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Evaluation of bio formulations against *Colletotrichum truncatum* and *Macrophomina phaseolina* in soybean

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ABSTRACT

Soybean crop is known to be infected by several seed and soil borne pathogens affecting the seed quality and viability in field and during storage. Colletotrichum truncatum and Macrophomina phaseolina are the important seed and seedling pathogens affect the germinating seed. Since intensive use of synthetic pesticides is unsafe, bio formulations can be an alternative as they are eco-friendly and sustainable without any residual effects. Thirteen commercially available bio formulations were evaluated for their antagonistic activity against these two test pathogens. The population density of the bio agents ranged from 1x10^5 to 6x 10^8 cfu/g. The dual culture assay of C.truncatum and M. phaseolina revealed that, highest per cent inhibition was recorded by formulation T5, T. viride + P. flourescens followed by T6, T. harzianum+ B.subtilis with 88.16% and 88.01%; 62.00% and 53.64 per inhibition of mycelial growth, respectively. The fungal bio agents T.viride and T.harzianum were found effective over the bacterial bio agents P.fluorescens and B. subtilis for their suppressing activity. However, the bio fertilizer, Rhizobium sp. was least effective with 72.92% and 32.73% mycelial inhibition of both the pathogens, respectively. While, the formulation T10 basil oil @ 5ml/l was observed equally effective as that of fungicidal check carboxin+thiram @3g/l in poisoned food technique in suppressing cent per cent mycelial growth of both the test pathogens. However, the formulations T8 biopolymer and T9 neem oil were least effective with (21.18%, 2.32%) and (23.66%, 10.44%) inhibition, respectively. The response of these bio formulations for seed quality and seed health against C. truncatum stated that seed treatment formulation T5, T. viride + P. flourescens @10g/kg was most effective with 85.50 per cent seed germination followed by T10 basil oil @5 ml /kg with 84.50 per cent which were statistically on par with each other. T5 has shown 34.79% increase of seed germination over untreated control (55.75%). Formulation T10 basil oil has recorded minimum 9.75% seed infection, maximum SV-I and SV-II (2355, 98 g) respectively. All the bio formulations except for T8 bio polymer and T13 GA3 have showed per cent seed germination above IMSCS against M. phaseolina. Formulation T5 has recorded highest 85.75 % seed germination and was on par with T10 basil oil, 85.00 per cent. However, T5 has exhibited 33.81% increase of seed germination over the control (56.75%). Seed treatment with T10 has reported minimum 7.00% seed infection followed by T5 with 8.00 per cent. Significantly highest 2190, 96.61g SVI-I and SVI- II, respectively was observed in T5. However, formulations, T8 biopolymer and T13 GA3 have showed maximum (27.25%, 24.00%) seed infection, lowest 1068, 48.62g, 1125, 55.14g, SV-I and SV-II, respectively. The results of the present investigation states that formulations, T5 T. viride + P. fluorescens, T1 10 and T6 T. harzianum+ B.subtilis were found effective for their antagonistic potential against C.truncatum and M. phaseolina.

Keywords: *Bio-formulations, C. truncatum and M. phaseolina. soybean, seed quality parameters*

Introduction

Soybean [*Glycine max* (L.) Merill] commonly known as "Golden bean" is native to East Asia. Among leguminous species, the crop is valued for its relatively high-quality oil (20%) and protein (40%) contents. It has many industrial uses such as a biodiesel product, its meal in livestock and poultry feed, as cooking oil *etc*. Globally, Brazil, United States, Argentina, China, Paraguay, India and Canada are the major soybean producing countries. The crop is infected by several seed and soil borne pathogens greatly affecting the seed quality and seed health. The most common are Anthracnose (*Colletotrichum* spp.), Charcoal rot (*Macrophomina* spp), Fusarium root rot (*Fusarium* spp), Phomopsis seed decay (*Phomopsis* spp.), Cercospora purple seed stain (*C. kikuchii*); Frogeye leaf spot on seed (*C. sojina*) and secondary fungal invaders of injured pods including *Alternaria* spp., *Cladosporium* spp. and *Penicillium* spp etc. Economic yield losses in soybean due to various diseases include *M. phaseolina* (77%), *C. truncatum* (16-25%), *Fusarium* spp (64%), *C. sojina* (22%), *Alternaria* spp (15%), etc. (Sharma *et al.,* 2014) [14].

Most of the seedborne fungi affect the seeds directly or indirectly resulting in seed rot and/or discoloration influencing reduction in crop yields. Several pathogens have been known to be associated with legumes where, in certain cases, serious diseases and significant economic losses have been documented (Dell'Olmo *et al*., 2023) [12]. Both as a soil borne and seed borne pathogen, *M. phaseolina* colonises seeds primarily under the seed coat, causing seed degradation and germination failure (Sharma *et al*., 2023) [24]. Seed treatment is the best option to protect the crop from various seed and soil borne infections at early stages of the crop growth. The disease infected seed

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reduce germination per cent, seedling vigour, loss of seed weight and decreased meal and oil quality. As a common practice use of chemical fungicides for the management of the diseases is an easier and cheaper source, but is unsafe to the environment and soil health. Several methods are suggested to reduce the use of synthetic products, such as physical, microbial treatment and treatment with natural agents, Koch and Roberts (2014)[17]and Ayilara *et al*. (2023)[3].

Bio pesticides are the integral part of the sustainable agriculture. They usually contain beneficial organisms which produce toxins, enzymes, vitamins and plant hormones that can act antagonistically towards disease causing pathogens additionally enhancing the plants immune systems there by their resistance. Due to greater awareness of their potential and increased focus on the environmental and health risks related to the use of synthetic pesticides, the market for biopesticides has expanded drastically in recent years. Only 4-5% of the world's pesticide market currently uses biopesticides, but it is predicted that this number will rise to 20% in the near future (Isman, 2020) [15]. While, some experts predict that the importance of biopesticides in agricultural productivity will be on par with that of chemical pesticides by 2050 (Olson, 2015)[21].

In soybean, the fungicide, carboxin+thiram was found very effective seed dresser against *Colletotrichum truncatum* and *Macrophomina phaseolina*. Over a time this practice could not cause to develop fungicidal resistance and it is crucial to find a replacement. Therefore, using biocontrol and biopesticides, non-chemical, environmentally benign methods of control has become a viable alternative under these circumstances. Even though the existence of naturally occurring microorganisms with antifungal properties is well known and well-documented, only a small number of them have been thoroughly investigated in the context of soybean seed borne diseases. Hence the present study was focused to evaluate the efficacy of commercially available bio formulations including bio agents, biopesticides along with fungicide (Vitavax power) against seed borne pathogens *M. phaseolina* and *C. truncatum* in soybean.

