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Bio-formulation based on plant oil of the future *Beauveria bassiana* (Balsamo) for fruit borer control in tomatoes

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Abstract
The plant oil based bio-formulation of *Beauveria bassiana* (B2 and TBb8) strains and its mixture amended with and without plant derived oils were developed and tested for their efficacy against the lepidopteran fruit borer insect pest (*Helicoverpa armigera*) in tomato under glasshouse and field conditions. Application of oil based bio-formulation of strains mixture (amended with or without oil) through seed, soil and foliar spray effectively reduced the incidence of fruit borer in tomato compared to individual control treatments both under glasshouse and field conditions. Tomato leaves when treated with this bioformulation mixture, altered the feeding behavior of fruit borer larvae and increased larval mortality under *in vitro*. Further, a significant increase in growth parameters and yield were observed in tomato plants treated with compared to untreated control. Increased accumulation of defence related enzyme was observed in tomato plants treated with against *H. armigera* when compared to other treatments.

Keywords: Bio-formulation, *Beauveria bassiana,* fruit borer, *Helicoverpa armigera,* tomato

Introduction

Tomato (*Solanum lycopersicum* Mill.) is one of the most important vegetable crops in the world. It is cultivated for its fleshy fruits and the area under its cultivation is increasing day by day due to its nutrient value, demand and high yield. The production and productivity of the crop is greatly hampered by the fruit borer, *Helicoverpa armigera* (Hubner) which causes damage to the developing fruits and results in yield loss ranging from 20 to 60 per cent (Tewari and Krishnamoorthy 1984 [27]; Lal and Lal, 1996 [12]. The pest management in tomato is widely practiced using pesticides Singh *et al.* 2001 [23]. However, indiscriminate use of chemical insecticides triggered the insect to develop resistance to insecticides, caused a resurgence of sucking pests, reduced the population of natural enemies and increased chemical residues in the environment. Biocontrol approaches help to develop ecofriendly sustainable management strategy for controlling the pest and pathogens. Development of biological control for insect pests is accepted as a durable and eco friendly alternative for agrochemicals. Entomopathogenic microorganisms provide an alternative method to reduce the use of synthetic pesticide in integrated pest management for sustainable agriculture Liu *et al.* 2002 [14]; Al-maza awi *et al.* 2006 [1]. Entomopathogenic fungi are key regulatory factors of insect populations in nature Charnley 2003 [6] and are attracting attention as biocontrol agents for insect pests Clarkson and Charnley 1996 [7]. There are 400 to 500 species of fungi known to have insect pathogenic properties Whitten and Oakeshott 1991 [30] Starnes *et al.* 1993 [25]. Among them, only 10 species were developed commercially as mycoinsecticides. Especially, the deuteromycetous fungi *Beauveria bassiana* (Balsamo) Vuillemin Sandhu *et al.* 2001 [15] has been used extensively for the control of wide range of insect pests. *Beauveria bassiana* is an imperfect entomopathogenic fungus that attacks a wide range of agricultural pests Feng *et al.* 1994 [10] and also grows on soil as saprophyte Bidochka *et al.* 1998 [4]. The mass propagation of fungi for use as biopesticide is a goal for investigators, because include advantages as mainly on the processes of scaling which are relatively easy, as well as the control of parameters such as temperature, aeration and pH. In addition, production costs can be much lower than other formulations.

The present research was carried out to highlight the implication of bio-pesticide in pest management of vegetable crops, optimization of media for growth of *Beauveria bassiana* and bioassay of different oil based formulations for their efficacy as sellable and easily applicable biopesticide. The investigation accomplished the purpose of *Beauveria bassiana* oil based bio-formulation as an potential biocontrol agent against the most destructive pest of tomato *Helicoverpa armigera* (Hubner).

