Prospects of Botanicals and Microbial Bioagents on Seedling vigour of Brinjal *vis-a-vis* combating Damping off pathogen *Pythium aphanidermatum*

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J. Mycopathol. Res. 61(1) : *21-30, 2023;*
ISSN 0971-3719

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J. Mycopathol. Res. **61(1)** : 21-30, 2023; ISSN : 0971-3719 (Print), 2583-6315 (Online) doi. 10.57023/JMycR.61.1.2023.021

Prospects of Botanicals and Microbial Bioagents on Seedling vigour of Brinjal *vis-a-vis* combating Damping off pathogen *Pythium aphanidermatum*

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Received : 20.10.2022 Accepted : 29.12.2022 Published : 27.03.2023

Effects of seven botanicals (essential oils and plant extracts), Citronella oil, Palmarosa oil, Lemon grass oil, Neem seed oil, Babul leaf extract, Tamarind leaf extract and Neem leaf extract and five microbial bio-agents (three fungal, *Trichoderma viride*, *T. harzianum*, *Aspergillus niger* and two bacterial, *Pseudomonas fluorescens*, *Bacillus subtilis*) were tested for induction of seedling vigour of brinjal (*Solanum melongena* cv. *pusa swani*) prior to application for management of pre- and post- emergence Damping off disease of nursery seedlings of brinjal, caused by *Pythium aphanidermatum* (Edson) Fitz. Seed soaking with plant extracts and seed-coating with bioagents in particular doses, screened through laboratory experiment, were used in both *in vitro* and *in vivo* conditions to observe the effects of those requisite doses of botanicals and bio-agents on growth and vigour of brinjal seedlings. The results revealed that, both botanicals and bioagents significantly increased the growth and vigour of seedlings as well as reduced disease incidence. The best results were exhibited by 0.025% Palmarosa oil or 0.020% Citronella oil (@10 ml suspension per 10 g seed) among the botanicals and by *T. viride* or *T. harzianum* (@10 ml suspension containing 4 x 10^6 spores / ml per 10g seed) among the microbial bio-agents in this regard.

Keywords: Bio-agents, *Cymbopogon winterianus*, essential oils, germination, plant extracts, seed treatment, seedling vigour

INTRODUCTION

Brinjal (*Solanum melongena*) is one of the most widely known and utilized species of the family Solanaceae and is suitable for cultivation as a garden crop as well as on large commercial farms throughout the year in India.

Now-a-days, hybrid varieties are being extensively used for maximum return and raising of seedlings in seedbed faces maximum threat and sometimes total loss of costly seeds owing to various notorious soil-borne pathogens. In an attempt to prevent the loss, farmers are accustomed in using chemical pesticides in field with no concern to ecology and non- target effects. Hence, mitigation of chemical substances using natural pesticides is the best path to solve these problems. Over the last two decades, using the biological control has been proved to be beneficial and alternative measure in developing an eco-friendly management (Gawande, *et. al*., 2009 and Waghunde *et. al.* 2016). The plant extracts is one of the interesting ways to substitute chemical substances for inhibition of plant pathogens which does not render wicked problems to environment and living being in the world. Prospects of use of plant products or botanicals for plant disease control have been explored by different workers in management of Damping off (Pandey *et al.* 2017; Rajput *et. al.* 2018) and some other soil-borne, foliar and post harvest diseases (Kulkarni, 2009; Sreenivasa *et. al*. 2011). Botanical seed treatment is a liquid formulation and it has synergistic effect on early and uniform seed germination as well as in inducing seedling vigour and enhances tolerance to pest and disease during early crop stage. Several workers have found that different botanicals (Patil *et. al*. 2015, Sinha and Kumar ,2019) can induce plant growth parameters. The objective of the present investigation was aimed to determine the

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effect of some common biofriendly plant products on germination as well as vigour of the treated seeds which may help them to combat with the Damping-off pathogen *P. aphanidermatum*.

MATERIALS AND METHODS

Laboratory experiment was conducted to evaluate the efficacy of seven botanicals (essential oils and plant extracts), Citronella (*Cymbopogon winterianus*) oil, Palmarosa (*C. martini* var. *motia*) oil, Lemon grass (*C. citratus*) oil, Neem (*Azadirachta indica*) seed oil, Babul (*Acacia nilotica*) leaf extract, Tamarind (*Tamarindus indica*) leaf extract and Neem (*Azadirachta indica*) leaf extract for a synergistic effect of growth promotion as well as management of pre- and post-emergence Damping off disease of nursery seedlings of brinjal (*Solanum melongena* var. *pusa swani*) caused by *Pythium aphanidermatum* (Edson) Fitz.

