:

Virulent culture of *P. aphanidermatum* was isolated on potato dextrose agar (PDA) from naturally infected brinjal seedlings collected from farmer's field. Its pathogenicity was tested on tomato (cv Panjab Kesari), brinjal (cv Pusa Anupam), Chilli (cv Pusa Sadabahar) cauliflower (cv Pusa Early Synthetic) and cabbage (cv Pusa Ageti) plants in greenhouse. From the diseased seedlings re-isolation of pathogen was carried out and this culture was maintained for further studies. Three species namely: *Trichoderma viride* (Tv) *Trichoderma harzianum* (Th), and *Trichoderma koningii (*Tk***)** and a *Pseudomonas fluorescence* were isolated from disease suppressive soils. The identity of *Trichoderma* spp. was confirmed through the microbial Type Culture Collection (MTCC), Chandigarh, India. Talk-based powder formulations of these microbial agents were developed as described by Vidhyasekaran *et al.*, 1997.

Seed treatment with neem formulations and bio-control agents

Two neem formulations, namely, oil, based and kernel based were used in the study. Two chemical fungicides Ridomil MZ 72 (metalaxyl 8% mancozed 65% WP) and captra 50 WP (Captan 50% WP) were also included for comparison. Seeds of all tested crops were dip treated with neem formulation (0.5%) and fungicides (0.25%) for an hour. The seed treatments with the powder formulations of *Trichoderma viride* (Tv) *Trichoderma harzianum* (Th), and *Trichoderma koningii (*Tk***)** and *Pseudomonas fluorescence* were carried out by coating @ 10 g/kg seed.

Greenhouse experiment and observations

The greenhouse experiments were conducted using earthen pots (30 cm diam). The inoculum of *P. aphanidermatum* grown on corn-meal sand medium was added @ 50 g/pot and mixed well. For each treatment, four replications were, maintained. Treated seeds were sown in pots (Brinjal. Tomato, Chilli, cauliflower and cabbage @30 seed/pot. Neem formulations and fungicides were also drenched twice at 15 and 30 days of sowing @ 50 ml/pot. Observation for germination and mortality due to damping-off were recorded up to 45 days. The plants were then uprooted and dried to constant weight at 60 C and dry weight was recorded. Experiments on the all the five crops were repeated once, using fresh pots and soil to confirm the results. For the two repeate data on the greenhouse experiments, homogeneity of error mean square from analysis of variance was tested through application of F test (Gomez and Gomez, 1984) and the error variance were found homogeneous. There, pooled analysis was made.

Results and discussion

Seed germination percentage varied in five crops evaluated as could be seen (Table 1) in control treatments of brinjal (39), tomato (38), Chilli (13), cauliflower (26), and cabbage (56) and it improved significantly and substantially in all the treatments involving microbial agents, neem preparations and fungicides. The range of improvement in germination over control was observed to the tune of 48-79% in brinjal with captra 50 WP and Ridomil MZ 72 as the best, 46-61% in tomato with *Trichoderma harzianum* and Ridomil MZ 72 as the best, 20-40% in Chilli with kernel based neem formulation and Ridomil MZ 72 as the best. 36-52% in cauliflower with *Trichoderma viride* and kernel based neem formulation as the best, 70-91% in cabbage with kernel based neem formulation and captra 50 WP as the best,

The data indicate that all the treatments effectively suppressed the pre-emergence damping-off of seed resulting in significantly higher seed germination of the all crops tested. Kernel based neem formulation was superior to the two chemical fungicides in respect of germination of cauliflower, cabbage and Chilli seeds.

Table 1: Effect of bio-control agents and neem formulations on seed germination in solanaceous and cruciferous vegetable

Treatment	Germination (%)				
	Brinjal	**Tomato**	**Chilli**	**Cauliflower**	**Cabbage**
Trichoderma viride-ST	58.9	56.0	20.5	52.5	87.0
Trichoderma harzianum -ST	48.3	61.3	35.5	44.2	78.0
Trichoderma koningii-ST	65.9	46.0	30.6	36.0	80.0
Pseudomonas florescence.- ST	56.6	56.3	27.0	46.0	70.0
Oil based neem	63.3	54.0	33.3	44.0	88.0
Kernel based neem	65.6	57.0	37.8	46.6	91.0
Ridomil MZ 72 (0.25%) ST and drench	74.6	60.6	39.6	46.0	83.0
Captra 50 WP (0.25%) ST and drench	79.3	59.0	26.5	43.0	90.0
Control (untreated)	39.3	38.3	23.5	26.0	56.0
CD (P= 0.05)	7.3	11.5	7.9	11.1	10.4

Figures were angular transformed before analysis; *Trichoderma viride* (Tv), *Trichoderma harzianum* (Th), and *Trichoderma koningii (*Tk*)* and *Pseudomonas fluorescence* as ST = seed treatment @ 10g prowder formulation/kg seed; Oil based neem or kernel based neem formulation @ (0.5%) as ST= seed treatment and drench (at 15 and 30 days of sowing).