Materials and Methods

Collection of Plant Oils and Chemicals

Plant oils were purchased from local market (Table 1)

Table 1: Influence of ingredient combination on the stability and viscosity of oil formulations

S. No.	Per cent ingredients of invert emulsion formulations (ml)					Time(min) and speed of mixing (rpm)	Stability(%) emulsion layer (V/V)[b]	Viscosity (cps)[c]
	Oil phase (50% W/W)		Aqueous phase(50% W/W)					
	Tween 20	Oil(s)[a]	Tween80	Sterile Dis. H_2O	Water Soluble wax			
1.	10	Corn (40.0)	5.0		1.0	10, 6000	94.0	23±0.44
2.	10	Soyabean (40.0)	5.0	44.0	1.0	10, 6000	93.0	18±0.48
3.	10	Castor (40.0)	5.0	44.0	1.0	10, 6000	93.0	14±0.51
4.	10	Rice bran 40.0)	5.0	44.0	1.0	10, 6000	93.0	18±0.42
5.	10	Mustard (40.0)	5.0	44.0	1.0	10, 6000	96.0	25±0.66
6.	10	Mahuva (40.0)	5.0	44.0	1.0	10, 6000	93.0	38±0.32
7.	10	Pinnai (40.0)	5.0	44.0	1.0	10, 6000	93.0	21±0.74
8.	10	Neem (40.0)	5.0	44.0	1.0	10, 6000	94.0	23±0.32
9.	10	Groundnut (40.0)	5.0	44.0	1.0	10, 6000	93.0	20±0.15
10.	10	Palm (40.0)	5.0	44.0	1.0	10, 6000	93.0	26.4±0.64
11.	10	Coconut (40.0)	5.0	44.0	1.0	10,6000	93.0	6.65±0.23
12.	10	Gingelly (40.0)	5.0	44.0	1.0	10,6000	93.0	23.0

[a] ***Source*:** Vegetable Oil Industries Co. Ltd., Coimbatore, Tamil Nadu, India.
[b] Stability was measured 0–10 days after preparation on volume basis; changes in stability after this period were very slight.
[c] Viscosity was measured 0–10 days after preparation using viscometer with ±3% accuracy; viscosity after this period remained constant

Insect culture

Establishment of stock culture of *H. armigera*

Nucleus culture

H. armigera larvae were collected from the tomato experimental farm, Department of vegetables, Tamil Nadu Agricultural University (TNAU), Coimbatore to establish the stock culture. Field collected insects were reared and closely watched for any disease symptoms. Inactive insects, sluggish caterpillars and larvae with disease symptoms, which exhibited slow growth as well as malformed pupae were discarded.

Formulations

Preparation of oil based formulation of *B. bassiana* (TBb8)

Preparation of oil bioformulation of TBb8

Oil based bioformulations were prepared according to modified method of Batta 2004 [3]. Thirteen types of oils with different combinations were used for preparation of oil formulation of *B.bassiana* isolate TBb8. The aqueous phases as well as oil phase of different oils were prepared as explained in below the Table. The ingredients of each phase of the oil being investigated were first mixed separately and then combined in a 50: 50 per cent ratio by adding the aqueous phase into the oil phase to obtain a water- in-oil formulation. The mixture was then homogenized mechanically using a homogenizer. The aqueous phase and oil phase were mixed in a round bottom flask of one lit and sterilized under autoclave at 120°C, 15 lb pressure for 20 min. The sterilized mixtures containing oil and aqueous phases were inoculated with a conidia suspension of 7 days old cultures of TBb8 in laminar air flow chamber and kept for incubation at 28 ± 2ºC in optical shaker with a speed of 120 rpm under storage conditions. Thirteen samples representing 3 replicates per formulation were set up and the experiment was repeated twice to confirm the reproducibility of the results. The stability of the different emulsions evaluated by holding samples in capped graduated test tubes, in which the volume of the emulsion layer was measured 0-10 days after preparation in relation to the sample volume (10 ml). The viscosity of the emulsions was measured at the same time using a viscometer. The most stable formulation with low viscosity was selected for subsequent stages of the glasshouse and field conditions.

