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In vivo and in vitro assessment of *Trichoderma* species and *Bacillus thuringiensis* integration to mitigate insect pests of brinjal (*Solanum melongena L.*)

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Abstract

The present study was performed to assess the individual and combined potential of 3 different concentrations of *Trichoderma* sp. (*Ts*) and *Bacillus thuringiensis* (*Bt*) to control brinjal insect pests. Tested formulations were applied on larval and adult stages of the pest. The *Trichoderma* laboratory bioassays revealed 73% mortality of the aphid species, *Aphis gossypii* (Glover), and 53% mortality of the cotton leafhopper (Jassids) *Amrasca bigutulla bigutulla* (Ishida), while opposite results were observed in case of *Bt* at the highest concentration (1×10^8 cfu ml^{-1}) used. In vivo results revealed that *Ts* caused a significant population reduction of the aphid (87%) than the jassid (72%), 7 days of post-treatment, at the highest concentration, while non-significant results were observed at the lowest concentrations. *A. gossypii* was significantly found more susceptible to the mixture of *Trichoderma* + *Bt* than the jassid (62%) and brinjal shoot and fruit borer (65%) even after 48 h of treatment application. The combined application (*Trichoderma* + *Bt*) showed maximum population reduction of jassid (88%), aphid (95%), and BSFB (96%), respectively, 7 days post-applications. The positive correlation among time and concentration was observed. The result may imply that consortium of these microbial organisms could be effective and can be incorporated in IPM programs for effective control of sucking and chewing insect pests of brinjal.

Keywords: Brinjal, Biological control, *Trichoderma* spp., *Bacillus thuringiensis*, IPM, Insect pests

Background

Brinjal (*Solanum melongena* L.) is cultivated throughout the world on more than 1.8 million ha with about 50.9 million tonnes production annually. In Pakistan, the cultivated area under brinjal crop is 8575 ha with annual production of 87,587 tonnes (FAO, 2018). It is grown in all cropping seasons. Aphid (*Aphis gossypii*), jassid (*Amrasca bigutulla bigutulla* Ishida), white fly (*Bemisia tabaci* Gennadius), and brinjal shoot and fruit borer (BSFB) (*Leucinodes orbonalis* Guenee) are the major pests of brinjal crop. These insect pests attack the crop at different growth stages and results in a significant decrease in yield quantity and quality. Among all the sucking and chewing insect pests and brinjal shoot and fruit borer, *L. orbonalis* is the major pest of brinjal crop worldwide (Chakraborty and Sarkar 2011; Dutta et al. 2011). It is the most serious pest of brinjal in Asia, especially in Pakistan, India, Nepal, Bangladesh, Sri Lanka, Thailand, the Philippines, Cambodia, Vietnam, Cambodia, Laos, Southeast Asia, Africa, and Sahara. In South Asia, brinjal shoot and fruit borer causes severe yield losses (85–90%) (Misra 2008; Jagginavar et al. 2009).

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Pesticides usage for the control of the insect pests in brinjal is high (Shetty 2004). For example, it has been reported that in certain areas of the Philippines and Bangladesh, the farmers sprayed 56 and 180 times, respectively, during a single cropping season. Therefore, it is needed to switch for other environmentally safe pest control methods, such as the bioagents/biopesticides for pest management (Kabadwa et al. 2019). The entomopathogenic fungi and bacteria are the most important and auspicious candidates for pest management (Afzal et al. 2013; Xiaoman et al. 2019).