Materials and methods

The experiment was conducted at Seed Pathology laboratory, Seed Research and Technology Centre, PJTSAU, Telangana State. Thirteen commercially available bio-based antifungal formulations *viz.,* Bhoomika (*T. viride)*, Hariz (*T.harzianum)*, Agnee (*P. fluorescens)*, Knassa *(B. subtilis)*, Anoka (*T.viride + P. fluorescens*) and (*T.harzianum + B.subtilis*), Rhizopower biofertilizer (*Rhizobium* sp.), and bio formulations *viz.*,Neemazol (neem oil) @10ml/L, basil oil@5ml/L, chitosan@5ml/L, silicon based chitosan @5ml/L, (Pro Gibb) gibberellic acid@5g&2g/L, and biopolymer @5ml/L along with one check fungicide, vitavax power (carboxin+thiram) @3g/L were collected from different agro markets of Telangana State during, 2022-23. Among the bio formulations, the bioagents were isolated following serial dilution plate method (Johnson and Curi, 1972), where an aliquot of 1 ml from 10^{-4} and 10^{-8} dilutions were transferred on to the Potato Dextrose Agar (PDA) medium for fungal bio agents and nutrient agar (NA) for bacterial bio agents including *Rhizobium* sp, respectively. For enumerating colony forming units of *T. viride*, *T. harzianum*, (*T.viride + P. fluorescens*), (*T.harzianum + B.subtilis*), *P. fluorescens, B.subtilis* and *Rhizobium* sp., six replications in each were maintained. The number of colonies developed from each plate were recorded after incubation of 48 hrs for bacterial and 120 hrs for fungal bio agents. Following single colony isolation and hyphal tip methods, the isolates were sub cultured and preserved for further studies to test their efficacy against test pathogens under *in vitro* conditions by adopting dual culture technique (Dennis and Webster, 1971) [11] and poisoned food technique (Nene and Thapliyal, 1993)[20].

The test pathogens, *M. phaseolina and C. truncatum* were isolated following standard seed health methods given by ISTA, 2022 from soybean seed samples collected from farmers fields of major soybean growing areas of Telangana.

Seed borne fungi detected in Standard seed health tests

Agar plate method Standard blotter method

***In vitro* evaluation of bio agents for antagonistic activity against test pathogens:**

The antagonistic activity of bio agents *T. viride*, *T.harzianum*, (*T.viride + P.fluorescens*), (*T.harzianum + B.subtilis*) and bacterial bio agents *P. fluorescens, B.subtilis* including biofertilizer *Rhizobium* sp. against test pathogens *M. phaseolina* and *C. truncatum* was evaluated on PDA and Nutrient agar, respectively. For assay, all the fungi and consortia bio agents were cultured for 120 hr on PDA medium and bacterial bioagents for 48- 72 hrs on NA. While, the test pathogens, *M. phaseolina* and *C. truncatum* were cultured at 28 ± 1 ºC for 4 days and 8 days, respectively on PDA medium in petri plates.

Five mm disc of *C. truncatum* and *M. phaseolina* were cut separately using sterilize cork borer and transferred towards periphery of PDA plates. In case of *T. viride*, *T.harzianum,* (*T.viride + P. fluorescens*) and (*T.harzianum + B.subtilis*) from the four day old culture plates, 5 mm mycelial discs were placed towards opposite end of the respective test pathogens and incubated at 25 ± 2 ºC in a BOD incubator. Whereas, for *P. fluorescens, B.subtilis* and *Rhizobium* sp., a loop full of retrieved inoculum was streaked on other side of the pathogen mycelial disc. All the plates were incubated at 28 ± 2 ºC in BOD incubator. Petri plates with mycelial disc for each of the test pathogens was maintained as control. When full growth was achieved in the control plate, the mycelial growth of the test pathogens was measured in each Petri dish separately and expressed in mm. The per cent growth inhibition of test pathogens was calculated using the formula given below.

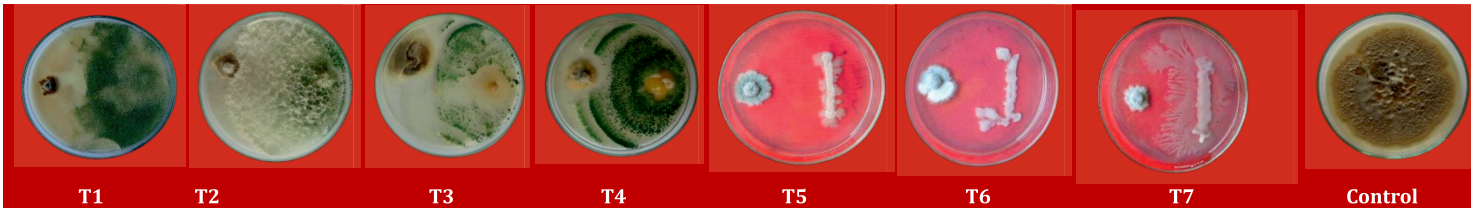
$$I = \frac{C-T}{C} X100$$

Where, I = Per cent inhibition of pathogen

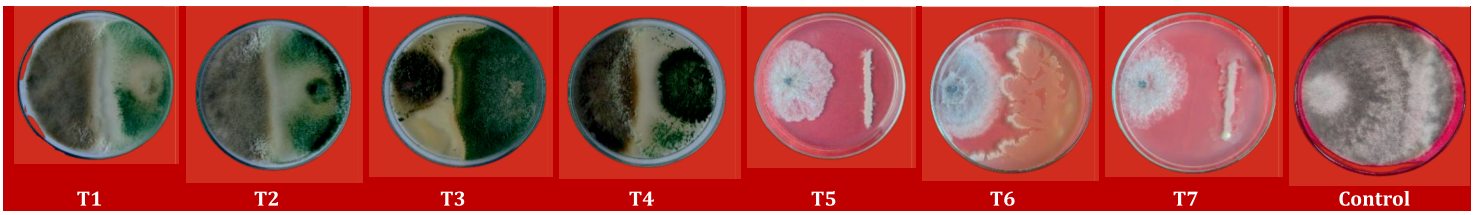
C = mycelial growth of pathogen in control (mm)

T = mycelial growth of pathogen in treatment (mm)

Antagonistic activity of bio agents against C. truncatum



Antagonistic activity of bio agents against M.phaseolina



T1-Trichoderma viride, T2- T.harzianum ,T3-Pseudomonas fluorescens, T4- Bacillus subtilis,T5-T.viride + P.fluorescens,T6-T.harzianum +B.subtilis, T7- Rhizobium spp.