The experiment was conducted in two parts- In the first part, seed-soaking with botanicals and seed coating with the bio-agents were done and both *in vitro* and *in vivo* experiment were carried out to observe the growth promotion effect and seedling vigour. Next part was the management part, where seed coating with bioagents and seed soaking with botanicals were applied against the test pathogen in artificially inoculated soil condition.

Isolation of pathogen

The pathogen was isolated from the infected seedlings of brinjal by tissue segment method (Rangaswami, 1958) and identified as *Pythium aphanidermatum* from Indian Type Culture Collection, Division of Plant Pathology, IARI. Pathogenecity test was conducted following Koch's postulates.

Screening of botanicals

Bioassay of seven botanicals viz. Citronella (*Cymbopogon winterianus*) oil, Palmarosa (*C. martini* var. *motia*) oil, Lemon grass (*C. citratus*) oil, Neem (*Azadirachta indica*) seed oil, Babul (*Acacia nilotica*) leaf extract, Tamarind (*Tamarindus indica*) leaf extract and Neem (*Azadirachta indica*) leaf extract against the test pathogen was carried out to evaluate the tolerance limit by poisoned food technique at different dosages. The oils were extracted from the aromatic grasses, Citronella and Palmarosa, Lemon grass and Neem seed through hydro-distillation and the leaf extracts were obtained through methanolic extraction of leaves of Tamarind, Neem and Babul in Centre for Aromatic and Medicinal plant, Central Regional Research Farm, Gayeshpur, Nadia, West Bengal. Tween- 80 (0.1%) was used as emulsifier for preparing the aqueous solution of the essential oils and preserved as 100% stock solution. Percent inhibition was measured following "Poisoned food technique" and the ED_{50} was calculated for each botanical except for Neem (*Azadirachta indica*) seed oil and leaf extract, as both the botanicals failed to show any growth inhibition of the test fungus even @ 15% and 10% respectively.

Collection of microbial bioagents

The fungal bioantagonists *Trichoderma harzianum*, *Trichoderma viride* and *A. niger* (AN-27, Kalisena) were collected from Indian Type Culture Collection, Division of Plant Pathology, IARI, New Delhi. Among the two bacterial antagonists, *Pseudomonas fluorescens* was collected from Centre of Advanced Faculty Training in Plant Pathology, Gobind Ballabh Pant University of Agriculture and Technology, Uttarakhand and *B. subtilis* from Department of Plant Pathology, BCKV. *In vitro* screening of the antagonistic property against the pathogen were done using dual culture plate method (Dennis and Webster, 1971) and the degree of hyper-parasitism was rated on Bell's scale 1- 5.

Seed soaking with botanicals

Brinjal seeds were soaked in requisite dosages of botanicals (@ 0.025% palmarosa oil, 0.020% lemon grass oil, 0.020% citronella oil, 4.0% *A. nilotica* leaf extract and 1.5% *T. indica* leaf extract) for 30 minutes at their different concentrations selected through ED_{50} value obtained from the earlier experiment and then shade-dried. Neem (*Azadirachta indica*) seed oil and leaf extract were discarded for their poor efficacy to control *P. aphanidermatum*. To determine the effects of botanicals on the growth and vigour of brinjal seedlings, both laboratory and greenhouse experiments were conducted.

Seed coating with microbial bio-agents

Seed coating with fungal spore was done by dipping the surface sterilized seeds in spore

suspension of fungal agents containing 4× 10^6 spores/ ml and 0.1% carboxy-methyl cellulose (CMC) for 1 hr and then shade-dried. 10 ml suspension was used for 10 g of seeds. CMC was used as sticking agent. Seed coating with bacteria was done by dipping the surface sterilized seeds in bacterial suspension containing 3× 10^8 cfu/ ml and 0.1% carboxy-methyl cellulose for 1 hour and then shade-dried. 10 ml suspension was used for 10 g of seeds. Seed coated with 0.01% CMC without any bioagents served as control.