Trichoderma strains colonize and infect the outer layers of roots making a zone of chemical interaction (Mishra et al. 2018). Chemical elicitors from *Trichoderma* interact with putative plant receptors (Harman and Shoresh 2007). They induce systemic resistance on a wide range of plant/pathogen combinations as described in previous studies in both axenic and field soil systems (Yedidia et al. 2003). Similarly, *Bacillus thuringiensis* (*Bt*), the naturally occurring, spore-forming bacterium, is present in soil and has been utilized more efficiently for protection of food crops, forest trees, ornamentals, and stored grains (Meadows 1993). Being safe to environment and highly specified, Bt spores and crystals have been used successfully as bioinsecticide for control of different lepidopteran, coleopteran, and dipteran insect pests (Schnepf et al. 1998). Similarly, once infection with *Trichoderma* strains occur, a zone of chemical interaction develops at these sites. Within this zone of chemical interaction, the *Trichoderma* hyphae are walled off on the plant but do not kill it (Harman and Shoresh 2007). Chemical elicitors from *Trichoderma* produced by the walled off hyphae interact with putative plant receptors (Harman and Shoresh 2007).

The present study was conducted to assess individual and combined potential effects of *Trichoderma* spp. and *Bt* on brinjal productivity and insect pest infestation.

Materials and methods

Trichoderma and *Bt* formulations were obtained from the Soil and Environmental Microbiology Laboratory, Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan, and mass cultured in the Integrated Pest Management Laboratory, Department of Entomology, UAF, for experimentation.

Bacterial formulation

For bacteria, inoculum was prepared in TSB (tryptic soy broth) media (Paik et al. 1997) in 1000 ml Erlenmeyer flask and autoclaved at 121 °C for 20 min. After preparation of broth, a loopful of bacterial colony from the Petri plate was added into the broth under the laminar air flow chamber and kept in shaking incubator (Firstek Scientific, Tokyo, Japan) at 180 rpm for 48 h at 28 ± 1 °C. An OD of 0.5, measured with an optical density meter (Biolog® Model-21907; Biolog Inc.) at λ 600 nm, was achieved by dilution to maintain a uniform cell density (10^8 cfu ml^{-1}) prior to application (Naveed et al. 2014). The *Bt* inoculum was further diluted to prepare formulations of different cell density (10^7 and 10^6 cfu ml^{-1}). Inoculum and saline buffer (0.85% NaCl w/v) at ratios 1:9 and 2:18 were mixed to prepare *Bt* suspensions containing 10^7 and 10^6 cfu ml^{-1}. To achieve these populations, $OD_{0.4,\ 0.3}$ were adjusted prior to application.

Fungal formulation

For fungus inoculum, PDB (potato dextrose broth) media was used (Meyer et al. 2000) in 1000 ml Erlenmeyer flask and autoclaved at 121 °C for 20 min. After preparation of media, a 5-mm disc of fungus from the Petri plate was added into the prepared media under a laminar airflow chamber and kept at 25 ± 1 °C for 5 days. The same procedure (as described for *Bt*) was followed to prepare fungal formulations before application.

Leaf dip bioassays

Laboratory experiments, using leaf dip bioassay method against brinjal insect pests, were carried out. The brinjal plants were sown at the Entomological Research Farm, University of Agriculture, Faisalabad, to collect the leaves and to sample aphid, jassid, and fruit borer larvae. The collected leaves of brinjal plants were rinsed by distilled water to remove contamination (if any). A disc of 7 cm diameter was cut from the collected leaves. Then, leaf discs were dipped into the bio-pesticide formulations (Table 1) for 3 min and room dried. The leaf discs were placed in Petri dishes. The 20 specimens of aphid and jassid were fed on leaf discs, while 5 larvae of fruit borer (collected from the field plants) were fed on brinjal fruits treated with biopesticide formulations (Table 1). Mortality rates of aphid, jassid, and larvae of fruit borer were recorded 24, 48, and 72 h post-treatment of biopesticides (Mazra 2007).