2.2 *In vitro* evaluation of biopesticides for antagonistic activity against test pathogens:

The efficacy of biopolymer @ 5ml, chitosan @5g, silicon based chitosan@5ml, neem oil@10ml, basil oil @5ml and growth hormone, gibberellic @ 0.2 and 0.5% concentrations along with fungicidal check (carboxin + thiram) @3g L-1per litre were evaluated against the test pathogens on PDA medium.

For each treatment, 100 ml of PDA medium was taken in 250 ml conical flask and sterilized. To this, required quantity of bio pesticides was added at luke warm state to get desired concentration of each formulation and four replications were maintained for each treatment. Five mm discs of seven days old test fungal culture of *C. truncatum* and *M.phaseolina* was obtained with sterilized cork borer and transferred to the centre of the poisoned medium in each of the petri plates. Similarly, controls were maintained by placing 5 mm disc of test fungal cultures in the centre of unpoisoned medium in the plates. All the petri plates were incubated at 28± 1ºC in BOD incubator. The diameter of fungal colony was measured in each of the treatment when the fungal colony growth in control plate was full and the per cent mycelial inhibition was calculated in each treatment by comparison with control plates by the following formula.

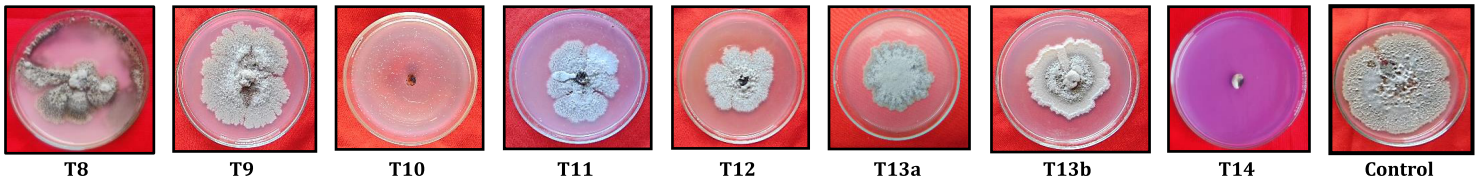
$$I = \frac{C - T}{C} X\ 100$$

Where, I =Per cent inhibition of pathogen

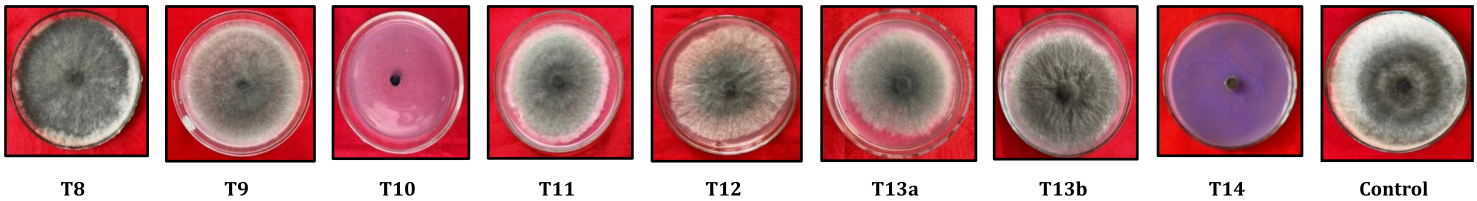
C= Colony diameter of the pathogen in control (in mm),

T = Colony diameter of the pathogen in treatment (in mm)

Antagonistic activity of biopesticides against C. truncatum



Antagonistic activity of biopesticides against M.phaseolina



T9 – Neem oil, T10 – Basil oil, T11- Chitosan, T12 – Silicon based chitosan, T13a – Gibberellic acid @ 2g/L,T13b-Gibberellic acid @ 5g/L, T14 – Carboxin + thiram, T8- Biopolymer

Evaluation of bio formulations against test pathogens *in vitro* using rolled paper towel method:

The efficacy of biopesticides was evaluated against *C.truncatum* and *M.phaseolina* using rolled paper towel method (ISTA, 2022) for seed health and seed quality parameters, germination, seed infection, seedling vigour index- I and II.

Seeds of popular soybean cultivar JS 335 were surface sterilized with 0.1% NaOCl for 30 sec. followed by washing with sterile distilled water. For seed inoculation, the sterilized seeds were treated with the respective individual pathogens. In case of *C.truncatum*, 5 ml sterile distilled water was transferred to the 10 day old culture plates and with the help of camel hair brush, the conidia were harvested. Using serial dilution method and calibration with hemocytometer, the spore suspension was prepared to $5x10^5$ spores/ml per kg of soybean seeds. While, the seeds were rolled on 7 days old culture of *M.phaseolina* thriving on PDA in petri plates. The pathogen treated seeds were dried for overnight followed by treatment with respective bio formulations at their dosages. After 24hr

of treatment, the treated seeds were assayed for germination and vigour tests by adopting rolled paper towel method.
The paper towels were initially soaked in sterile distilled water and excess water was drained out. From each treatment along with fungicidal check and control, 100 seeds were randomly taken and placed at equidistance in between two layers of moistened paper towels. Later, the paper towels were rolled up along with polythene sheet placed on the top paper towel to retain moisture and placed vertically in walk-in germinator at constant temperature (25±0.5°C) and relative humidity (90±3%). Four replications for each bio formulation treated seeds were maintained for the two test pathogens. The observations on per cent seed germination, seed infection, SVI-I and II were recorded on the 8th day and calculated using the following formula:

Number of normal seedlings Germination (%) = x 100
Total number of seeds

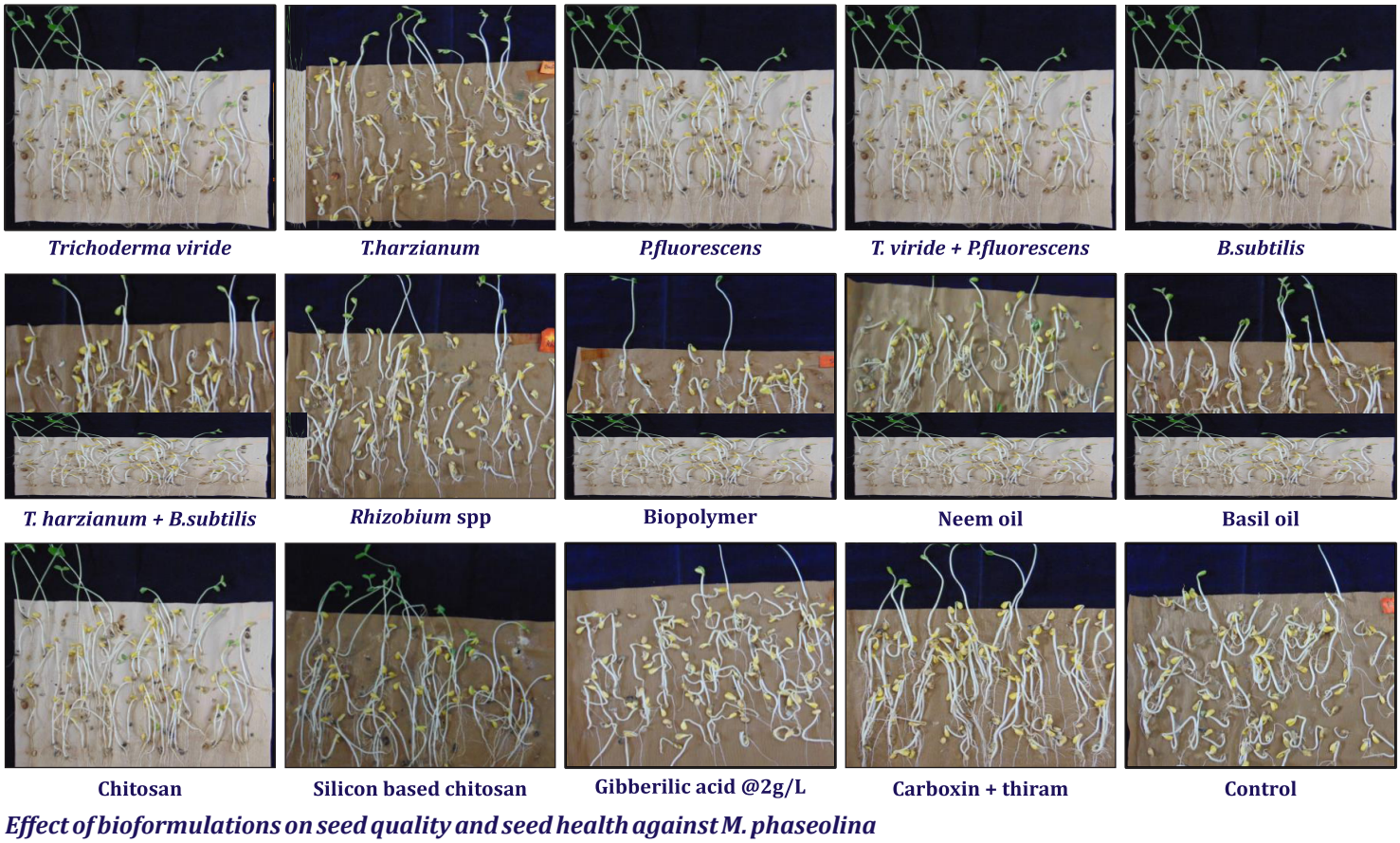
Number of seeds infected Percent seed infection (PSI) = x100
Total number of seeds

Seedling vigour indices, SVI-I and SVI-II were evaluated as per the procedure suggested by Abdul Baki and Anderson (1973)[1]. On 8th day, in each treatment ten randomly selected seedlings were measured for seedling length from tip of the primary leaf to the tip of the primary root using measuring scale and expressed in centimeters (cm). The seedling vigour index - I was calculated as per the formula

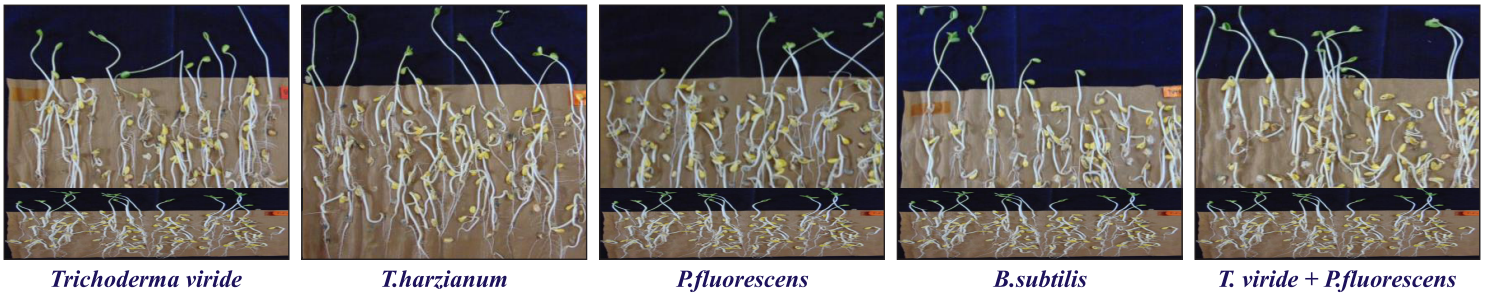
SVI-I = Germination (%) x Average of 10 no. seedling length (cm)
Similarly, SVI-II, was carried out by selecting ten seedlings at random and were oven dried at 90ºC for overnight and dry weights were recorded and computed using the formula

SVI- II = Germination (%) x Average of 10 seedlings dry weight (g).