Laboratory bioassay

In vitro* efficacy of *B. bassiana* (TBb8) isolate against *H. armigera

For testing the insect feeding preference *in vitro* conditions, detached leaves collected from the *Beauveria* oil formulation treated plants were used. The tomato leaves (2g) were cut and kept in Petri dishes (9 cm diameter) lined with damp filter papers to maintain the humidity. Young larvae were collected from the laboratory culture, starved for 5 h and then allowed for feeding the leaves. The plates were incubated at 25 ±2 ^{0}C in the dark in 70 per cent relative humidity. After 24 h, larval mortality, pupal and adult malformations were recorded.

Glasshouse studies

Efficacy of *P. fluorescencs* (TPf12) and *B. bassiana* (TBb8) bioformulations against *Fusarium* wilt and fruit borer of tomato under glasshouse conditions

Two sets of pot culture experiments were conducted to test the efficacy of *P. fluorescens* (TPf12) and *B. bassiana* (TBb8) separately as seed, soil, seedling dip and foliar applications in controlling *Fusarium* wilt disease and fruit borer incidence in tomato. The isolate of *F. oxysporum* f.sp. *lycopersici* was mass multiplied in the sand-maize medium and was mixed with the sterilized pot soil @ 15 g/kg of soil and filled in earthen pots. The seeds were treated with entomopathogenic fungus and PGPR bioformulations. Treated seeds were raised in pots. The treatments were compared with fungicide control (Carbendazim @ 2 g/kg of seed) and insecticide control (Quinalphos 25% EC @ 2 ml/lit). The seeds not treated with entomopathogenic fungus or PGPR strain served as control. Larvae of *H. armigera* collected from tomato field and cultured in lab were inoculated into plants at the time of flowering. The experiment was conducted in a completely randomized block design three plants/pot and replicated three times.

Field studies

Field experiments

Two field trials were conducted at Thazhiyur and Malumachanpatti, Coimbatore (District) with three replications during from December 2012 and March 2013 to test the efficacy of entomopathogenic fungus and PGPR bioformulations against *Fusarium* wilt and fruit borer under drip irrigation systems. The experiments were laid out in Randomized Block Design with nine treatments. Three replications for each treatment were maintained. Tomato PKM1 variety was used for field experiments.

Yield and yield attributes

The liquid and oil based formulations of *P. fluorescens* (TPf12) and *B. bassiana* (TBb8) were tested for their efficacy on growth promotion. The yield of ripened fruits per plant was also observed.

Statistical analysis

The data were statistically analyzed using the IRRISTAT version 92 developed by the International Rice Research Institute Biometrics Unit, the Philippines Gomez and Gomez,1984 [11].

Prior to statistical analysis of variance (ANOVA) the percentage values of the disease index were arcsine transformed. Data were subjected to analysis of variance (ANOVA) at two significant levels ($P< 0.05$ and $P< 0.01$) and means were compared by Duncan's Multiple Range Test (DMRT).

Results

Shelf life of *B. bassiana* (TBb8) in oil based formulation

Among thirteen different plant oils evaluated for sustaining *B. bassiana* populations, corn oil was found superior by maintaing a population of 7.0 x10^9 on 210th days after storage. Pinnai oil also recorded 3.67 x10^8 population after 210day storage, Neem oil was found to sustain the *B. bassiana* population up to 180 days only. All other oils were found not suitable for making bioformulation, since they could not maintain the population of *B. bassiana* for more than 60 days (Table 2)