Potted plant bioassay (field bioassay)

The plants were sown in earthen pots at Entomological Research Farm, Department of Entomology, University of Agriculture Faisalabad. Healthy plants were placed in open field for insect pests attack. The plants were irrigated regularly to fulfill their requirement of water and nutrients as well. When the infestation of brinjal insect pests reached near their threshold levels, i.e., aphid 5/leaf, jassid 2/leaf, and BSFB 10% damage (http://www.pestwarning.agripunjab.gov.pk/economic-thresholds), the biopesticide formulations (Table 1) were applied by foliar application method. The data of aphid and jassid were collected from upper-middle and lower leaves of each pot, and the data

Table 1 Treatments and their details for leaf dip bioassay in laboratory and potted plant bioassay in open field conditions

T. no.	Treatments	Composition of the inoculum		Concentration
		Laboratory	Field	
T1	*Trichoderma spp.*	60 ml	600 ml	10^8 cfu ml^{-1}
T2	*Bacillus thuringiensis*	60 ml	600 ml	10^8 cfu ml^{-1}
T3	*Trichoderma + Bacillus thuringiensis*	30 ml + 30 ml	300 ml + 300 ml	10^8 cfu ml^{-1}
T4	*Trichoderma spp.*	10 ml + 90 ml water	60 ml + 540 ml water	10^7 cfu ml^{-1}
T5	*Bacillus thuringiensis*	10 ml + 90 ml water	60 ml + 540 ml water	10^7 cfu ml^{-1}
T6	*Trichoderma + Bacillus thuringiensis*	(10 ml + 10 ml) + 80 ml water	(60 ml + 60 ml) + 480 ml water	10^7 cfu ml^{-1}
T7	*Trichoderma spp.*	5 ml + 95 ml water	30 ml + 570 ml water	10^6 cfu ml^{-1}
T8	*Bacillus thuringiensis*	5 ml + 95 ml water	30 ml + 570 ml water	10^6 cfu ml^{-1}
T9	*Trichoderma + Bacillus thuringiensis*	(5 ml + 5 ml) + 90 ml water	(30 ml + 30 ml) + 540 ml water	10^6 cfu ml^{-1}
T10	Control	Water application	Water application	

of the fruit borer were estimated by its damage symptoms (shoot and fruit damage). The data was recorded after 24, 48, and 72 h and 1 week post-application.

Data analysis

Population reduction percentage and percentage mortality of insects in the leaf dip bioassay were calculated by Abbott's formula (Abbott 1925). The collected data were analyzed using a software, Statistics version 8.1, and means were compared, using Tukey's honestly significant difference (HSD) test at 5% significant level.

Results and discussion

The results presented concentration (cfu ml^{-1}) and time-dependent efficacy of the *Trichoderma* and *Bt* in vivo as well as in vitro experiments. The highly significant difference was observed when entomopathogens were applied both alone and in combination. The maximum population reduction percentage of the target insects was observed after 1 week of field application, while the maximum mortality was after 72 h of treatment application in laboratory bioassay.

In laboratory experiments (Table 2), the results of *Trichoderma* alone are in accordance with Ganassi et al. (2001). The time and dose-dependent mortality was recorded. The significantly high percentage aphid mortality was observed (44, 36, and 13%) while 33, 13, and 6% jassid mortality was observed at 10^8, 10^7, and 10^6 (cfu ml^{-1}) concentrations, respectively, after 24 h of post-treatment application (Pacheco 2017). The active movements of jassids may be a factor of getting less mortality than the aphids. Therefore, the jassids may be more resistant to such fungus than the aphids. Maketon et al. (2008) used *Metarhizium anisopliae* at the high concentration at 10^{13} cfu ml^{-1} and recorded > 70% mortality

Table 2 Means percentage mortality of jassid, aphid, and brinjal shoot and fruit borer after entomopathogen treatments with different time intervals in leaf dip bioassay under laboratory condition. (values with different lower case letters show significant difference at 0.05 significance level)