In vitro growth promotion experiment in Laboratory

The experiment was carried out under Laboratory condition in Department of Plant Pathology, BCKV, Mohanpur, Nadia, West Bengal. For *in vitro* experiment, Roll Towel method (ISTA, 1976) was used. Seventy five of treated seeds were placed on two moist paper towels of 23× 30 cm size per replication and kept over a butter paper of 25× 37 cm size. Seeds were kept at an appropriate spacing and covered with another moist paper towel and rolled up. These rolled paper towels were kept at an upright position in incubator at 28± 2º C and 95.2% RH. For each treatment, three replications were maintained. A parallel control was also maintained, where instead of plant extract, only distilled water was used.

Observations were recorded on germination percentage, root length and shoot length of the seedlings after 7 days and 10 days after sowing. Vigour index were calculated following the procedure, suggested by Abdul- Baki and Anderson (1973).
Vigour Index= (Mean root length+ Mean shoot length) × Germination percentage.

In vivo growth promotion experiment in greenhouse

For Greenhouse experiment, sand method was used. Enamel coated iron trays of 45× 30× 8 cm size were filled with fresh and clean white sand. Seventy five treated seeds were sown in rows at an equal distance in small drill and then covered with sand. Trays were kept in complete randomized block design (CRBD) with three replications for each of the test botanical. Sand was kept moist by watering twice a day by sprayer. Data recording was done up to 14 days after sowing at a regular interval. On 14th days after sowing, the top layer of the sand was sieved through a wire-mesh. Number of seeds germinated, root and shoot length and seedling vigour were determined.

RESULTS AND DISCUSSION

All the test extracts, except *Azadirachta indica* (both seed oil and leaf extract) exhibited fungitoxic property significantly, suppressing the mycelial growth of *P. aphanidermatum*. Neem oil and extract showed no inhibition against the test pathogen even at the concentration of 15% and 10% respectively. The ED_{50} values obtained were 0.021%, 0.017%, 0.017%, 3.381%, and 1.253% for Palmarosa oil, Lemon grass oil, Citronella oil, *A. nilotica* leaf extract and *Tamarindus indica* leaf extract respectively.

Effect of botanicals on growth parameters in Laboratory (Roll Towel method)

The *in vitro* experiment (Roll Towel method) showed that, seed soaking with different plant products had increased the germination percentage at 7& 10 DAS, as compared to untreated control (70% and 89.44% respectively), no test plant product had adverse effect on the seed germination (Table 1). Maximum germination was noticed in citronella oil (93.33 at 7 DAS and 98.33 at 10 DAS) followed by palmarosa oil (90.0 at 7 DAS and 96.67 at 10 DAS) and *A. nilotica* leaf extract (85.0 at 7 DAS and 96.67 at 10 DAS). Minimum germination was noticed in *T. indica* leaf extract (75.0 at 7 DAS and 90.0 at 10 DAS) which was statistically at par with control (70% and 89.44%).

Seed soaking with most of the plant extracts increased (37.32% to 4.87%) the root length of seedlings significantly, as compared to untreated control (58.67 mm at 10 DAS). Maximum root length was recorded in palmarosa oil (80.57 mm) followed by citronella oil (79.67 mm) at par with lemon grass oil (76.78 mm) at 10 DAS. Minimum root length was recorded in *T. indica* leaf extract (61.53 mm). *A. nilotica* leaf extract (67.83 mm) exhibited intermediate result. Seed soaking with botanicals also increased (18.67% to 10.84%) the shoot length of brinjal seedlings significantly as compared to untreated control (32.21 mm at 10 DAS). Maximum shoot length was recorded in citronella oil (50.88 mm) followed by palmarosa oil (45.14 mm) at 10 DAS. Minimum shoot length was

recorded in *T. indica* leaf extract (32.33 mm) statistically at par with control (32.21 mm). Lemon grass oil (36.07 mm) and *A. nilotica* leaf extract (35.70 mm) exhibited intermediate result at 10 DAS (Table 1).