The damping-off in control treatments was maximum and expected and it varied (Table 2) brinjal (57%), tomato (70%), Chilli (38%), cauliflower (44%) and cabbage (41). In various microbial, neem formulations and fungicidal treatment, damping-off disease was significantly reduced to the extent of 16-22% in brinjal with *Trichoderma viride* and kernel based neem formulation as the best, 22-49% in tomato with *Trichoderma harzianum* and Captra 50 W as the best, 15-37% in chilli with *Trichoderma harzianum* and *Trichoderma koningii* as the best, 7-17% in cauliflower with Captra 50 WP and *Pseudomonas florescence* as the best, 14-26% in cabbage with Captra 50 WP and *Trichoderma koningii* as the best (Table 2). These results clearly reveal that microbial agents as seed treatment and neem formulations and fungicidal treatment as well as soil drench significantly reduced the incidence of damping-off and improved plant stand. The least damping-off incidence in brinjal and Chilli was observed in oil based and kernel based neem formulations as compare to Ridomil MZ 72 and Captra 50 WP.

Table 2: Effect of bio-control agents and neem formulations on the incidence damping-off in solanaceous and cruciferous vegetable

Treatment	Damping-off (%)				
	Brinjal	**Tomato**	**Chilli**	**Cauliflower**	**Cabbage**
Trichoderma viride-ST	16.6	32.7	20.0	14.7	22.5
Trichoderma harzianum -ST	20.6	22.1	16.9	10.1	17.3
Trichoderma koningii-ST	19.8	49.4	16.8	11.7	16.4
Pseudomonas florescence.- ST	17.5	45.9	19.7	9.1	17.5
Oil based neem	16.1	38.9	15.6	17.6	26.2
Kernel based neem	16.3	33.7	15.1	9.6	22.2
Ridomil MZ 72 (0.25%) STand drench	21.7	31.8	37.3	11.4	19.0
Captra 50 WP (0.25%)STand drench	20.6	24.9	33.3	7.1	14.5
Control (untreated)	56.9	70.3	38.1	43.9	40.9
CD (P= 0.05)	9.3	15.6	15.5	16.6	20.8

Figures were angular transformed before analysis; *Trichoderma viride* (Tv), *Trichoderma harzianum* (Th), and *Trichoderma koningii (*Tk*)* and *Pseudomonas fluorescence* as ST = seed treatment @ 10g prowder formulation/kg seed; Oil based neem or kernel based neem formulation @ (0.5%) as ST= seed treatment and drench (at 15 and 30 days of sowing).

The dry biomasses in control treatments were min. as expected in and varied in the different crops evaluated: brinjal-8.10g, tomato-0.192g, Chilli-0.92g cauliflower-0.446g, and cabbage-1.421g and (Table 3). In various bio-control agent, neem formulation and fungicidal treatments, the dry biomass was significantly increased to the level of 11.21-12.64g in brinjal with oil based neem formulation and Ridomil MZ 72 as the best, 0.410-0.891g in tomato with Captra 50 WP and *Trichoderma harzianum* as the best, 1.21-2.87g in Chilli with kernel based neem formulation and oil based formulation as the best, 0.660-1.56g in cauliflower with *Trichoderma koningii* and Captra 50 WP as the best. 1.662-3.034g in cabbage with Captra 50 WP and *Trichoderma koningii* as the best (Table 3). All the treatments resulted in enhanced dry biomass of all crops tested. The two neem based formulations were equally effective or superior to chemical fungicides in respect of dry biomass of brinjal and Chilli.

Table 3: Effect of bio-control agents and neem formulations on the dry biomass of solanaceous and cruciferous vegetable.

