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Evaluation of *Trichoderma*-based biopesticides against plant pathogens and agronomic crop response

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ABSTRACT

Biopesticides are the part of bio-inputs driven agriculture, popularly define the success of organic agriculture, especially in northeast India. Even today, many of the disease causing pathogens (*Fusarium oxysporum* f. sp *lycopersicum*, *Alternaria brassicae, Colletotrichum lindemuthianum, Rhizoctonia solani and Sclerotium rolfsii*) are beyond their control with conventional management strategy. In the year 2019-20, an evaluation was carried out in the department of Plant Pathology, Assam Agricultural University, Jorhat, Assam where we collected as many 10 commercial formulations marketed widely as biopesticides, and observed the population of *Trichoderma* sp. ranging from 1.0×10^5 to 2.0×10^8 cfu/g, inclusive of two predominant species *T. harzianum* and *T. viride*. As many five formulations, viz. Biogreen, Biofor –PF2, Biolime, Bioveer, Biozium and Biozin-PTB were observed effective in suppressing the growth of these five pathogens upto 91.2-97.1% under challenge inoculation *in-vitro* studies, which corroborated the response on tomato and rice seeds with regard to germination and vigour index (I and II). Our studies warranted greater effectiveness of these formulations carrying multiple antagonists in addition to strong seed priming effectsof *Trichoderma* sp.

Keywords: Biopesticides, Germination, Pathogen, Rice, Tomato, Vigour index

In India, production of microbial biopesticides initiated in early 1990s, and by now more than 400 units are commercially engaged. Till date, more than 500 biopesticides are registered by the Central Insecticide Board (CIB) and available across Indian market (Gautam *et al.* 2018).We attempted to study some important plant pathogens, viz. *Alternarnia brassicae* (Berk.) Sacc. (*Alternaria* blight in mustard group of crops), *Colletotrichum lindemuthianum* (Sacc.&Magn.) Bri & Cav. (Anthracnose or black spot disease of bean), *Rhizoctonia solani*, *Sclerotium rolfsii* (attacking the roots and stem base of tomato) and *Fusarium oxysporum* f. sp. *lycopersicum* (wilt of tomato) causing some devastating and widespread disease throughout the world. Unfortunately, not many of the management options of these pathogens are available. Of late researches have focused on developing long lasting environmentally safe and effective biocontrol methods for plant diseases management (Kloeopper 1991, Paulitz *et al.* 2006, Ignjatov *et al.* 2012, Cho 2015, Padderi *et al.* 2017). Due to these reasons, most of the works done during recent past has been directed towards biological management, use of antagonistic fungi like *Trichoderma* spp. in this context appeared logical and safe (Bora *et al.* 2013, 2019, 2020).

Trichoderma spp. have long been identified and characterized as potential opportunistic, virulent plant symbiont and a effective biological agent against different soil borne pathogens antagonistic to wide range of soil borne plant pathogens (Howell 2002, Harman *et al*. 2004). Northeast agriculture, known as corridor of organic hub, bordering with south Asian countries, is likely to turn into trade and business hub of southeast Asia, thereby, offering a great market of biopesticides for organic agriculture. In this background, the present investigation was carried out with three objectives: i. estimate the effective population of some commercial *Trichoderma*-based formulations, ii. study the efficacy *Trichoderma*-based formulations on some important pathogens and iii. effect of *Trichoderma* formulations on germination and vigor index of tomato and rice seeds to identify biopesticides carrying effective *Trichoderma* bioagent strain.

MATERIALS AND METHODS

Isolation of microbial biogents from biopesticides: The experiment was conducted at the Department of Plant Pathology, Assam Agricultural University, Jorhat, Assam. A total of 10 commercial biopesticide products (T_1,Taglife V;T_2, Taglife H; T_3, Trigen; T_4, Panther TV; T_5, Biogreen;T_6,

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Biofor-PF-2; T_7, Biotime; T_8, Bioveer; T_9, Biozium and T_{10} Biozin-PTB) were collected during 2019-20 from different agromarkets of Assam. Isolation of the microbial bioagent was carried out following the standard protocol of serial dilution plate method as described by Johnson and Curl (1972). An aliquot of 1 ml from 10^{-4} dilution were spread on Potato Dextrose Agar (PDA) for counting the colony forming units of *T. viride* and *T. harzianum* through five replicates. The numbers of colonies formed on each medium were counted following 120 hr of incubation. The bioagents isolated were preserved for their further studies to evaluate the bioactive potential against important plant pathogens.