The seed treatment effect finally was also reflected on vigour index of seedlings. All the botanicals increased the vigour index significantly in comparison to untreated control. Maximum vigour index was recorded in citronella oil (12827.50, 60.29% increase over control) followed by palmarosa oil (12169.67, 52.07% increase over control). Intermediate results were noticed in lemon grass oil (10530.50, 31.59% increase) and *A. nilotica* leaf extract (10013.73, 25.13% increase). However, *T. indica* leaf extract (8461.85, 5.73% increase) failed to show any significant difference in vigour index as compared to untreated control (8002.0). Interaction between plant-extracts × days after sowing showed no significant difference among themselves in respect to germination percentage, root length, shoot length and vigour index of tomato seedlings (Table 1).

Above all, the results suggested that the test plant extracts used as seed soaking of brinjal showed no phytotoxicity but revealed growth promoting effects.

Effect of botanicals on growth parameters in greenhouse (Sand method)

In *in vivo* condition (Table 2), most of the botanicals exhibited superior germination (37.84% to 21.78%) effect over control. Best result was obtained in palmarosa oil (68.67%) followed by citronella oil (64.33%), lemon grass oil (64.0%) and *A. nilotica* leaf extract (60.67%). *T. indica* (54.88%) showed at par result with control (49.82%) regarding germination at 10 DAS.

The results of the relative performances of different plant extracts revealed that, most of the plant extracts increased (49.60% to 20.98%) the root length of seedlings significantly as compared to untreated control. Maximum root length was recorded in palmarosa oil (65.33 mm) statistically at par with citronella oil (64.33 mm), followed by lemon grass oil (61.67 mm). Minimum root length was recorded in *T. indica* leaf extract (46.87 mm) at par with control (43.67 mm). *A.nilotica* leaf extract (52.83 mm) exhibited intermediate result. Similarly, different plant extracts also increased (95.78% to 24.04%) the shoot length significantly, as compared to untreated control. Maximum shoot length was observed in citronella oil (40.88 mm) followed by palmarosa oil (35.20 mm) at 10 DAS. Lemon grass oil (26.13 mm) and *A. nilotica* leaf extract (25.90 mm) exhibited intermediate result. No significant difference in shoot length was observed in and *T. indica* leaf extract (22.50 mm) in relation to untreated control (20.88 mm).

All the botanicals significantly increased the vigour index of brinjal seedlings as compared to untreated control (3193.52). Maximum vigour was observed in palmarosa oil (6889.87, 115.75% increase over control), closely followed by citronella oil (6778.50, 112.24% increase). Intermediate result was observed in case of lemon grass oil (5612.07, 75.33% increase) and *A. nilotica* leaf extract (4791.90, 50.02% increase). *T. indica* leaf extract (3833.59, 20.04% increase), however, was not found to be promising in increasing seedling vigour. All results are presented in Table 2.

Interactions between plant extracts × days after sowing showed no significant difference among themselves in respect to increase in germination percentage, root length, shoot length and vigour index of brinjal seedlings.

The results therefore suggested that, plant extracts showed no phytotoxic effects but also promoted the growth of the seedlings compared to untreated control. The present study is an important step in developing plant based pesticides which are ecofriendly for the management of the plant pathogenic fungi. The best result was shown by citronella oil and palmarosa oil followed by lemon grass oil. Similar result was also observed by Patil *et. al.* (2015), who found the germination and growth promotion effect of botanicals like neem and vekhand powder on stored pulses. Sinha *et. al.* (2019) found superior germination percentage and seedling vigour of maize plants, when seed treatment were done with different combinations of five botanicals, *viz*., onion, tulsi, neem, zinger and garlic. This needs further studies, to test the efficacy of these crude extract under field conditions. Furthermore, the allelochemicals present in these extracts responsible for germination and growth promotion should be identified and isolated. There is a possibility of using these allelochemicals directly.

Table 1 : Effect of different botanicals as seed soaking on germination percentage, root length, shoot length and vigour index of Brinjal in Laboratory condition (Roll Towel method)

