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Evaluation of bio-control agents and new fungicides to control Pythium damping off in nursery of solanaceous vegetable crops

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Abstract

Nursery of solanaceous vegetable crops severely infected by damping - off. Commercial cultivation of solanaceous vegetables are depends on healthy and disease free seedlings under nursery condition in fields. Seed treatments are most cost effective and commonly used delivery system for bio-agents and fungicides. Nine modules comprises three talc based fungal [*Trichoderma viride*- 1 (IIVR–BATF-39-1), *T. viride*-2 (IIVR-BATF-43-1), *Trichoderma harzianum* -Kalyanpur)], two bacterial (*Bacillus subtilis* (IIVR-BS2, *Pseudomonas fluorescens* -Kalyanpur) formulations and four fungicides (carbendazim 12% + mancozeb 63% WP, pencycuron 250 SC, fosetyl-Al 80% WP, fenamidone 10% + mancozeb 50% (WP) were evaluated under nursery of tomato, brinjal and chilli in *kharif* season during 2016-2018. Among tested bio-formulations of bio-agents *Bacillus subtilis* (IIVR-BS2) was found effective for highest germination percentage in tomato (84.77) and brinjal (66.81) and lowest incidence of damping off 15.22% and 33.18%, repectively whereas cost benefit ratio (CBR) 1: 79.98 (tomato var. Kashi Aman) and 1: 36.69 (brinjal var Kashi Taru) were recorded. However, among all tested modules, a fungicide carbendazim 12% WP + mancozeb 63% WP was found most effective in chilli for highest germination percentage (86.58), vigour index (479.52) and lowest incidence of damping off (13.30) with cost benefit ratio (CBR) 1: 90.79. Hence, use of bio-agent *Bacillus subtilis* (IIVR-BS2) 3.92×10^{11} cfu/g in tomato and brinjal; however, combiproduct of carbendazim (12%) and mancozeb (63%) in chilli may be recommended for management of damping off in nursery.

Keywords: Biocontrol agents; Seed treatment; Fungicide; Solanaceous vegeables, Phytopathogens, Soil borne diseases

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Introduction

Damping off is a wide host range soil borne disease infecting crops like brinjal, chilli, canola, dry pea, tomato, sugar beet and safflower (Tripathi et al. 2019, 2020, 2021). Commercial cultivation of solanaceous vegetables are depends on healthy and disease free seedlings. Nursery of brinjal, chilli and tomato severely infected by damping off, root rot, bacterial blight and bacterial rot and wilt. Several strains of fungal and bacterial bio-agents have been found to control plant diseases in vegetable crops. Formulations of *Pseudomonas, Stenotrophomonas* and *Bacillus* intensively and extensively documented as plant growth promoting bacteria that have been successfully used as biological seed treatments against plant pathogens and plant growth promotion (Chen et al. 2009; El-Sayed et al. 2014). Biological control is an effective option to minimize the use of expensive synthetic fungicides in modern agriculture, as they are the main cause of resistance development in pathogens besides polluting the environment. Broad spectrum mechanisms of bio-control action viz. competition with phytopathogens for an space and nutrition, production of broad spectrum antibiotic compounds and cell wall degrading enzymes against phytopathogens very well documented by researchers (Harman et al. 1989; Manjula et al. 2004; Loganathan et al. 2016). There is an urgent requirement to find sustainable strategy for the control of nursery diseases. In this study, we investigated that the use of talc based formulation of fungal and bacterial bio-agents were evaluated for nursery disease management and growth promotion of seedlings in brinjal, chilli and tomato.

Materials and Methods

Five talc based formulation of fungal and bacterial bio-agents including *Trichoderma viride*- 1 (IIVR –BATF-39-1), *T. viride*-2 (IIVR-BATF-43-1), *Trichoderma* *harzianum* (Kalyanpur), *Bacillus subtilis* (IIVR-BS2), *Pseudomonas fluorescens* (Kalyanpur) and four fungicides namely (carbendazim 12% + mancozeb 63% WP, pencycuron 250 SC, fosetyl-Al 80% WP, fenamidone 10% + mancozeb 50% (WP) were evaluated against damping off disease in brinjal, chilli and tomato under nursery condition in *kharif* season during 2016-2018.

Nursery plots: Plots sizes were 1m×1m. One thousand seeds of *Solanum lycopersicon* (tomato cv. Kashi Aman, 2g), *Solanum melongena* (brinjal cv. Kashi Taru, 2g), *Capsicum annum* (chilli cv Kashi Anmol, 2.5 g) were sown in each plot. Nursery sowing of brinjal and chilli was done on dates viz. 16 August 2016, 20 September 2017, 19 September 2018 however in case of tomato seed sown in nursery on 2 September 2016, 26 September 2017 and 19 September 2018. The experiment was planned as a randomized block design with 3 replications for each treatment.