Antagonism of Trichoderma *spp*: *In vitro* test was carried out to evaluate the inhibitory effect of antagonists against the fungal pathogens using PDA as basal medium (Moorhouse *et al.* 1992). Culture plates of five important pathogens, viz. P_1 (*Fusarium oxysporum* f. sp *lycopersicum*), P_2 (*Alternaria brassicae)*, P_3 (*Colletotrichum lindemuthianum*), P_4 (*Rhizoctonia solani*) and P_5 (*Sclerotium rolfsii*) were prepared by growing on PDA in petri plates. Mycelial disc (5 mm diameter) were inoculated at the centre of PDA plates and incubated at 28±1°C for 72 hr. Assay plates of the fungal pathogens were prepared by transferring pure culture of the respective pathogens to near the periphery of the PDA plates. A 0.5 cm diameter of *Trichoderma* sp in TSM was scooped out with the help of a sterilized cork borer and transferred to either side of the PDA plates containing growth of the pathogens, one opposite the other. The percentage of growth inhibition was calculated according to method suggested by Hokkanen and Kotiluoto (1992). The mycelia growth inhibition was calculated as: I = C-T/C ×100, where, C and T stand for mycelial growth of pathogen in control and mycelial growth of pathogen in dual culture plate, respectively.

Response of Trichoderma *formulations*: The response of *Trichoderma* spp. isolated from 10 commercial formulations were tested *in vitro* for seed germination and vigour index of tomato (*Lycopersicon esculentum* Mill) and rice (*Oryza sativa* L.).

Seed germination: The seed germination test with *Trichoderma* based formulations was undertaken with tomato seeds surface sterilized with 30% sodium hypochlorite solution for 8 min followed by rinsing with distilled water. While, rice seeds were surface sterilized with 70% ethanol followed by 5% sodium hypochlorite, and finally washed by with sterilized distilled water. The sterilized seeds were then subjected to germination test following the between paper method. Three replicates of 100 seeds for each of the 10 *Trichoderma* based formulations plus as control were placed between two moist Whatman filter papers and then rolled up. A 0.3 ml of sterilized culture medium as a control and 0.2 ml of *Trichoderma* spp from

Table 1 *Trichoderma* isolates from different biopesticide products and their suppressive effect against five important pathogens (P_1-P_5)

Treatment	Carrier bioagents	Population (cfu/g)	Mycelial growth inhibition (%)				
			P_1	P_2	P_3	P_4	P_5
T_1	*T. viride*	$1.0×10^5$	95.0 (77.08)	88.6 (70.27)	57.7 (49.43)	76.7 (61.14)	76.1 (60.73)
T_2	*T. harzianum*	$1.0×10^5$	87.7 (69.47)	75.0 (60.00)	71.1 (57.86)	91.8 (73.36)	88.0 (69.73)
T_3	*T. viride*	$3.0×10^6$	92.5 (74.11)	74.4 (59.60)	89.8 (75.00)	91.1 (72.64)	77.6 (61.75)
T_4	*T. viride*	$2.0×10^5$	91.4 (72.95)	90.1 (71.66)	93.1 (66.97)	76.2 (35.24)	57.5 (49.31)
T_5	*T. viride* + PGPR	$5.0×10^5$	96.2 (78.76)	93.2 (74.88)	94.2 (76.06)	95.3 (77.48)	91.2 (72.74)
T_6	*T. harzianum* + *P. fluorescens*	$6.0×10^5$	96.8 (79.70)	94.0 (75.82)	90.7 (72.24)	94.2 (76.06)	91.0 (72.54)
T_7	*T.harzianum* + *P. fluorescens* + *M. anisopliae*	$7.0×10^7$	95.3 (77.48)	95.2 (77.34)	91.6 (73.15)	93.3 (75.00)	93.3 (75.00)
T_8	*T. viride*	$5.0×10^5$	95.6 (77.89)	93.8 (75.58)	93.8 (75.59)	94.5 (76.44)	94.2 (76.06)
T_9	*T. harzianum*	$7.0×10^5$	95.8 (78.17)	92.5 (74.11)	92.9 (74.55)	94.4 (76.31)	92.2 (73.38)
T_{10}	*T. harzianum* + *P. fluorescens* + *B. brevis*	$2.0×10^8$	97.1 (80.20)	96.1 (78.61)	94.2 (76.06)	96.5 (79.22)	94.5 (76.44)
CD (P=0.05)			1.9	2.0	3.1	4.8	3.4