Effect of bioformulations on seed quality and seed health against C.truncatum



Effect of bioformulations on seed quality and seed health against M. phaseolina



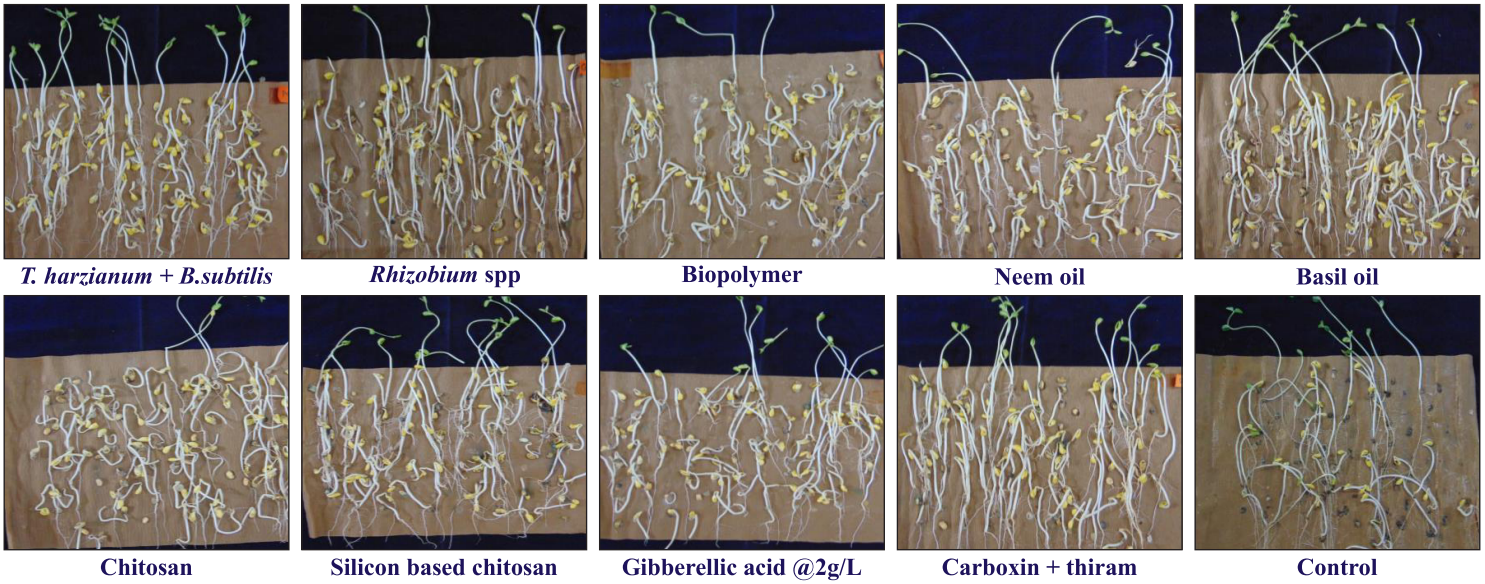


Table 1. Effect of bio agents against seed borne pathogens C. truncatum and M. phaseolina

S.No.	Bio pesticide	Colony forming units (cfu /g)	*C. truncatum*		*M. phaseolina*	
			Radial growth (mm)	Mycelial growth inhibition (%)	Radial growth (mm)	Mycelial growth inhibition (%)
1.	*Trichoderma viride*	$3x10^6$	15.86	81.34 (64.39)	45.20	46.82 (43.15)
2.	*T. harzianum*	$4x\ 10^6$	11.97	85.92 (67.96)	42.40	51.03 (45.57)
3.	*P.fluorescens*	$2\ x\ 10^6$	20.17	76.26 (60.82)	50.33	40.79 (39.67)
4.	*B.subtilis*	$2x\ 10^9$	22.05	74.05 (59.35)	55.60	34.58 (36.00)
5.	*T.viride + P.fluorescens*	$5\ x\ 10^8$	10.07	88.16 (69.86)	32.30	62.00 (51.92)
6.	*T. harzianum +B.subtilis*	$6x\ 10^8$	10.19	88.01 (69.74)	39.41	53.64 (47.06)
7.	*Rhizobium* sp.	$1x10^5$	23.01	72.92 (58.62)	57.18	32.73 (34.88)
	CD (P=0.05)			1.60		1.08
	SE(m)			0.54		0.36
	C.V.			1.68		1.72

Table 2. Effect of bio pesticides against seed borne pathogens C. truncatum and M. phaseolina

S.No.	Bio-based antifungal formulations	Dosage per litre	*C. truncatum*	*M. phaseolina*
			Per cent growth inhibition (%)	Per cent growth inhibition (%)
1.	Neem oil	10 ml	23.66 (29.07)	10.44 (18.82)
2	Basil oil	5 ml	100.00 (90.00)	100.00 (90.00)
3	Chitosan	5 g	38.58 (38.37)	26.63 (31.05)
4	Silicon based chitosan	5 ml	44.68 (41.92)	30.88 (33.74)
5	Gibberilic acid	2g	42.22 (40.50)	37.03 (37.46)
6	Gibberilic acid	5g	35.38 (36.48)	20.82 (27.12)
7	Biopolymer	5 ml	21.18 (27.37)	2.32 (8.15)
8	Carboxin + thiram	3 g	100.00 (90.00)	100.00 (90.00)

	CD(P=0.05)		1.28	2.16
	SE(m)		0.43	1.04
	C.V.		1.77	3.50

3. Effect of bio agents and bio formulations for seed quality and seed health against C. truncatum

Treatment	Bio-based antifungal formulations	Dosage per kg seed	Germina-tion (%)	Seed infection (%)	Seedling Vigour Index-I	Seedling Vigour Index-II
T1	*Trichoderma viride*	10g	74.50 (59.65)	16.00 (23.54)	1606	73
T2	*T. harzianum*	10g	77.25 (61.49)	13.50 (21.53)	1693	73
T3	*P.fluorescens*	10g	72.00 (58.03)	16.50 (23.94)	1558	68
T4	*B.subtilis*	10g	68.75 (55.99)	19.50 (26.18)	1318	67
T5	(*T.viride* + *P.fluorescens*)	10g	85.50 (67.60)	10.50 (18.87)	1964	83
T6	(*T. harzianum* +*B.subtilis*)	10g	80.00 (63.43)	14.50 (22.32)	1759	78
T7	*Rhizobium* sp.	10g	75.50 (60.31)	17.25 (24.51)	1427	78
T8	Bio Polymer	5ml	62.00 (51.92)	29.00 (32.55)	980	47
T9	Neem oil	10 ml	64.50 (53.41)	25.75 (30.47)	1087	50
T10	Basil oil	5 ml	84.50 (66.80)	9.75 (18.17)	2355	98
T11	Chitosan	5 g	67.25 (55.07)	24.25 (29.47)	1126	55
T12	Silicon based chitosan	5 ml	70.75 (57.24)	23.75 (29.13)	1283	61
T13	GA3	2g	64.00 (53.11)	27.50 (31.60)	1019	47
T14	Carboxin + Thiram	3g	89.75 (71.34)	5.50 (13.48)	2696	113
	Control		55.75 (48.28)	36.00 (36.85)	594	9
	CD(P=0.05)		1.49	1.83	89.89	10.58
	SE(m)		0.52	0.64	31.4	3.70
	C.V.		1.77	5.02	4.20	11.10