Table 2: Population of *Beauveria bassiana* in different oil formulations

Days	Population (cfu/ml)								
	GN	Coconut	Neem	Musturd	Pinnaigai	Mahuva	Pungam	Gingelly	Corn
0th day	4.5x10^8	4.6x10^8	4.1x10^8	4.3x10^8	4.2x10^8	4.35x10^8	4.3x108	4.5x10^8	4.6x10^8
2nd day	7.2x10^8	7.8x10^8	6.9x10^8	7.4x10^8	7.1x10^8	7.5x10^8	7.0x108	7.2x10^8	6.5x10^8
5th day	2.2x10^9	4.5x10^8	4.5x10^{10}	5.2x10^8	1.1x10^{10}	4.5x10^7	3.8x107	5.6x10^7	3.0x10^{10}
15th day	5.0x10^7	6.2x10^7	3.5x10^{10}	5.8x10^5	6.5x10^9	2.5x10^5	3.4x105	6.1x10^4	2.6x10^{10}
30th day	4.5x10^6	5.5x10^6	2.5x10^{10}	-	5.1x10^9	-	-	-	1.2x10^{10}
45th day	1.5 x10^6	2.5X10^6	1.2x 10^{10}	-	1.5 x 10^{10}	-	-	-	2.2x10^{10}
60th day	7.5 x10^5	6.5X10^5	1.0×10^{10}	-	1.1x10^{10}	-	-	-	2.1x10^{10}
90thday	-	-	9.2×10^9	-	9.1x 10^9	-	-	-	2.2x10^{10}
120th day	-	-	9.2×10^9	-	8.9x 10^8	-	-	-	2.0x10^{10}
150th day	-	-	8.8×10^9	-	8.5 x10^8	-	-	-	1.5x10^{10}
180th day	-	-	8.0-×10^9	-	8.0 x 10^8	-	-	-	1.0x10^{10}
210th day	-	-	-	-	3.67x10^8	-	-	-	7.0x10^9

*Means of values three replications

Efficacy of *B. bassiana* (TBb8) oil formulation against *H. armigera in vitro*

Oil formulation of TBb8 isolate (1x10^8 spore/ml) treated *H. armigera* larval mortality of 70.0%, pupation period of 13.7 days, pupal malformation of 17.9, and adult malformation was recorded as 16.2%. Whereas, quinolphos (0.15%) recorded larval mortality (%), pupation period (days), pupal malformation (%) and adult malformation as 96.7,0.0 and 0.0 respectively (Table 3).

Table 3: *In vitro* efficacy of *B. bassiana* (TBb8*)* Oil formulation against fruit borer under condition

Treatments	Larval mortality (%)	Pupation period (days)	Pupa malformed (%)	Adult malformed (%)
Oil formulation (1×10^8)Spores/ml	70.0 (58.01)^b	13.7^b	17.9 (28.02)^b	16.2 (25.14)^b
Quinalphos (0.15%)	96.7 (78.86)^a	0.0^a	0.0^a	0.0^a
Control	0.0^c	0.0^c	0.0^c	0.0^c

* Mean of seven replications.

Figures in parentheses represent arcsine transformation.

Means in a column followed by same superscript letters are not significantly different according to DMRT at $P\leq 0.05$.

Efficacy of *B. bassiana* (TBb8) and *P. fluorescens* (TPf12) bioformulations against *H.armigera* under glasshouse conditions

Oil / liquid bioformulations of *B. bassiana* and *P. fluorescens* containing TBb8 and TPf12 strains individually and in combination were developed based on the results obtained from the mortality percentage (*B. bassiana*). Application of oil based formulation of *B. bassiana* (TBb8) alone and in combination with TPf12 were evaluated for their efficacy against *F. oxysporum* f.sp. *lycopersici* in tomato cv. PKM 1 plants under glasshouse conditions.

Wilt incidence

Among the various treatments, seed treatment+ seedling dip+soil application of TBb 8 + TPf 12 has significantly reduced the incidence of wilt under glasshouse conditions.

The observations indicated that the wilt incidence was less in plants treated with ST+SA+SD TBb8 + TPf12 (14.0%) followed by TPf12 alone (36.0%). In chemical treatment (Carbendazim), 58.8 per cent wilt incidence was recorded and maximum incidence of 90 per cent was recorded in control (Table 4).