Treatments	DAYS AFTER SOWING							
	Germination percentage		Root length (mm)		Shoot length (mm)		Vigour Index	
	7 DAS	10 DAS	7 DAS	10 DAS	7 DAS	10 DAS	7 DAS	10 DAS
Palmarosa oil	90.0 (75.0)	96.67 (83.86)	67.17	80.57	35.73	45.14	9294.70	12169.67
Lemon grass oil	86.67 (68.86)	93.33 (75.24)	63.98	76.78	30.08	36.07	8163.97	10530.50
Citronella oil	93.33 (77.71)	98.33 (85.69)	67.47	79.67	43.23	50.88	10306.03	12827.50
A. nilotica leaf extract	85.0 (67.40)	96.67 (81.39)	57.23	67.83	27.62	35.70	7267.23	10013.73
T. indica leaf extract	75.0 (60.07)	90.0 (74.53)	48.53	61.53	23.11	32.33	5383.83	8461.85
Control	70.0 (56.84)	89.44 (71.27)	41.47	58.67	20.53	32.21	4354.57	8002.0
Mean	83.33 (67.65)	94.07 (78.33)	57.74	70.84	30.05	38.72	7461.72	10334.21
	SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%
Treatment	3.05	8.90	1.25	3.66	1.23	3.59	646.44	1886.91
DAS	1.76	5.14	0.72	2.11	0.71	2.08	373.22	1089.41
Treatment x DAS	4.31	NS	1.77	NS	1.74	NS	914.20	2668.49

* Figures are mean values of three replicas.
** Figures in the parenthesis are indication of angular transformed value.

Effect of microbial bioagents on growth parameters in Laboratory (Roll Towel method)

The *in vitro* experiment (Roll Towel method) revealed that (Table 3), seed coating with the microbial bio-agents had no such effect on germination percentage, as compared to untreated control (76.0% and 88.33% at 7& 10 DAS respectively). Only 10.69% to 3% increase has been noticed.

But seed coating with all the bio-agents increased (67.50% to 34.20%) the root length of seedlings significantly as compared to untreated control (40.08 mm) at 10 DAS. Maximum root length was recorded in *T. harzianum* (67.14 mm) followed by *B. subtilis* (65.23 mm). Minimum root length was recorded in *A niger* (53.79 mm) and intermediate results was obtained in *Ps. fluorescens* (61.57 mm) statistically at par with *T. viride* (60.59 mm). Seed treatment also proved to be superior (111.67% to 55.0% increase) regarding the shoot length of brinjal seedlings as compared to untreated control (20.98 mm) at 10 DAS. Maximum shoot length was recorded in *T. harzianum* (44.41 mm) followed by *T. viride* (43.58 mm)> *A. niger* (41.89 mm) > *Ps. fluorescens* (37.31 mm) > *B. subtilis* (32.52 mm) and their differences were statistically significant.

Seed coating treatment increased the vigour index significantly as compared to untreated control (6494.01) at 10 DAS. The result in increase was recorded in an order of *T. harzianum* (9920.15, 52.76% increase)> *Ps. fluorescens* (9687.14, 49.17% increase)> *T. viride* (9490.93, 46.13% increase) statistically at par with *B. subtilis* (9455.17, 45.60% increase) > *A. niger* (9349.38,

Table 2 : Effect of different botanicals as seed soaking on germination percentage, root length, shoot length and vigour index of Brinjal in *in vivo* condition (Sand method)

Treatments	DAYS AFTER SOWING							
	Germination percentage		Root length (mm)		Shoot length (mm)		Vigour Index	
	7 DAS	10 DAS	7 DAS	10 DAS	7 DAS	10 DAS	7 DAS	10 DAS
Palmarosa oil	52.67 (46.53)	68.67 (56.03)	55.0	65.33	25.83	35.20	4254.73	6889.87
Lemon grass oil	48.0 (43.85)	64.0 (53.17)	51.67	61.67	20.15	26.13	3441.83	5612.07
Citronella oil	48.84 (44.33)	64.33 (53.34)	55.33	64.33	33.23	40.88	4289.50	6778.50
A. nilotica leaf extract	40.0 (39.12)	60.67 (51.20)	45.33	52.83	17.70	25.90	2544.83	4791.90
T. indica leaf extract	35.41 (36.33)	54.88 (47.84)	36.53	46.87	13.27	22.50	1773.95	3833.59
Control	33.07 (34.87)	49.82 (44.89)	29.47	43.67	11.17	20.88	1390.45	3193.52
Mean	42.99 (40.84)	60.39 (51.08)	45.56	55.78	20.23	28.58	2949.22	5183.24
	SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%
Treatment	2.11	6.15	1.24	3.62	1.15	3.35	264.23	771.28
DAS	1.21	3.55	0.72	2.09	0.66	1.93	152.56	445.29
Treatment x DAS	2.98	NS	1.75	NS	1.62	NS	373.68	NS

* Figures are mean values of three replicas.
** Figures in the parenthesis are indication of angular transformed value.