4. Effect of bio agents and bio formulations for seed quality and seed health against M. phaseolina

Treatment	Bio-based antifungal formulations	Dosage per kg seed	Germina-tion (%)	Seed infection (%)	Seedling Vigour Index- I	Seedling Vigour Index- II
T1	*Trichoderma viride*	10g	77.50 (61.66)	11.75 (20.01)	1716	68.59
T2	*T. harzianum*	10g	81.25 (64.34)	10.00 (18.41)	1852	74.06
T3	*P.fluorescens*	10g	74.50 (59.65)	13.00 (21.11)	1586	83.93
T4	*B.subtilis*	10g	73.00 (58.68)	16.25 (23.74)	1449	63.39
T5	(*T.viride* + *P.fluorescens*)	10g	85.75 (67.82)	8.00 (16.40)	2190	96.61
T6	(*T. harzianum* +*B.subtilis*)	10g	81.25 (64.35)	9.50 (17.91)	1914	85.17

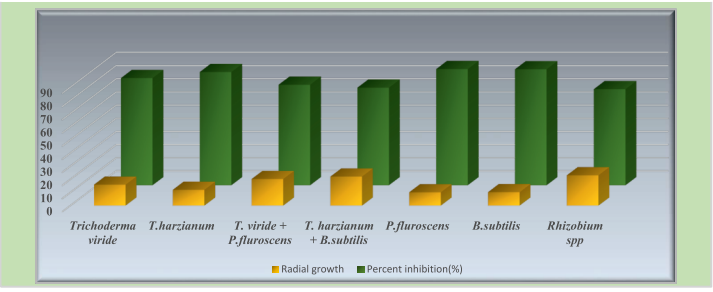
T7	*Rhizobium* sp.	10g	78.50 (62.38)	15.25 (22.95)	1521	68.08
T8	Bio Polymer	5ml	69.00 (56.45)	27.25 (31.44)	1068	48.62
T9	Neem oil	10 ml	71.00 (57.40)	21.00 (27.24)	1239	57.78
T10	Basil oil	5 ml	85.00 (67.24)	7.00 (15.25)	2404	81.86
T11	Chitosan	5 g	71.75 (57.87)	21.50 (27.59)	1285	58.84
T12	Silicon based chitosan	5 ml	75.25 (60.16)	19.00 (25.82)	1391	63.26
T13	GA3	2g	69.00 (56.15)	24.00 (29.29)	1125	55.14
T14	Carboxin+ Thiram	3g	91.75 (73.37)	3.50 (10.63)	2703	105.14
	Control		56.75 (48.86)	34.00 (35.63)	646	4.53
	CD(P=0.05)		2.23	2.00	223.92	7.81
	SE(m)		0.78	0.70	78.35	2.73
	C.V.		2.56	6.13	9.75	8.08

Results

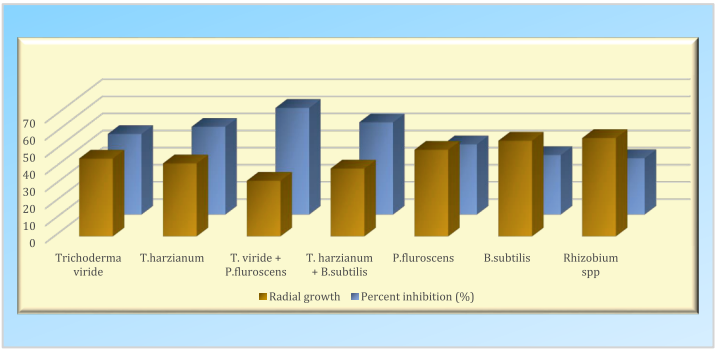
The results of the present studies stated that the antagonistic activity of bio formulations evaluated differed significantly in inhibiting the mycelial growth of the two test seed borne pathogens, *C.truncatum* and *M. phaseolina* in soybean.

The population density of different bio agents varied in different proportions *i.e T. viride* ($3x10^6$cfu/g), *T.harzianum* ($4x10^6$cfu/g), *P. fluorescens* ($2 x 10^6$cfu/g), *B. subtilis* ($2x 10^9$cfu/g), *T.viride + P. fluorescens* ($5 x 10^8$ cfu/g), *T.harzianum + B.subtilis* ($6x10^8$cfu/g) and *Rhizobium* sp. ($1x10^5$ cfu/g). The dual culture assay of *C. truncatum,* has revealed that among the bio agents, highest per cent inhibition was recorded by the formulation T5, *T. viride + P. flourescens* with 88.16 per cent followed by T6, *T. harzianum+ B.subtilis* with 88.01% which were on par with each other. These were followed by T2, *T. harzianum* (85.92%) and T1, *T.viride* 81.34% The bacterial bio control agents, T3, *P. fluorescens* and T4, *B. subtilis* has showed 76.26% and 74.05 per cent inhibition, respectively. However, the bio fertilizer, *Rhizobium* was least effective with 72.92% mycelial inhibition of the pathogen. In case of suppressing *M. phaseolina,* the per cent inhibition of mycelial growth ranged from 32.73% to 62.00%. The bio formulation T5, *T. viride + P. flourescens* has showed highest 62.00% mycelial growth inhibition followed by T6, *T. harzianum+ B.subtilis* 53.64 per cent. The two fungal bio agents *T.harzianum* and *T. viride* has recorded 51.03% and 46.82 per cent inhibition, respectively, while, the bacterial bio agents *P. flourescens* has shown 40.79% and *B. subtilis* with 34.58 per cent inhibition and were differed significantly among them. The bio fertilizer, *Rhizobium sp.* has recorded lowest 32.73 per cent growth inhibition of the pathogen (Table 1).

Fig.1. Effect of bioagents on radial growth and percent inhibition of C. truncatum