Treatment	Dry biomass (g/pot)				
	Brinjal	**Tomato**	**Chilli**	**Cauliflower**	**Cabbage**
Trichoderma viride-ST	12.27	0.632	2.16	0.740	1.954
Trichoderma harzianum -ST	11.81	0.842	2.18	0.660	1.662
Trichoderma koningii-ST	12.01	0.410	2.28	1.560	2.234
Pseudomonas florescence.- ST	11.75	0.628	2.19	1.205	2.041
Oil based neem	12.63	0.442	2.43	0.686	1.797
Kernel based neem	11.21	0.698	2.87	0.830	2.095
Ridomil MZ 72 (0.25%) ST and drench	12.53	0.727	1.55	1.105	2.127
Captra 50 WP (0.25%) ST and drench	11.67	0.891	1.21	1.167	3.034
Control (untreated)	8.10	0.192	0.92	0.446	1.421
CD (P= 0.05)	1.7	0.432	0.62	0.422	0.62

Figures were angular transformed before analysis; *Trichoderma viride* (Tv), *Trichoderma harzianum* (Th), and *Trichoderma koningii (*Tk*)* and *Pseudomonas fluorescence* as ST = seed treatment @ 10g prowder formulation/kg seed; Oil based neem or kernel based neem formulation @ (0.5%) as ST= seed treatment and drench (at 15 and 30 days of sowing).

Present study indicated that all the tested bio-control agents and neem formulations had significant impact on seed germination, plant biomass yield and damping-off incidence in solanaceous and cruciferous vegetable and their effects were comparable with the chemical fungicides. *Trichoderma viride* resulted in highest germination of cabbage and least damping-off in cauliflower and max dry biomass in brinjal. *Trichoderma harzianum* resulted in highest germination of cabbage and least damping-off in cauliflower and max dry biomass in brinjal. *Trichoderma koningii* resulted in highest germination of cabbage and least damping-off in cauliflower and max dry biomass in brinjal. *Pseudomonas florescence* resulted in highest germination of cabbage and least damping-off in cauliflower and max dry biomass in brinjal. Oil based neem formulation resulted in highest germination of cabbage and least damping-off in chilli and brinjal and max dry biomass in brinjal. Kernel based neem formulation resulted in highest germination of cabbage and least damping-off in cauliflower and chilli and max dry biomass in brinjal. Ridomil MZ 72 resulted in highest germination of cabbage and least damping-off in cauliflower and brinjal and max dry biomass in brinjal. Captra 50 WP resulted in highest germination of cabbage and least damping-off in cauliflower and cabbage and max dry biomass in brinjal.

Application of bio-control agents in plant disease control are of unique importance because of reducing environmental pollutions. The potential of *Trichoderma* species as biocontrol agents in plant disease control was first recognized in the early 1930s (Weindling, 1932) and subsequently they were applied successfully as biocontrol agents against several plant diseases in commercial agriculture (Howell, 2003). Several superior strains have been identified and formulated into commercial biopesticides (Agrios, 1997). Control may be achieved by competition, production of antibiotics or by mycoparasitism (Campbell, 1989). It exhibits good fungicidal properties and improves plant growth and yield. The present study demonstrates that Three species *Trichoderma* namely: *Trichoderma viride* (Tv) *Trichoderma harzianum* (Th), and *Trichoderma koningii (*Tk***)*** reduced the damping-off and increased dry biomass in the 5 crops examined. These are equal or superior in effectiveness as compared to the chemical fungicides. Similar observations have been reported in earlier studies against damping-off in tomato (Bohra and Mathur 2005) and brinjal and chilli (Bohra et^al 2006). Thus, these

organisms have high potential for the control of damping-off diseases in nurseries and fields.

Among the plant growth promoting Rhizobacteria (PGPR), *Pseudomonas fluorescence* is the most exploited bacteria for biological control of soil-borne and foliar plant pathogens. In the past three decades, numerous strains of *Pseudomonas fluorescence* have been isolated from the rhizosphere soil and plant roots by several workers and their biocontrol activity against soil-borne and foliar pathogens were reported (Vivekananthan *et al.,* 2004). *Pseudomonas fluorescence* are non-pathogenic rhizobacteria which suppress the soil-borne pathogens through rhizosphere colonization, antibiosis, iron chelation by siderophore production and ISR. In present study, seed treatment with *Pseudomonas florescence* resulted in substantial decrease in damping-off incidence and increase in plant dry biomass in all the crops studied. Due to its specific attributes like production of broad-spectrum antibiotics and ability to grow under dry conditions, the use of *Pseudomonas florescence* has immense potential for biological control of damping-off.

Results from the present study indicated that both Neem oil and kernel based formulations were highly effective in controlling the damping-off and increase the dry biomass yield in brinjal, tomato, Chilli, cauliflower and cabbage. Since neem products have several active principles such as azadirachtin, nimbin, nimbidin, nimbinene, nirnbridic acid and azadirone, which are antifungal and insecticidal, these would be useful for eco-friendly management of 'damping-off. Mariappan (1995) has reported that extracts of Neem seeds or oil contain azadirachtin, meliantriol, salannin and other limonoids having inhibitory effect on the growth and sporulation in fungi and as such are effective in controlling the diseases.