Treatments	Jassid			Aphid			Brinjal shoot and fruit borer		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
T1	33.33 ± 1.33^{i}	46.67 ± 1.47^{g}	53.33 ± 1.57^{ef}	44.00 ± 0.74^{er}	60.00 ± 1.51^{c}	73.33 ± 1.57^{b}	22.22 ± 0.78^{fg}	44.44 ± 1.26^{d}	66.67 ± 1.77^{c}
T2	40.00 ± 1.34^{g}	60.00 ± 1.52^{ef}	73.33 ± 1.58^{e}	36.67 ± 0.74^{fg}	48.67 ± 1.47^{e}	53.33 ± 1.58^{d}	33.33 ± 0.78^{g}	55.56 ± 1.25^{f}	77.78 ± 1.78^{e}
T3	53.33 ± 1.48^{ef}	73.33 ± 1.68^{d}	93.33 ± 1.86^{b}	60.00 ± 0.78^{c}	73.33 ± 1.48^{b}	95.33 ± 1.86^{a}	44.44 ± 0.77^{d}	66.67 ± 1.31^{c}	88.89 ± 1.76^{a}
T4	13.33 ± 0.85^{g}	33.33 ± 1.33^{ef}	46.67 ± 1.49^{e}	36.67 ± 0.85^{fg}	46.67 ± 1.13^{e}	53.33 ± 0.87^{d}	11.11 ± 0.85^{g}	22.23 ± 0.93^{f}	55.56 ± 0.79^{e}
T5	33.33 ± 1.39^{f}	46.67 ± 1.56^{de}	53.33 ± 1.75^{c}	33.33 ± 0.79^{g}	34.00 ± 1.16^{g}	40.00 ± 0.85^{f}	22.23 ± 0.79^{gh}	44.47 ± 0.76^{gh}	66.33 ± 0.75^{fg}
T6	46.67 ± 1.61^{e}	66.67 ± 1.67^{c}	86.67 ± 1.86^{a}	43.33 ± 0.81^{ef}	60.00 ± 1.17^{c}	76.67 ± 0.86^{b}	33.34 ± 0.81^{f}	55.57 ± 0.87^{d}	77.78 ± 0.76^{b}
T7	06.67 ± 0.85^{j}	26.67 ± 1.36^{gh}	32.97 ± 1.24^{g}	13.33 ± 0.85^{j}	26.67 ± 1.66^{h}	50.00 ± 1.84^{de}	0.00 ± 0.65^{k}	22.23 ± 0.93^{hi}	33.33 ± 1.64^{ef}
T8	26.67 ± 0.99^{gh}	33.33 ± 1.57^{g}	46.67 ± 1.33^{ef}	15.00 ± 0.99^{ij}	23.33 ± 1.57^{hi}	33.33 ± 1.83^{g}	11.11 ± 0.49^{jk}	33.34 ± 1.15^{i}	44.44 ± 1.44^{gh}
T9	33.33 ± 1.31^{g}	53.33 ± 1.77^{e}	73.33 ± 1.82^{c}	19.67 ± 0.81^{i}	30.00 ± 1.77^{gh}	60.33 ± 1.82^{c}	22.23 ± 0.51^{j}	44.45 ± 1.29^{h}	66.67 ± 1.78^{d}
T10	0 ± 0^{k}	0 ± 0^{k}	0 ± 0^{k}	0 ± 0^{k}	0 ± 0^{k}	0 ± 0^{k}	0 ± 0^{l}	0 ± 0^{l}	0 ± 0^{l}

rate in Jassids, while in the obtained results, 33% mortality rate was recorded at 10^8 cfu ml^{-1}. After 48 and 72 h, the percentage mortality of jassids was 46 and 53%, and 60 and 73% in the case of aphids, at the highest concentration (10^8 cfu ml^{-1}) of *Trichoderma sp.* Similar trend was observed in percentage mortality of aphids and jassids at the lowest concentrations (10^7 and 10^6 cfu ml^{-1}). In comparison to the field results, the population reduction trend was similar in the field pots after 48 h of post-treatment application. In case of *Bt*, an opposite mortality trend than *Trichoderma* was observed for aphids and jassids. Greater percentage mortality of jassid was observed, i.e., 40, 33, and 26%, than the aphids 36, 33, and 15% at the 3 concentrations (10^8, 10^7, and 10^6 cfu ml^{-1}), respectively. *Bt* has known to have some genes to control the sucking pest (Kaur and Subash 2014) and may be jassid is not more resistant to those genes. In comparison to the field results, the opposite trend was observed, as more reduction was observed in aphids' population.