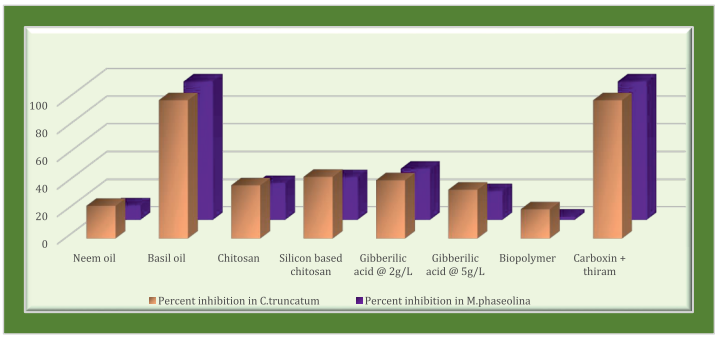


Effect of bioagents on radial growth and percent inhibition of M.phaseolina



The poisoned food technique assay revealed significant differences in inhibiting the mycelial growth of the two test pathogens. Among the bio pesticides evaluated, basil oil @ 5ml/l has recorded cent per cent inhibition of mycelial growth of two test pathogens *i.e., C. truncatum* and *M. phaseolina* and was found equally effective to carboxin+thiram @3g/l, the check fungicide which also has shown cent per cent inhibition against the two pathogens in the study. In case of *C.truncatum* this was followed by silicon based chitosan @5 ml/l and gibberellic acid @2g/l with 44.68% and 42.22%, respectively. The formulations neem oil @ 10 ml/l and T8, biopolymer @ 5 ml/l with 23.66 and 21.18 per inhibition was found to be least effective in suppressing the pathogen. While in *M.phaseolina*, of the bio formulations next to basil oil, gibberellic acid @ 2g/L was found effective in inhibiting the mycelial growth of the pathogen. However, there was no significance between the treatments silicon based chitosan (30.88%) and gibberellic acid @2g/L (37.03%) towards the per cent inhibition of *M.phaseolina*. In both the pathogens *C.truncatum* and *M.phaseolina,* the per cent inhibition observed was least with the formulations biopolymer (21.18%, 2.32%), followed by neem oil (23.66%, 10.44%), gibberellic acid @5g/L (35.38%, 20.82%) and chitosan (38.58%, 26.63%) inhibition, respectively (Table 2).

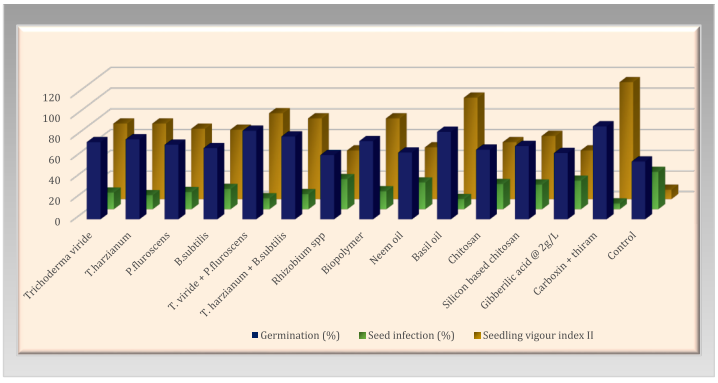
Fig.2. Effect of biopesticides on percent inhibition of C. truncatum and M.phaseolina



The efficacy of bio formulations tested against *C.truncatum* using paper towel method were significantly differed for the seed quality parameters tested. The bio formulations, T5 (*T.viride + P.fluorescens*), T6 (*T. harzianum +B.subtilis*), T1 (*T. viride)*, T2 *(T. harzianum)*, T3 (*P.fluorescens)*, T7 (*Rhizobium* sp.), T10 (basil oil) and T12 (silicon based chitosan) have recorded per cent seed germination above Indian Minimum Seed Certification Standards (IMSCS) i.e >70%. Soybean seed treated with T5 was most effective with 85.50% seed germination followed by T10 and T6 with 84.50 and 80.00%, respectively, where T5 and T6 were statistically on par with each other. Among the individual bio agents, the per cent seed germination recorded by fungi, T2 (77.25%) and T1 (74.50%) was high over the bacterial bio agents, T3 (72.00%) and T4 (68.75%). The T8 bio fertilizer *Rhizobium* sp. recorded 75.50 per cent seed germination which was superior over T3 and T4 and was found on par with T1. However, the check fungicide carboxin+thiram @3g/kg which recorded 89.75% seed germination has showed 4.73 per cent improvement over the formulation T5 (85.50%). While, the per cent increase of seed germination shown by T5 was 34.79% over untreated control (55.75%).

Among the bio formulations, T10 has recorded minimum 9.75 per cent seed infection followed by T5 with 10.50% and were on par with each other compared to the untreated control which was 36.00 per cent. The check fungicide carboxin+thiram has showed 5.5 per cent seed infection with 43.58 % reduction over T10. The SV-I and SV-II were high in the treatment, T10 (2355, 98g) followed by T5 and T6 with 1964, 83g and 1759, 78 g, respectively. The per cent increase of SV-I and SV-II recorded by fungicidal check was 12.64 and 13.27 per cent over the formulation T10. While, it was 74.77 and 90.81 per cent for T10 over untreated control (594, 9 g). However, the bio formulations, T13 and T8 have recorded lowest germination (64.00%, 62.00%), maximum per cent seed infection (27.50%, 29.00%), minimum SV-I and SV-II (1019, 47g; 980, 47g), respectively (Table 3).

Fig.3. Effect of bioformulations on seed germination, infection and SVI-II against C.truncatum



In case of *M. phaseolina,* all the bio formulations except for the formulation T8 and T13 have recorded per cent seed germination above IMSCS. The formulation, T5 has recorded significantly highest seed germination per cent of 85.75 and was on par with T10, 85.00%. The formulations, T6 and T2 have recorded similar 81.25 per cent of seed germination. Seed treatment with T7 and T1 were on par with 78.50% and 77.50% seed germination, respectively. The check fungicide which recorded 91.75 per cent germination has showed 6.53% improvement of seed germination over T5. While the per cent increase of seed germination of T5 was 33.81 per cent over the control (56.75%).

Minimum seed infection of 7.00 per cent was observed in the soybean seeds treated with T 10 followed by T5 with 8.00 per cent. No significant differences were observed between the seed treatments with T10 (7.00%) and T5 (8.00); T5 (8.00), T6 (9.50%) and T2 (10.00%). The fungicidal check T14 has shown 50.00 per cent reduction of seed infection over T10. The SV-I was highest in seed treatment with T10 (2404) and was on par with T5 (2190) but differed significantly with T6 (1914) and T2 (1852). The bacterial formulations T3, T4 and T7 bio fertilizer, *Rhizobium* sp. were on par with 1586, 1449 and 1521 SV-I, respectively. The fungicidal check has recorded highest of 2703 SV-I and 11.06 per cent improvement over the formulation T10 (2404). The bio formulation T5, has recorded significantly highest 2190, 96.61 g SVI-I and SVI- II, respectively which was followed by T6 (85.17g) and T3 (83.93g) for SVI-II. However, seed treatment with T8, and T13 have recorded maximum per cent seed infection (27.25%, 24.00%), lowest SV-I and SV-II of 1068, 48.62g, 1125, 55.14g, respectively (Table 4).