Bio-control and chemical modules: Bi-ocontrol module comprises five talc based formulation of bio-agents viz *Bacillus subtilis* (IIVR-BS2) $3.92x10^{11}$ cfu/ g; *Trichoderma viride*- 1 (IIVR –BATF-39-1); *T. viride*-2 (IIVR-BATF-43-1); *Trichoderma harzianum* (Kalyanpur) $1.5x10^{8}$ cfu/g; *Pseudomonas fluorescens* (Kalyanpur) $2x10^{7}$ cfu/g were evaluated by application of 4g/kg seed, soil application as 10g/m^{2} and soil drenching @ 5% and chemical module consisted four fungicides namely (carbendazim 12% + mancozeb 63% WP) @ 1.5g/kg seed treatment with drenching @ 0.1%, (pencycuron 250 SC) @ 1ml/litre drenching, (fosetyl-Al 80% WP) @ 0.1% drenching, (fenamidone 10% + mancozeb 50% WP) @ 0.25% drenching under nursery of tomato, brinjal and chilli in *kharif* season from 2016-2018. Benefit cost ratio was assessed for each treatment.

Germination and vigour index: In order to determine the eûect of seed treatment on seed germination in each replication germination percentage was recorded on the 7 day after seed sowing in the nursery. Root and shoot length were measured approximately 21 days old 10 seedlings from each replication. Germination percentage and vigour index were calculated by following of formula given as: Germination (%) = number of seeds germinated/total number of seeds ×100; Vigour index = % germination × total plant length. Moreover, the number of healthy seedlings was counted in each plot on 15 days after sowing. Thereafter, diseased seedlings were collected from each replication of the treated and untreated control. Infected plant portion were disinfected with sodium hypochlorite (0.5% a.i.) for 1 min, blotted dry on a tissue paper and plated on 4% potato dextrose agar. Fungal colonies growing from roots were identified on the basis of morphology.

Results and Discussion

Effect of bio-agents & fungicides on Damping off: Seed and soil treatment of *Bacillus subtilis* (IIVR, BS-2) revealed that lowest incidence of damping off in tomato (15.22%) and in brinjal (33.18%), however, in case of chilli treatment with combiproduct of carbendazim (12%) and mancozeb (63%) recorded lowest incidence of damping off (13.30) over than control (Table 1). Benefit cost ratio (BCR) was sown that it was maximum with treatment *Bacillus subtilis* (IIVR- BS-2) which was 1: 7.99 in tomato cv Kashi Aman and 1: 3.66 in brinjal cv Kashi Taru however in chilli (cv. Kashi Anmol) it was 1: 9.07 recorded with combiproduct of carbendazim (12%) and mancozeb (63%).

Results from our study indicate that bio-agents and fungicides applied as a seed treatment and soil application are signiûcantly reduced damping off severity on tomato, brinjal and chilli nursery. Among the tested treatments, *Bacillus subtilis* (IIVR-BS2) was effective for highest germination and lowest damping off incidence with highest benefit cost ratio in tomato cv. Kashi Aman and brinjal cv. Kashi Taru. Seed treatment with combiproduct of carbendazim (12%) and mancozeb (63%) found most effective for highest germination percentage, vigour index and lowest incidence of damping off with highest cost benefit ratio (CBR) 1: 9.07 in chilli cv. Kashi Anmol. Significant progress has been made in the past many researcher reviewed that biological seed treatments were found effective against damping-off in vegetable crops (Harman et al. 1989). Bacillus and Pseudomonas are the most commonly used seed treating plant growth promoting rhizobia. Seed treatment are easy, cost effective and potential delivery system for bio-agents such as *Bacillus subtilis* (IIVR-BS2), *Pseudomonas fluorescens* (Kalyanpur), *P. fuorescens* 700, *Trichoderma viride*- 1 (IIVR –BATF-39-1)], *T. viride*-2 (IIVR-BATF-43-1), *Trichoderma harzianum (*Kalyanpur) which were found effective for the control of damping – off disease of various vegetable crops (Tripathi et al. 2019). The fungal antagonists *Pseudomonas stutzeri, P. fluorescens*, *B. subtilis, B. amyloliquifaciens, S. maltophilia, Trichodderma* spp have been shown to be eûective biocontrol agents against soil borne diseases (Tripathi et al. 2019; Dal Bello et al. 2002; Erlacher et al. 2014). Biological control of soil borne phytopathogens can also result from antibiosis by the bacteria (Nakayama et al.

1999; Chung et al. 2008; Islam et al. 2016). Biological control is an effective option to minimize the use of expensive chemical fungicides in modern agriculture (Plan-Paulitz 1992; Loganathan et al. 2016; Pandey et al. 2016). In past, many researchers found that *P. fluorescens* 54/96 compete for nutritional requirement with *Pythium* sp. and produces antifungal compound.