-Figures given in parenthesis indicate their angular transformation values

-P_1,P_2,P_3,P_4 and P_5 represent pathogens such as *Fusarium oxysporum* f.sp *lycopersicum, Alternaria brassicae, Colletotrichum lindemuthianum, Rhizoctonia solani* and *Sclerotium rolfsii*, respectively.

each the products was collected after growing for 12 days, and poured into each petri dish. After five days, germinated seeds were determined.

Seed vigour: In continuation to seed germination test, the vigour indices were evaluated according to the protocol proposed by Abdul Baki and Anderson (1973). Post-germination test (8^{th} day of seed germination), 10 normal seedlings from all the treatments covering each replication were selected at random and their average seedling length (plumule tip to root tip) was recorded. Similarly, randomly selected 10 normal seedlings were oven-dried for an overnight at 90°C and their dry weights we rerecorded. The vigour indices were calculated using the formula as: Vigour index I = Germination (%) × Average seedling length of 10 cm seedlings and Vigour index II = Germination (%) × Average seedling weight of 10 (g) seedlings

RESULTS AND DISCUSSION

Population denisty of Trichoderma *spp. in different biopesticides*: Amongst different biopesticides, *Trichoderma* is most exploited and carved a niche as important bioagent for management of various diseases (Bora *et al*. 2019, Bora and Bora 2020). In our study, different biopesticides analysed for presence of *Trichoderma* spp. showed predominantly the abundance of *T. harzianum* and *T. viride* to varying proportions (Table 1). Out of the 10 formulations, the formulations, viz. Tagalife V(T_1), Trigen (T_3), Panther TV (T_4), Biogreen (T_5) and Bioveer (T_8) were observed as *T. viride* based formulations. While, other formulations such as Taglife H (T_2), Biofor-PF-2 (T_6), Biotime (T_7), Biozium (T_9) and Biozin-PTB (T_{10}) showed *T. harzianum* based formulations. These results revalidated the fact that *T. harzianum* and *T. viride* are the widely used species. According to Sharma *et al.* (2014), *Trichoderma* sp. have been evaluated on about 87 different crops and about 70 soil borne and 18 foliar pathogens, signifying the pivotal role of *Trichoderma* against soil borne fungal diseases.

The population density of two different species of *Trichoderma* was also observed to be contrastingly varying, being maximum in T_{10} carrying Biozin-PTB (2.0×10^8 cfu/g) followed by T_7 as Biotine (7.0×10^7 cfu/g) and T_3 as Trigen (3.0×10^6 cfu/g), regardless of *Trichoderma* sp. And, rest of the formulations (T_1 as TaglifeV, T_2 as Taglife H, T_4 as Panther TV, T_5 as Biogreen, Biofor-PF-2 as T_6, Bioveer as T_8 and Biozium as T_9) were observed having population density of 1.0-7.0 $\times10^5$cfu/g (Table 1).