Table 4: Effect of *P. fluorescens* (TPf 12) and *B. bassiana* (TBb 8) bioformulation on the incidence of *Fusarium* wilt in tomato under glass house conditions

Treatments		Plant height (cm)	Per cent disease incidence (%)	Per cent reduction over control (%)	Fruit yield g/plant
T_1 -	ST with TPf 12 liquid formulation	78.2^{bc}	36.0^{c}	60.0^{c}	420.05^{e}
T_2 -	ST with TBb 8 oil formulation	77.1^{bc}	52.0^{de}	42.2^{e}	400.21^{ef}
T_3 -	ST+SD with TPf 12 liquid formulation	85.5^{a}	22.6^{b}	74.8^{b}	485.75^{cd}
T_4 -	ST+SD with TBb 8 oil formulation	84.3^{ab}	43.0^{d}	52.2^{d}	455.00^{d}
T_5 -	ST+SD+SA with TPf 12 liquid formulation	90.0^{a}	14.8^{ab}	83.5^{ab}	595.50^{b}
T_6 -	ST+SD+SA with TBb 8 oil formulation	88.5^{a}	26.0^{bc}	71.1^{bc}	499.50^{bc}
T_7 -	ST+SD+SA with TPf 12 liquid formulation +FS with TBb8 oil formulation	91.5^{a}	14.0^{a}	84.4^{a}	605.10^{a}
T_8 -	ST+SD+SA+FS with TBb 8 oil formulation	84.8^{ab}	37.3^{cd}	58.5^{cd}	495.68c
T_9 -	Seed treatment with Carbendazim @2g/kg of seeds	77.5^{bc}	58.8^{e}	34.6^{f}	225.55^{h}
T_{10}-	Foliar spray with Quinolphos @ 25%EC	24.5^{e}	85.0^{ef}	5.5^{g}	210.00^{hi}
T_{11}-	Inoculated control	62.7^{d}	90.00^{j}	-	175.67^{i}
T_{12}-	Healthy control	67.8^{d}	-	-	340.50^{g}

ST- Seed treatment (10 ml/kg)
SD- Seedling dip (1000 ml/ha of seedlings)
SA- Soil application (1000 ml/ha)
FS – Foliar spray (1000 ml/ha)
Values are mean of three replications
Means followed by a common letter are not significantly different at 5% level by DMRT

Fruit borer damage

Among the various treatments, Seed treatment+seedling dip + soil application with TPf12 + foliar spray with TBb8 oil formulation significantly reduced damage of *H. armigera* under glasshouse conditions. The observations indicated the lowest fruit borer damage in tomato plants treated with ST+SD+SA with TPf12 bioformulation+FS with TBb8 oil formulation (13%) followed by TBb8 alone (45.2%). In chemical treatment (Quinalphos), 15.45 per cent damage was noticed. In control, 60.55 per cent damage was recorded (Table 5).

Table 5: Effect of *P. fluorescens* (TPf12) and *B. bassiana* (TBb8) bioformulation on the incidence of fruit borer in tomato under glass house condition

Treatments		Plant height (cm)	Fruit Infestation (%)	% reduction over control	Larval mortality (%)	Fruit yield g/plant
T_1 -	ST with TPf 12 liquid formulation	76.5^{e}	50.12^{f}	17.2^{f}	20.10^{f}	220.05^{gh}
T_2 -	ST with TBb 8 oil formulation	74.2^{g}	45.21^{e}	17.0^{f}	22.50^{de}	200.21^{h}
T_3 -	ST+SD with TPf 12 liquid formulation	83.2^{bc}	38.21^{de}	36.8^{de}	24.54^{d}	464.75^{d}
T_4 -	ST+SD with TBb 8 oil formulation	80.3^{cd}	35.45^{d}	41.4^{d}	28.72^{b}	445.00^{e}
T_5 -	ST+SD+SA with TPf 12 liquid formulation	88.2^{ab}	28.22^{bc}	53.3^{bc}	29.41^{bc}	495.50^{c}
T_6 -	ST+SD+SA with TBb 8 oil formulation	84.3^{bc}	20.22^{b}	66.5^{b}	31.45^{b}	529.50^{b}
T_7 -	ST+SD+SA with TPf 12 liquid formulation +FS with TBb8 oil formulation	90.4^{a}	13.00^{a}	78.5^{a}	90.21^{a}	625.10^{a}
T_8 -	ST+SD+SA+FS with TBb 8 oil formulation	83.8^{b}	14.95^{a}	75.2^{a}	88.12^{a}	595.68^{b}
T_9 -	Seed treatment with Carbendazim @2g/kg of seeds	74.5^{fg}	55.21^{fg}	8.7^{fg}	12.00^{h}	225.55^{g}
T_{10}-	Foliar spray with Quinolphos @ 25%EC	75.5^{f}	15.45^{a}	74.4^{a}	78.45^{a}	380.00^{f}
T_{11}-	Inoculated control	72.7^{fgh}	60.55^{h}	-	-	155.67^{i}
T_{12}-	Healthy control	77.8^{de}	-	-	-	340.50^{fg}