In the case of brinjal shoot and fruit borer, *Trichoderma* alone caused 22, 44, and 66% mortality after 24, 48, and 72 h at the highest concentration (10^8 cfu ml^{-1}), while the same trend was observed in the field that with the passage of time population reduction percentage increased, i.e., 27, 36, 39, and 49% after 24, 48, 72 h, and 1 week of post-applications, respectively (Table 3). Time-dependent efficacy of *Trichoderma* was observed in both field and laboratory experiments (Ganassi et al. 2001). At lower concentrations (10^7 and 10^6 cfu ml^{-1}), the results were similar in laboratory bioassay when compared to the field experiment. *Bt* showed up to 33, 55, and 77% mortality rates of brinjal shoot and fruit borer after 24, 48, and 72 h, respectively, at the highest concentration (10^8 cfu ml^{-1}) in laboratory bioassay (Table 2). Similarly, the lower concentrations (10^7 and 10^6 cfu ml^{-1}) showed less mortality after 24 and 48 h, respectively.

The results obtained with *Trichoderma* alone are in accordance with Ganassi et al. (2001). The significantly higher population reduction of jassid (45%) than aphid (32%) was observed after 24 h in the pots, at the highest concentration used (10^8 cfu ml^{-1}). In contrast, with the passage of time, the population reduction of aphid was significantly higher, i.e., 66, 78, and 87% than the jassid 47, 58, and 72% after 48, 72, and 168 h, respectively (Table 3). The entomopathogenic fungi are slow in action to cause an effect on target insects (Hafiza et al. 2014), and jassids movement was faster than the aphids. Therefore, the jassid may move from one place to other due to some repellent action of plant after treatment application. The population reduction of aphid increased with the passage of time because aphids remain stick to the target site and cannot change their place quickly (Ali et al. 2010). Overall, at the lowest concentrations (10^6 and 10^7 cfu ml^{-1}), the population reduction of aphids was lower than the jassids. This may be due to the reason that aphids are resistant to *Trichoderma* at low concentration (Ganassi et al. 2001). Similarly, in case of *Bt*, the overall jassid population reduction was higher, i.e., 38, 26, and 19% than aphids 33, 26, and 26% after 24 h of all treatment application. The population reduction of aphids increased than the jassids with increase in treatment period, i.e., 48, 72, and 168 h. The reason behind that could be the same as in the case of *Trichoderma*. Similarly, field study also revealed time-dependent population reduction of BSFB after 24 (46%), 48 (56%), 72 (61%), and 168 (87%) h post-application. Rehab et al. (2020) also reported more than 80% population reduction of spiny bollworm *Earias insulana* after 1 week of post-application of five different bio-insecticides including entomopathigenic fungi and *Bt*. In addition, Joshi et al. (2010) and Nayak et al. (2013) reported that *Bt* significantly reduced the brinjal shoot and fruit borer population. The *Bt* was significantly more effective in vivo than in vitro conditions which is also in accordance with the previous studies (Nayak et al. 2013).

The pathogenicity of *Trichoderma* and *Bt* consortium was significantly higher in laboratory than in the field. However, a significant population reduction of aphids (73%) was recorded after 48 h at the highest concentration than jassids (62%). In accordance with individual application of entomopathogen, the aphids become significantly more susceptible to the combination of *Trichoderma* and *Bt* with the increasing time of application of the highest concentration. The population reduction of aphids was 90–95% and jassid was 79–88% after 72–168 h, respectively, at the highest concentration (10^8 cfu ml^{-1}). Low concentration of *Trichoderma* and *Bt* consortium showed non-significant population reduction difference of target insects (aphids and jassids). The correlation coefficient values (r) and scatter diagram (Fig. 1) showed a positive correlation with mean percent mortality of target insect pests in laboratory as well as under field conditions.