Antagonistic response of Trichoderma *formulations*: The isolated *Trichoderma* spp from different biopesticides subjected to qualitative assessment for inhibition of mycelial growth of five pathogens through dual culture method showed differential magnitude of growth inhibition (Table 1). The formulation T_{10} was observed most effective with 97% mycelial growth inhibition of pathogen P_1, statistically on par with T_6, T_5, T_9,T_8 and T_7 with 95.3-96.8% growth inhibition followed by 95.01% growth inhibition with T_1, 92.5% growth inhibition with T_3 and 91.4% growth inhibition with T_4. In case of suppressing pathogen P_2, T_{10} and T_7 were observed most effective with 95.2-96.1% growth inhibition and T_1, T_2 and T_3 as least effective with 74.4-88.6% growth inhibition. Likewise, formulations T_{10} and T_5 were observed most effective against pathogen P_3 with 94.2% growth inhibition and formulations T_{10} and T_5 against P_4 with 95.3-96.5% growth inhibition. Again, the formulations T_{10} and T_8 displayed a growth inhibition of 94.2-94.5% being most effective against pathogen P_5. However, formulation T_{10} was observed invariably effective against all the five pathogens due to cumulative effect of multiple bioagents having different modes of action. These observations showed that *Trichoderma*-based formulations have gone a long way from a common soil borne fungi to an effective biocontrol agent for destructive plant pathogens (Christos *et al.* 2018) employing several mechanism, viz.

Table 2 Response of different *Trichoderma*- based formulations on germination and vigour index of tomato and rice seeds

Treatment	Tomato seeds			Rice seeds		
	Germination (%)	Vigour index-I	Vigour index-II	Germination (%)	Vigour index-I	Vigour index-II
T_0 (Control)	70.00	2940	2128	75.00	1125	829
T_1 (Taglife V)	84.66	4938	3699	85.66	1724	842
T_2 (Taglife H)	83.33	4665	3083	88.00	2029	1604
T_3 (Trigen)	86.66	5198	4101	77.66	1567	1048
T_4 (Panther TV)	80.66	4355	3113	88.66	1612	1622
T_5 (Biogreen)	95.00	6175	4759	93.00	2119	1813
T_6 (Biofor-PF-2)	96.66	7249	5831	96.66	2402	1955
T_7 (Biotime)	94.33	7263	5907	95.66	2387	1973
T_8 (Bioveer)	97.00	8148	7086	95.00	2413	2092
T_9 (Biozium)	96.00	7200	5878	96.66	2213	1730
T_{10} (Biozin PTB)	93.00	7905	5976	94.33	2354	1870
CD (P=0.05)	2.3	510	214	2.83	89	56

mycoparasitism, antibiotic production and production of lytic enzymes (Bora and Bora 2008, 2014, Bora *et al.* 2016a, 2016b).

Response on seed germination and vigour index: All the *Trichoderma* formulations displayed a significant influence on germination and vigour index (I and II) of tomato and rice seeds (Table 2). The seed germination was maximum (95.0-97.00%) with treatments T_5, T_6, T_9 and T_{10} (statistically on par amongst each other). However, vigour index (I and II) out responded with treatment T_8 compared to rest of other treatments. Likewise, rice seeds responded differentially with regard to germination (95.00-96.66% with T_6, T_7, T_8 and T_9) and vigour index (Vigour index I was 2387-2413 and Vigour index II as 1955-2092 with T_6, T_7 and T_8).These observations suggested greater vigour index of tomato seeds than rice seeds in response to different formulations. The microbial bioagents, hence, have strong seed priming effect. *In-vitro* response of seven isolates of *Trichoderma* spp. showed increased rice seedling growth, germination rate, vigour index and speed of germination (Doni *et al*. 2014). While, Azarmi *et al*. (2011) observed that seed germination was not affected by *Trichoderma* application, but shoot height, shoot diameter, shoot fresh and dry, weight and root system and dry weight in tomato seedlings were interestingly increased in *Trichoderma harzianum* fortified soil.

Our studies, hence, suggested strong necessity of developing biopesticides having consortium of bioagents to understand the pathosystem and the strong seed priming effect of these *Trichoderma*-based formulations.

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