Fig.4. Effect of bioformulations on seed germination, infection and SVI-II against M.phaseolina

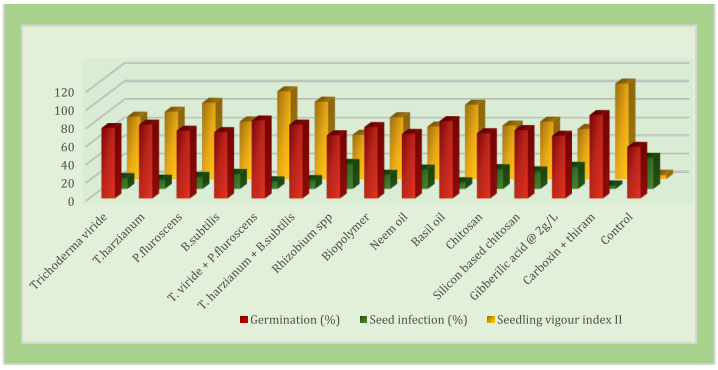
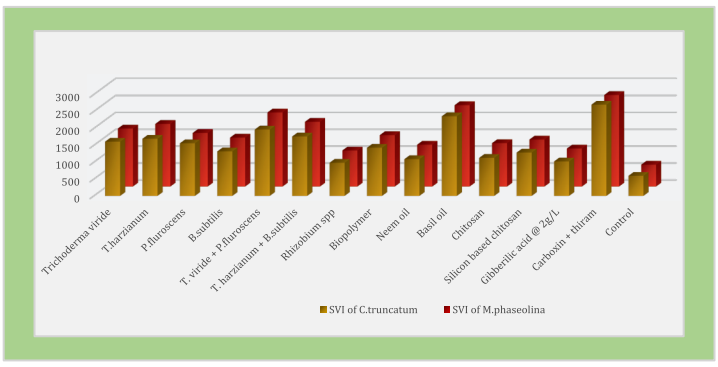


Fig.5. Effect of bioformulations on seedling vigour Index-I against C.truncatum and M.phaseolina



Discussion

Synthetic pesticides affect crop output and productivity positively, but they also have some adverse consequences on soil biodiversity, animals, aquatic life, and humans (Farooq *et al.*, 2019)[14].

In recent past, biopesticides have become widely available and have proven to be quite effective at controlling pests and diseases. The results of the study revalidate the facts that bioagents *T.viride* and *T.harzianum* either alone or in combination with bacterial bioagents *P.fluorescens* and *B.subtilis* are widely used microbials in combating the plant pathogens. However, the bio formulations T5, T6, T2 and T1 were found more effective than bacterial biocontrol agents against the test pathogens. The results are in harmony with the earlier reports of Jayalakshmi and Seetharamana (1998) [16] against *C.capsici*, Kulkarni (2009)[18] who observed that *T. harzianum* gave highest growth inhibition of *C.truncatum* (64.38%) followed by *T. viride* (50.46%). Kumar *et al*. (2013) [19] observed that maximum inhibition of the mycelial growth of *M. phaseolina* isolates was recorded by *T. harzianum* that varied from 61.1 to 70.1%. Doley and Jite (2012) [13] found that *T. viride* showed significant antifungal activity by inhibiting the mycelial radial growth of *M. phaseolina* by 71.42%. Ashwini *et al*. (2014) [2] reported that *P. fluorescens* showed best antagonistic activity against *M. phaseolina* (62.41%).

The poisoned food technique assay revealed that among the bio formulations, T10 basil oil was found equally effective with the check fungicide carboxin + thiram in inhibiting the mycelial growth of both the test pathogens. Natural products such as essential oils and biocontrol agents could be an effective alternative to synthetic pesticides against pathogens which decrease the negative impact of synthetic pesticides on humans and environment. From the present investigation, the antifungal activity of essential oil, basil oil is due to the presence of various antifungal components such as terpens, aliphatic and aromatic compounds especially alcohols, ethers, esters, aldehydes, ketones, lactones, phenols and phenol ethers Bakkali *et al*. (2008).[4]

The antagonistic response of bio formulations for seed quality and seed health parameters indicated that, the formulation T5 was most effective and superior followed by T6 in recoding highest per cent seed germination, minimum seed infection and maximum SVI-I and II comparatively with the remaining formulations. The microbial bioagents have exhibited significant impact on seed priming. This might be due to the cumulative effect of mixture of bio agents having different modes of action utilizing several mechanisms, such as mycoparasitism, production of antibiotics, lytic enzymes etc (Bora and Bora 2008[7], 2014[8], Bora et al.2016a[9], 2016b[10]). Tahia Benitez *et al*. (2004)[6] reported the antagonistic nature of *Trichoderma* spp. may be due to antibiosis, nutrient competition, production of specific compounds and metabolites, such as plant growth factors, hydrolytic enzymes, siderophores, Bakthavatchalu *et al.* (2012) [5] reported that antifungal activity of *P. fluorescens* might be due to IAA production, siderophore production, phosphate solubilizing activity. O.Sullivan and O.Gara, (1992) [25] proved that *Pseudomonas* spp. are well known for production of broad spectrum antibiotics such as 2,4-diacetyl phloroglucinol and antibiosis to be a major mechanism involved in their biocontrol activity and also stated that HCN and siderophores produced by *Pseudomonas* spp. were also involved in their antifungal activity. The present studies are in congruent with the findings of Rahman *et al.*, (2021) [22] , who conducted *in vitro* evaluation of ten commercial formulations of *Trichoderma* spp. as biopesticides against *Fusarium oxysporum* f. sp. *lycopersicum*, *Alternaria brassicae*, *Colletotrichum lindemuthianum*, *Rhizoctonia solani* and *Sclerotium rolfsii,* out of which five formulations, namely Biogreen, Biofor -PF2, Biolime, Bioveer, Biozium, and Biozin-PTB were found effective in suppressing the growth of these five pathogens up to 91.2 - 97.1%, which has supported the response on tomato and rice seeds in terms of germination and vigour index (I and II). Nevertheless, absolute replacement of chemical pesticides with biopesticides, is much difficult due to occurrence of abundant pest, disease, weed species etc and also climate variations. Therefore, it is reasonable to assume that both biopesticides and conventional pesticides will be included in the Integrated Plant Protection System.

Conclusion

The findings of the studies states that, development of bio formulations with a consortium of bioagents, botanical extracts, essential oils to comprehend different pathosystems and the potent seed priming effects of these bio formulations are strongly encouraged. Thus, an integration of various components of biological agents could be used as a technological innovation in soybean cultivation, as an alternative over chemical pesticides.

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Conflict of interest

All the authors have declared that they have no conflict of interest.

Ethical approval

The present investigation was the original research work carried by the authors and has not presented or published or submitted in any of the journals. The work performed and the results of the study does contain detrimental or harmful effects to the humans, animals and environment.

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