43.96% increase) and their differences were statistically significant.

Interaction between treatments × days after sowing showed no significant difference among themselves in respect to germination percentage, root length, shoot length and vigour index of brinjal seedlings. All results have been presented in Table 3.

Effect of microbial bioagents on growth parameters in greenhouse (Sand method)

In vivo experiment (Sand method) showed that (Table 4), at 7& 10 DAS, seed coating with all the bio-agents increased the germination percentage as compared to untreated control (26.28% and 15.71%). Maximum germination was obtained in *Ps. fluorescens* coated seed (67.12%) at 10 DAS, where *B. subtilis* exhibited minimum germination percentage (61.50%) in comparison to other bioagents.

Root length of seedlings increased significantly (107.94% to 54.67%) as compared to untreated control (25.08 mm) at 10 DAS. Maximum root length was recorded in *T. harzianum* (52.14 mm) statistically at par with *B. subtilis* (50.03 mm).

Minimum root length was recorded in *A. niger* (38.79 mm) and intermediate results were recorded by *Ps. fluorescens* (46.23 mm) followed by *T. viride* (44.92 mm). Seed coating with bio-agents also increased (69.55% to 26.18%) the shoot length of brinjal seedlings significantly as compared to untreated control (17.57 mm) at 10 DAS. The increase recorded was in an order of *T. harzianum* (29.79 mm) statistically at par with *B. subtilis*

Table 3 : Effect of different microbial bio-agents as seed coating on germination percentage, root length, shoot length and vigour index of Brinjal in Laboratory condition (Roll Towel method)

Treatments	DAYS AFTER SOWING							
	Germination percentage		Root length (mm)		Shoot length (mm)		Vigour Index	
	7 DAS	10 DAS	7 DAS	10 DAS	7 DAS	10 DAS	7 DAS	10 DAS
B. subtilis	96.67 (83.86)	96.67 (83.86)	45.32	65.23	21.79	32.52	6485.27	9455.17
Ps. fluorescens	97.22 (84.40)	97.78 (85.01)	44.85	61.57	27.18	37.31	7267.64	9687.14
T. harzianum	91.67 (73.45)	97.78 (85.01)	56.92	67.14	29.48	44.41	7912.23	9920.15
T. viride	86.38 (68.67)	91.11 (72.88)	49.15	60.59	27.30	43.58	6623.58	9490.93
A. niger	81.25 (64.55)	97.78 (85.01)	44.06	53.79	26.62	41.89	5713.04	9349.38
Control	76.0 (60.68)	88.33 (70.12)	28.33	40.08	14.83	20.98	3290.82	5394.43
Mean	88.19 (72.60)	94.91 (80.31)	44.77	58.07	24.50	33.45	6215.43	8733.14
	SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%
Treatment	2.95	8.61	1.83	5.33	1.04	3.04	256.6	748.76
DAS	1.70	4.97	1.06	3.08	0.60	1.76	169.0	493.14
Treatment x DAS	4.17	NS	2.58	NS	1.47	4.30	420.8	NS

* Figures are mean values of three replicas.
** Figures in the parenthesis are indication of angular transformed value.

(28.59mm) > *Ps. fluorescens* (26.42 mm) > *T. viride* (25.67mm) > *A. niger* (22.17mm).

Effect of seed treatment finally was reflected on vigour index of seedlings. All the treatments increased the vigour index significantly. The increase in vigour index was recorded in an order of *T. harzianum* (5161.01)> *Ps. fluorescens* (4837.67) statistically at par with *B. subtilis* (4822.99)> *T. viride* (4618.86)> *A. niger* (3986.39). Results have been presented in Table 4. Interaction between Treatments × days after sowing showed no significant difference among themselves in respect to germination percentage, root length, shoot length and vigour index of brinjal seedlings.

Above all, the results suggested that the test microbial bio-agents used as seed coating of brinjal showed growth promoting effects. Plant growth enhancement by some plant associated soil microorganisms is related to their ability to act as “biofertilizers” by increasing the availability of nutrients in the rhizosphere of plants (Vessey 2003). Adeniji *et. al*. (2022) observed plant growth promotion effect of bacterial bio-agents in their study with *Pseudomonas fulva* PS9.1 and *Bacillus velezensis* NWUMFkBS10.5.