Effect of bio-agents & fungicides on germination and vigour index: The eûect of seed treatments on seed germination and vigour index of brinjal, chilli and tomato varied with diûerent bio-agents and fungicides. All treatments had a signiûcant eûect on the germination rate and vigour index of seedlings compared to the untreated control. Result revealed that the seed germination percentage was highest in tomato cv. Kashi Aman (84.77) and brinjal cv. Kashi Taru (66.81) in treatment with *Bacillus subtilis* (IIVR-BS2) compared with the control. In tomato treatment with carbendazim 12% + mancozeb 63% WP (T6) @1.5g/kg + drenching @ 0.1% produced both the maximum shoot and root lengths of 4.75 and 5.64 cm however in case of brinjal maximum shoot length (4.80 cm) produced in *Pseudomonas fluorescens* (Kalyanpur) (T 5) @ 4g/kg seed, soil application as 10g/m^2 and soil drenching @5% and root length (4.17) in *Trichoderma harzianum* (Kalyanpur) (T4) @ 4g/kg seed, soil application as 10g/m^2 and soil drenching @5%; an compared to control treatments. However, maximum seedling vigour index in tomato cv. Kashi Aman (1035.67) and brinjal cv Kashi Taru (308.30) recorded with T6 and T4 respectively. In case of chilli (var Kashi Anmol) treatment T6 involving combiproduct of carbendazim (12%) with mancozeb (63%) was gave highest germination percentage (86.58) and maximum vigour index (545.83) revealed with T5 (Table 1). These results suggest that all treatments could improve the germination percentage and vigour index of tomato and brinjal seedlings.

All the treatment exhibited better germination; seed vigor and seedling stand in nursery. Plant growth promoting bacteria stimulate seed germination, plant growth and development (Glick et al. 1999; Chaurasia et al. 2018). Seed treatment with bio-agents control soil borne plant pathogens, enhance seed germination and vigour index by reducing the seed borne inoculum and incidence of pathogens (seed mycoflora) (Begum et al. 2003). Most commonly reported mechanisms are amylase hydrolyzes activity and phytoharmone (indole acetic acid) production to promote growth of roots and shoots in seedlings (Carrillo et al. 2002). Microbial bio-agents induced systemic resistance, produce antibiotics and lytic enzymes cause membrane damage and are particularly inhibitory to zoospores of Oomycete. Application of bio-agent *Bacillus subtilis* (IIVR) as seed treatment @ 4g/kg seed, soil application as 10g/m^2 and soil drenching @ 5%, in tomato and brinjal nursery however, in chilli nursery combiproduct of carbendazim (12%) and mancozeb (63%) @ 1.5g/kg with drenching @ 0.1% may be recommended for

Table 1: Effect of different bio-agents and chemicals on germination percentage, seedling vigour, disease incidence and benefit cost ratio in brinjal, chilli and tomato nursery

Treatment* Parameter		T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	SE(m)
Germination (%)	Brinjal	66.81	58.92	51.84	64.58	55.85	58.47	52.33	49.03	55.66	47.22	5.95
	Chilli	81.55	70.25	78.07	80.07	85.03	86.58	79.25	63.95	63.51	62.44	4.89
	Tomato	84.77	75.63	74.95	82.56	75.58	75.62	70.48	70.81	66.85	60.70	5.77
Seedling height (cm)	Brinjal	4.56	3.77	4.58	4.27	4.80	4.67	4.53	4.56	4.59	3.90	0.30
	Chilli	4.55	5.02	4.41	4.86	5.00	4.22	5.02	4.43	4.38	4.01	0.31
	Tomato	4.17	4.51	3.85	4.57	4.07	4.75	4.24	4.08	4.18	3.77	0.25
Root length (cm)	Brinjal	3.88	3.77	3.35	4.17	3.88	3.64	3.80	3.77	3.91	2.90	0.22
	Chilli	3.54	4.60	3.75	4.23	4.20	4.60	4.23	3.52	3.35	3.33	0.33
	Tomato	4.23	5.50	4.81	5.44	5.61	5.64	5.23	5.17	5.07	5.61	15.10
Vigour index	Brinjal	302.88	293.59	256.29	308.30	274.51	307.24	265.53	270.59	280.29	166.96	27.05
	Chilli	397.72	409.85	428.02	46.31	545.83	479.52	434.93	333.88	310.31	256.66	40.92
	Tomato	823.35	824.24	703.32	938.65	800.15	1035.67	775.30	859.06	847.31	766.12	81.92
Damping off	Brinjal	33.18	41.18	48.15	35.46	44.03	41.51	46.77	50.74	44.77	50.89	5.85
	Chilli	18.44	29.74	22.26	19.89	14.96	13.30	20.74	36.03	36.28	35.78	4.87
	Tomato	15.22	24.36	24.92	17.44	24.40	24.37	29.51	29.51	33.14	39.29	5.73
BCR	Brinjal	3.66	2.95	2.52	3.42	2.76	2.91	2.58	2.38	2.70	2.37	-
	Chilli	6.59	4.08	5.46	6.11	8.12	9.07	5.82	3.35	3.32	3.37	-
	Tomato	7.99	4.99	4.88	6.97	4.98	9.99	4.07	4.09	3.64	3.07	-