Generally, the BSFB is more susceptible to *Bt* than entomopathogenic fungi (Joshi et al. 2010 and Nayak et al. 2013) because *Bt* is more effective against borer than sucking insect pests (Krishna et al. 2002). The 51, 38, and 30% population reduction while 66, 55 and 44% mortality rate was observed after 24 h of *Bt* and *Trichoderma* consortium application at all concentrations (10^8, 10^7, and 10^6 cfu ml^{-1}). After 48 h of treatment application, similar trend of population reduction (65, 51, and 46%) and mortality (88, 77, and 66%) was recorded in field and laboratory conditions, respectively. The 96, 80, and 72% population reduction at all concentrations (10^8, 10^7, and 10^6 cfu ml^{-1}) was observed after 168 h respectively. These findings are also in

Table 3 Means population reduction percentage of jassid, aphid, and brinjal shoot and fruit borer after entomopathogen treatments with different time intervals under field conditions (values with different lower case letters shows significant difference at 0.05 significance level)

Treatments	Jassid				Aphid				Brinjal shoot and fruit borer			
	24 h	48 h	72 h	168 h	24 h	48 h	72 h	168 h	24 h	48 h	72 h	168 h
T1	45.68 ± 0.89^{gh}	47.00 ± 1.26^{g}	58.33 ± 1.36^{e}	72.00 ± 2.06^{c}	32.43 ± 0.40^{i}	66.82 ± 1.26^{d}	78.68 ± 0.52^{c}	87.77 ± 0.86^{b}	27.11 ± 0.83^{i}	36.04 ± 0.12^{gh}	39.00 ± 0.76^{gh}	57.55 ± 0.89^{e}
T2	38.46 ± 1.28^{hi}	40.66 ± 1.35^{h}	46.66 ± 1.38^{g}	53.00 ± 1.85^{f}	33.11 ± 0.40^{i}	52.12 ± 1.15^{f}	63.92 ± 0.53^{de}	71.62 ± 0.85^{cd}	46.52 ± 0.84^{fg}	56.60 ± 0.12^{e}	61.54 ± 0.88^{de}	87.52 ± 0.79^{ab}