Effects on germination, pre-emergence and post-emergence Damping off

Two years’ (2018-19) pooled mean data clearly revealed that (Table 5), at 28 DAS, every treatment significantly increased the germination (60.25% to 6.8% increase over control) percentage as compared to untreated control (52.48%). Microbial

Table 4 : Effect of different microbial bioagents as seed coating on germination percentage, root length, shoot length and vigour index of Brinjal in *in vivo* condition (Sand method)

Treatments	DAYS AFTER SOWING							
	Germination percentage		Root length (mm)		Shoot length (mm)		Vigour Index	
	7 DAS	10 DAS	7 DAS	10 DAS	7 DAS	10 DAS	7 DAS	10 DAS
B. subtilis	43.65 (41.34)	61.50 (51.67)	29.32	50.03	16.28	28.59	1983.15	4822.99
Ps. fluorescens	50.42 (45.24)	67.12 (55.07)	28.85	46.23	16.03	26.42	2234.45	4837.67
T. harzianum	41.78 (40.26)	63.10 (52.60)	40.92	52.14	22.73	29.79	2651.84	5161.01
T. viride	43.43 (41.22)	65.55 (44.07)	33.15	44.92	18.42	25.67	2221.99	4618.86
A. niger	39.79 (39.08)	65.53 (54.09)	28.06	38.79	15.59	22.17	1726.67	3986.39
Control	33.71 (35.49)	53.15 (46.81)	13.33	25.08	8.37	14.57	733.61	2116.61
Mean	42.13 (40.44)	62.66 (52.39)	28.94	42.87	16.24	24.53	1925.29	4257.26
	SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%
Treatment	1.12	3.27	1.77	5.17	0.97	2.83	116.92	341.28
DAS	0.65	1.89	1.02	2.98	0.56	1.63	67.50	197.04
Treatment x DAS	1.59	NS	2.50	NS	1.37	NS	165.35	482.64

* Figures are mean values of three replicas.
** Figures in the parenthesis are indication of angular transformed value.

bioagents recorded comparative better results than botanicals in this respect. Maximum germination was observed in *T. viride* (84.17%) followed by *T. harzianum* (81.13%).

Among the botanicals, however, palmarosa oil recorded higher germination (63.79%, 21.55% increase over control) to some extent. Minimum germination was observed in *A. nilotica* (56.05%) statistically at par with citronella oil (56.24%) and *T. indica* (56.90%).

While considering the pre-emergence Damping off, both botanicals and bio-agents showed promising results (55.67% to 41.08% reduction) and significant differences in disease reduction at 28 DAS. The pre-emergence mortality recorded in an order of *T. viride* (13.76%)> *T. harzianum* (14.59%)> citronella oil (14.99%)> Palmarosa oil (15.11%) and *Ps. fluorescens* (15.11%)>lemon grass oil (15.83%)> *A. niger* (16.72%)> *B. subtilis* (16.84%)> *A. nilotica* (17.49%)> *T. indica* (18.29%).

Every treatment significantly reduced the post-emergence mortality (46.87% to 36.16% reduction) as compared to untreated control (89.17%) at 28 DAS. Among the bio-agents, minimum mortality was observed in *T. viride* (47.50%) followed by *T. harzianum* (48.47%) and among the botanicals, minimum mortality exhibited by Palmarosa oil (47.38%), while maximum was observed in *A. nilotica* (56.93%) statistically at par with *A. niger* (56.91%) (Table 5).

Above all, the results suggested that all the test botanicals and microbial bio-agents used as seed treatment of brinjal showed growth promoting

Table 5: Effect of microbial bioagents and botanicals on germination, pre-emergence and post-emergence Damping off of brinjal in artificially inoculated soil (Two years' pooled mean)