*Treatment T_1- *Bacillus subtilis* (IIVR)-BS2, T_{2}-*Trichoderma viride*-1(IIVR) BATF-39-1, T3-*Trichoderma viride*-2 (IIVR)-BATF-43-1, T_{4}- *Trichoderma harzianum* (Kalyanpur), T_{5}- *Pseudomonas fluorescens* (Kalyanpur) @ 4g/kg seed, soil application as 10g/m^2 and soil drenching @5%; T_{6}- carbendazim 12% + mancozeb 63% WP@1.5g/kg + drenching @ 0.1%; T_{7}- Pencycuron 250 SC @1ml/litre drenching; T_{8}- Fosetyl-Al 80% WP@ 0.1%drenching; T_{9}- Fenamidone 10% + mancozeb 50% (WP) @0.25% drenching; T_{10}- untreated control management of damping off in tomato, brinjal and chilli nursery. This reveals that the potential of these bio-agents and fungicides exploit as seed treatments and soil applications in the nursery.

Hence, talc based formulation of *Bacillus subtilis* (IIVR) strain BS-2 WP (3.92×10^{11} cfu/g) could be commercially exploited as alternative of commonly used seed treating fungicides by the farmers/organic vegetable grower as seed treatment, soil amendment and soil drenching for raising of disease free healthy nursery of solanaceous vegetables namely tomato and brinjal. Future studies concerning multiplications testing of these bio-agents under ûeld condition and commercialization of potential bio-formulations are in progress.

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Lkkjka'k

टमाटर वर्गीय सब्जियों की व्यावसायिक खेती रोग मुक्त नर्सरी पौध पर निर्भर करती है। नौ मॉड्यूल में तीन [*ट्राईकोडर्मा विराइट*–1 (आई.आई.वी.आर.–बी.ए.टी.एफ.–39–1), 2 (आई.आई.वी.आर.–बी.ए.टी.एफ.–43–1) *ट्राईकोडर्मा हर्जियानम*–कल्याणपुर)] दो जीवाणु (*बैसिलस सबटिलिस*), आई.आई.वी.आर.बी.एस.–2, *स्यूडोमोनास फ्लोरेसेंस*–कल्याणपुर) संरूपण और चार कवकनाशी (कार्बेंडाजिम 12 प्रतिशत + मैंकोजेब 63 डब्ल्यू.पी., पेन्सीक्यूरॉन 250 एस.सी., फोसेटिल–ए.एल. 80 प्रतिशत डब्ल्यू.पी., फेनामिडोन 10 प्रतिशत + मैंकोजेब 50 प्रतिशत (डब्ल्यू.पी.) का मूल्यांकन टमाटर की पौधशाला में किया गया। वर्ष 2016–2018 (खरीफ मौसम) में बैंगन और मिर्च में परीक्षण किया गया। जैव–सूत्रों में *बैसिलस सबटिलिस* (आई.आई.वी.आर.–बी.एस.–2) टमाटर (84.77) और बैंगन (66.81) में उच्चतम अंकुरण प्रतिशत के लिये प्रभावी पाया गया जबकि लागतःलाभ अनुपात (सी.बी.आर.) 1:79.98 (टमाटर किस्म काशी अमन) और 1:36.69 (बैंगन किस्म काशी तरू) में दर्ज किया गया। कवकनाशी मॉड्यूल (कॉम्बीप्रोडक्ट कार्बेन्डाजिम 12 प्रतिशत डब्ल्यू.पी. और मैंकोजेब 63 प्रतिशत डब्ल्यू.पी.) मिर्च में उच्चतम अंकुरण प्रतिशत (86.58) के लिए सबसे प्रभावी पाया गया (6.58), ओज सूचकांक (479.52) एवं सबसे कम डैंपिंग आफ (13.30) और लागतःलाभ अनुपात (सी.बी.आर.) 1:90.79 पाया गया। इसलिए टमाटर और बैंगन में बायो–एजेंट *बैसिलस सबटिलिस* (आई.आई.वी.आर.–बी.एस.–2) का उपयोग, मिर्च में कार्बेंडाजिम (12 प्रतिशत) और मैंकोजेब (63 प्रतिशत) के संयोजन की सिफारिश पौधशाला में डैंपिंग ऑफ के प्रबंधन के लिए की जा सकती है।

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