T3	59.61 ± 1.07^{de}	62.75 ± 1.77^{de}	79.33 ± 1.37^{b}	88.40 ± 1.87^{a}	47.74 ± 0.41^{fg}	73.78 ± 1.37^{cd}	90.17 ± 0.54^{ab}	95.39 ± 0.87^{a}	51.72 ± 0.86^{f}	65.04 ± 0.13^{d}	85.15 ± 0.77^{ab}	96.12 ± 0.96^{a}
T4	33.68 ± 0.78^{i}	35.00 ± 0.88^{hi}	46.55 ± 0.55^{g}	53.00 ± 1.76^{f}	23.75 ± 1.73^{jk}	39.39 ± 0.84^{h}	45.31 ± 0.91^{g}	62.05 ± 1.76^{de}	19.67 ± 0.80^{k}	29.85 ± 0.70^{j}	30.66 ± 0.72^{i}	50.74 ± 0.89^{g}
T5	26.66 ± 0.77^{ij}	33.66 ± 0.87^{hi}	40.44 ± 0.54^{h}	46.00 ± 1.69^{g}	26.06 ± 1.75^{ij}	37.23 ± 0.85^{h}	49.62 ± 0.92^{fg}	52.79 ± 1.69^{f}	34.42 ± 0.81^{i}	48.37 ± 0.72^{h}	50.93 ± 0.73^{g}	66.12 ± 0.88^{e}
T6	40.84 ± 0.79^{h}	46.66 ± 0.87^{g}	58.33 ± 0.56^{e}	70.78 ± 1.66^{c}	38.57 ± 1.47^{h}	49.27 ± 0.83^{fg}	63.31 ± 0.92^{de}	71.70 ± 1.66^{cd}	45.76 ± 0.82^{h}	56.63 ± 0.70^{df}	74.63 ± 0.72^{de}	87.70 ± 0.87^{c}
T7	25.27 ± 0.80^{ij}	30.00 ± 0.80^{i}	39.33 ± 0.93^{h}	47.00 ± 1.24^{g}	19.15 ± 0.73^{k}	26.12 ± 0.84^{ij}	36.94 ± 0.78^{h}	43.27 ± 0.54^{g}	24.46 ± 0.88^{j}	29.00 ± 0.93^{hi}	32.85 ± 0.65^{h}	48.30 ± 1.76^{fg}
T8	19.86 ± 0.81^{j}	26.66 ± 0.81^{ij}	31.44 ± 0.93^{i}	40.33 ± 1.23^{h}	26.48 ± 0.71^{ij}	23.96 ± 0.84^{jk}	36.89 ± 0.78^{h}	42.35 ± 0.56^{g}	33.79 ± 0.87^{h}	42.66 ± 0.92^{g}	51.73 ± 0.64^{f}	69.90 ± 1.69^{cd}
T9	27.24 ± 0.82^{ij}	41.66 ± 0.82^{d}	49.00 ± 0.92^{fg}	61.40 ± 1.32^{d}	31.69 ± 0.72^{i}	40.46 ± 0.84^{gh}	52.72 ± 0.78^{f}	60.64 ± 0.61^{e}	38.27 ± 0.89^{gh}	51.66 ± 0.89^{f}	69.64 ± 0.66^{cd}	80.30 ± 1.66^{b}
T10	5.00 ± 0.80^{k}	5.00 ± 0.81^{k}	5.00 ± 1.26^{k}	5.00 ± 1.40^{k}	4.66 ± 0.71^{l}	5.00 ± 0.81^{l}	5.33 ± 0.93^{l}	5.00 ± 0.80^{l}	4.66 ± 0.73^{l}	5.33 ± 0.91^{l}	5.00 ± 0.83^{l}	5.00 ± 0.80^{l}