Treatments	Germination percentage		Pre-emergence Damping off	Post-emergence Damping off	
	14 DAS	28 DAS	28 DAS	14 DAS	28 DAS
B. subtilis	49.49 (44.71)	55.17 (47.97)	16.84 (24.23)	45.43 (41.01)	51.23 (45.71)
Ps. fluorescens	60.57 (51.13)	64.08 (53.23)	15.11 (22.87)	43.69 (41.37)	48.93 (44.38)
T. harzianum	76.44 (61.03)	81.13 (64.35)	14.59 (22.39)	43.39 (41.19)	48.47 (44.12)
T. viride	80.67 (64.02)	84.17 (66.58)	13.76 (21.76)	40.20 (39.34)	47.50 (43.57)
A. niger	64.32 (53.44)	71.67 (58.05)	16.72 (24.12)	47.88 (43.78)	56.91 (48.97)
Palmarosa oil	56.47 (48.73)	63.79 (53.05)	15.11 (22.87)	41.30 (39.99)	47.38 (43.50)
Lemon grass oil	54.69 (47.71)	62.34 (52.21)	15.83 (23.31)	42.22 (40.52)	49.50 (44.71)
Citronella oil	43.87 (41.41)	56.24 (48.59)	14.99 (22.69)	44.70 (41.95)	49.73 (44.84)
A. nilotica leaf extract	47.74 (43.70)	56.05 (48.50)	17.49 (24.71)	51.24 (45.72)	56.93 (48.98)
T. indica leaf extract	47.55 (43.59)	56.90 (48.99)	18.29 (25.31)	50.91 (45.52)	55.58 (48.21)
Control	47.55 (43.59)	52.48 (46.42)	31.04 (33.85)	78.71 (62.78)	89.17 (70.84)
Mean	57.21 (49.37)	64.0 (53.45)	17.25 (24.37)	48.15 (44.05)	54.67 (47.98)
	SEm±	CD at 5%		SEm±	CD at 5%
Treatment	0.84	2.36	0.48 1.36	0.46	1.29
Days after sowing (DAS)	0.36	1.01	- -	0.19	0.55
Treatment × DAS	1.19	NS	- -	0.65	1.82

* Figures in the parenthesis are indication of angular transformed value.

effects as well as reduction in disease incidence. Very little information is available about controlling the damping off disease using botanicals or plant essential oils in field condition owing to the following reasons of non standardized extraction methods, rapid degradation and need of proper formulations. *In vitro* study by different workers showed that, *Pythium* damping off of tomato and other crops can be minimized using plant products (Pandey *et. al.* 2017; Rajput *et. al.* 2018). Similarly, application of different bio-antagonists in disease management is also in practice. Seed pelleting and soil incorporation of *T. viride* gave highest seed germination and seedling survival of forest nursery plants, damaged by *P. aphanidermatum*, was reported by Sanjay *et. al*., (2001). *Pythium* - damping off of tomato can be minimized by antagonistic soil rhizobacteria has been studied by Al-Hussini (2019).

Actually, microbial biocontrol of plant pathogen is not very popular in field conditions as the crop is more open to a range of pests that may prevent the development of a specific biological control agent. Meteorological factors and soil factors also influence the potential of beneficial microbes against disease suppression. Many studies have discussed the low performance of fungal and bacterial-based products under open field conditions due to various climatic and soil factors. Here, an attempt was made to study the synergistic effect to induce plant vigour as well as plant defense mechanism in green house condition. Plant health and seedling vigour matters in case

of damping off, as the pathogen attacks the seedlings in a rudimentary stage. However, this study needs more exposure in different agro-climatic field conditions.

CONCLUSION

Results revealed that, application of every botanical as seed soaking and microbial bio-agent as seed coating individually helps to increase seedling vigour as well as to reduce disease incidence. However, use of *T. viride* or *T. harzianum* as seed coating or 0.025% Palmarosa oil or 0.020% Citronella oil as seed soaking gave the best results in comparison to other treatments used in this experiment. So from the present study, *T. viride* or *T. harzianum* (@10ml suspension containing 4 x 10^6 spores / ml per 10g seed) as seed coating or 0.025% Palmarosa oil or 0.020% Citronella oil (@10ml suspension per 10g seed) as seed soaking can be recommended for improvement of seedling growth parameters and management of *Pythium*- Damping off.

ACKNOWLEDGEMENT

Author is grateful to Dr. Srikanta Das, Professor in Department of Plant Pathology, Ex- Dean, Faculty of Agricultre, Bidhan Chandra Krishi Viswavidyalaya for guidance and encouragement. Author would also like thank the Department of Plant Pathology, BCKV, Mohanpur-741252, West Bengal, for providing laboratory facilities to carry out the research work.

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