References

1. Agrios GN. Plant Pathology. 4th Ed. Academic Press. San Diego. California. USA, 1997.
2. Alexander LJ. The causes and control of damping-off of brinjal, brinjal and chilli seedlings. Ohio Agr. Exp. Sta. Bull, 1931, 496.
3. Atkinson GF. Damping - off. N. Y. (Cornell) Agr. Exp. Sta. Bull. 1895; 94:233-272.
4. Bohra B, Mathur K. Ernluation of native isolates of biocontrol agents and neem based formulations for management of damping-off in brinjal. Indian Journal of Plant protection. 2005; 33:102-104
5. Bohra B, Vyas BN, Mistry KR. Biocontrol agents and neem formulations for management of damping-off in brinjal and chill. Indian Phytopath. 2006; 59:223-226.
6. Campbell R. Biological Control of Microbial Plant Pathogens. Cambridge Univ. Press. UK, 1989.
7. Copper AL, Campbell R. The effete of artificially in inoculated antagonistic bacteria on the prevalence of take all disease of wheal in field experiments. J Appl Bacterial. 1986; 60:155-160.
8. Elad Y, Chet I. Possible role of competition for nutrient in biocontrol of *Pythium* damping-off by bacteria. *Phytopathology*. 1987; 77:190-195.
9. Fageria MS, Choudhary BR, Dhaka RS. Vegetable Crops, Prodoction Tecnology. Kalyani Publishers, Ludhiana, 2003, 25-74.
10. Gomez KA, Gomez AA. Statistical Procedures far Agricultural Research.2*nd* ed, John Wiley and Sons, New York, 1984, 316-356.
11. Gupta VK, Paul YS. Fungal diseases of Brinjal, Chilli and Brinjal. In: Diseases of Vegetable crops. (Ed.) Gupta and Paul, 2001, 87-101.
12. Howell CR. Mechanism mployed by *Trichoderma* species in the biologicalcontrol of plant diseases: The history and evolution of current concepts. Plant Disease. 2003. 87:4-10.
13. Kado CI, Heskett MG. Selective media for isolation of *Agrobacterium, Corvnebacterium, Erwnia, Psendomonas* and Xamhomonas. Phytopathology. 1970; 60:969-976.
14. Manorangitham SK, Preakasam V, Rajoppan AK. Biocontrol of Damping off of Brinjal Caused by Pythium aphanidermatum. Indian Phytopathalogy. 2001; 54:59-61.
15. Mariappan V. Neem for the management of crop diseases. Associated Publishing Co., New Delhi, 1995, 220.
16. Mukhopadhyay AN, Shreshta SM, Mukherjee PK. Biological seed treatments for control of soil borne plant pathogens. *FAO PI Prot Bull* 1992; 40:110-115.
17. Ramesh R. Management of Damping-Off in Brinjal Using Biocontrol Agents. Journal of Mycology and Plant Pathology. 2004; 34:666-670.
18. Ranjan PP, Sharma YR, Anandraj M. Management of foot rot disease of black pepper with *Trichoderma* spp. Indian Phvtopathology. 2002; 55:34-38.
19. Tewari AK, Mukhopadhyay AN. Testing of different formulations of Glio*cladium virens* against chick pea wilt-complex. Indian Phytopathology. 2001; 54:67-71.
20. Tran TT. Antagonistic effectiveness of *Trichoderma* against plant fungal pathogens. Plant Protection. 1998; 4:35-38.
21. Vidhyasekaran P, Rabindran R, Muthamilan M, Nayar K, Rajappan K, Subramanian N *et al*. Development of a powder formulation of *Pseudomonas fluorescens* for control of rice blast. Plant Pathol. 1997; 46:291-297.
22. Vivekananthan R, Ravi M, Ramanathan A, Samiyappan R. Lytic enzymes induced by *Pseudomonas fluorescens* and other biocontrol organisms mediate defence against the anthracnose pathogen in mango. World J Micro Biotec. 2004; 20:235-244.
23. Weindling R. *Trichoderma lignorum* as a parasite of other soil fungi. Phytopathology. 1932; 22:838-845.
24. Whipps JM, Lumsden DR. Biological control of Pythium species. Biocont. Sci Technol. 1991; 1:75-90.