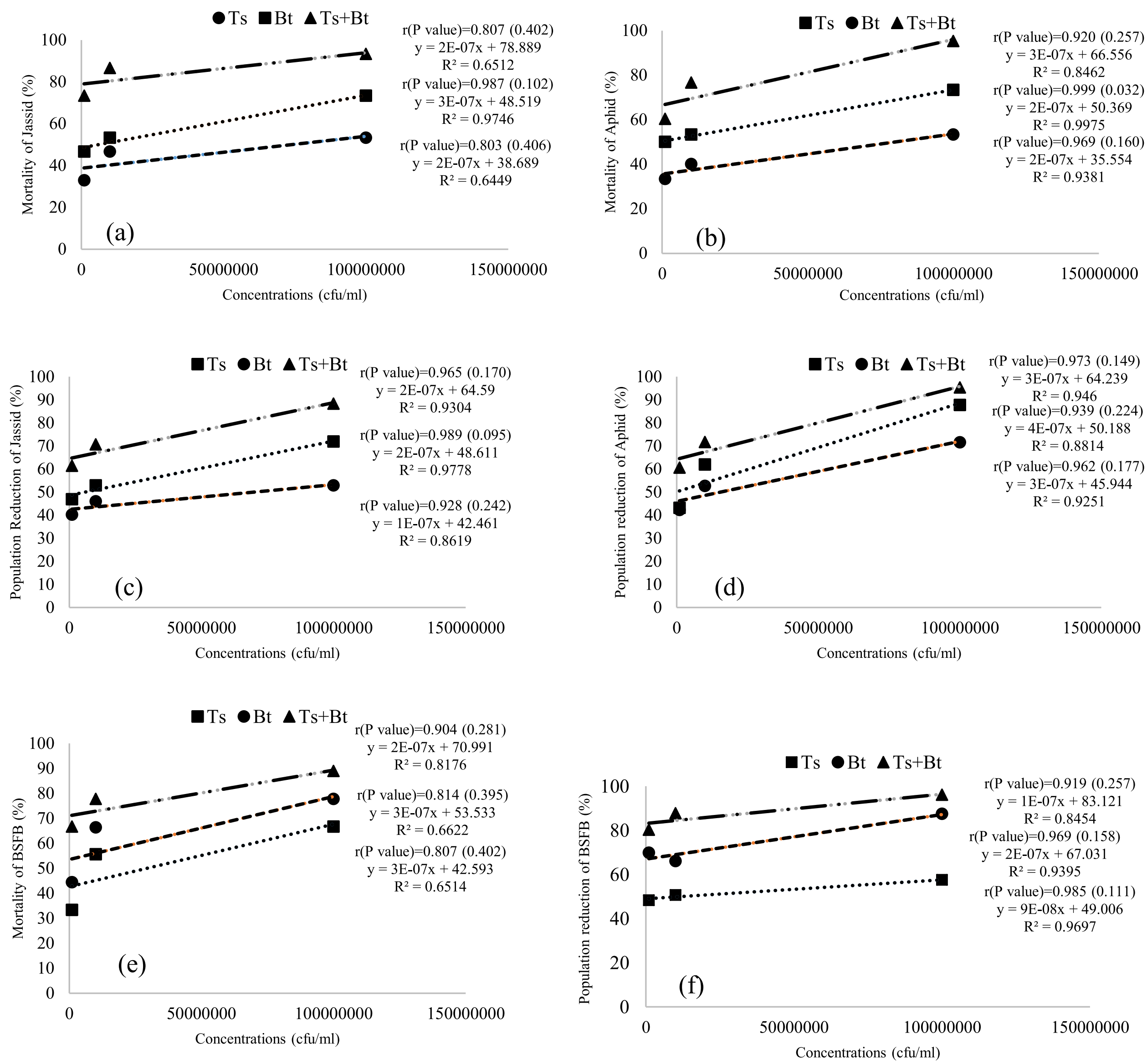

Fig. 1 The correlation coefficient values and scatter diagram showed a positive correlation with mean percent mortality of target insect pests in laboratory as well as under field conditions (R^2: coefficient of determination), ($\hat{Y}$: dose-dependent percentage mortality of target pests) (X: densities of aphid, jassid, and brinjal shoot and fruit borer)

accordance with recent findings of Rehab et al. (2020). The maximum mortality (88%) of BSFB was recorded after 72 h treatment application in laboratory by combined application of *Trichoderma* and *Bt* (Table 2). The maximum population reduction (96%) was observed after 168 h of treatment application at highest concentration used in the field (Table 3). These results revealed that combined application of entomopathogens can lead to a sustainable reduction in both chewing and sucking insect pests. The laboratory conditions always showed high mortality than pots in open field conditions, which may be the result of pest migration to other host plants and vice versa.

Conclusion

It may be concluded that the combination of *Trichoderma* spp. and *B. thuringiensis* showed a synergistic/additive effect and gave better control of brinjal insect pests. Single formulation provided also significant population reductions of brinjal insect pests. So, this microbial consortium could be used as safe potential biocontrol agent against insect pests of brinjal. However, field trial evaluation with this microbial consortium is required.

Abbreviations

IPM: Integrated pest management; Bt: Bacilllus thuringiensis; Ts: Trichoderma species; BSFB: Brinjal shoot and fruit borer; TSP: Tryptic soya broth;

PDA: Potato dextrose broth; HSD: Honestly significant difference; CFU: Colony-forming unit

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Authors' contributions
AN designed the experiment and completed the write up. MDG and MS helped to design the study, and MN and MB helped SI to conduct the experiment. MW and MJA reviewed the manuscript and made valuable changes, and HA helped in statistical analysis. All authors approved the final article after reading.

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Competing interests
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