Effect of bioformulations on growth and yield attributes of tomato cv. PKM 1 cultivar under glasshouse conditions

Application of *B. bassiana* and *P. fluorescens* bioformulations significantly enhanced the yield of tomato under glass house conditions compared to untreated control and chemical treatment. The growth and yield attributing characters *viz.*, plant height (91.5 cm), fruit yield (605.10 g/plant), were significantly higher in the ST+SD+SA with TPf12 bioformulation+FS with TBb8 oil formulation treated plants compared to control plants (62.7cm in plant height, yield 175.67g/plant). In case of treatment with+SD+SA with TPf12 bioformulation+FS with TBb8 oil formulation recorded the plant height of 90.4cm, fruit yield of 625.10g/plant. Comparatively in control 72.7 cm of plant height, 155.67 g/plant of fruit yield/plant was recorded. Whereas in TPf 12 alone and TBb8 alone treatments showed moderate growth and yield attributing characters.

Field experiments

Two field trials were conducted, one at Thazhiyur - Trial I and another at Malumichampatti - Trial II to evaluvate the bioefficacy of *B.bassiana* and *P. fluorescens* bioformulation individually and in combination against *Fusarium* wilt and fruit borer of tomato.

Wilt incidence

In trail I, among the all treatments, combinations of *P. fluroscens* and *B. bassiana* (ST+SD+SA TPf12 +FS TBb8) treatment 9.54 per cent which was significantly low from untreated control 48.41%. Further the incidences in carbendazim, TPf12 and TBb8 and 19.42%., 24.30% and 29.5% respectively (Table 6).

Table 6: Effect of *P. fluorescens* (TPf 12) and *B. bassiana* (TBb 8) bioformulation on the incidence of *Fusarium* wilt and fruit borer intomato under field conditions (Trial I)

Treatments		Plant height (cm)	Percent disease incidence (%)	Percent reduction over control (%)	Fruit borer Incidence (%)	Percent reduction over control (%)	Fruit yield t/ha
T_1 -	ST with TPf 12 liquid formulation	81.4^{c}	24.30 (29.53)^{de}	49.80^{ef}	34.12 (31.66)^{de}	17.48	37.40b^{cd}
T_2 -	ST with TBb 8 oil formulation	79.5^{c}	29.5 (32.89)^{d}	39.06^{g}	26.85 (28.49)^{d}	35.06	36.20^{cd}
T_3 -	ST+SD with TPf 12 liquid formulation	85.4^{b}	14.52 (22.39)^{a}	70.00^{c}	20.12 (26.74)^{b}	51.34	40.15^{bc}
T_4 -	ST+SD with TBb 8 oil formulation	80.4^{c}	26.44 (30.93)^{de}	45.38^{f}	22.15 (28.26)^{e}	46.43	39.25^{bc}
T_5 -	ST+SD+SA with TPf 12 liquid formulation	89.5^{a}	10.42 (18.82)^{a}	78.47^{ab}	24.85 (32.87)^{cd}	39.90	46.54^{a}
T_6 -	ST+SD+SA with TBb 8 oil formulation	84.4^{bc}	24.53 (29.67)^{de}	49.32^{e}	18.41 (25.03)^{b}	55.47	38.71^{c}
T_7 -	ST+SD+SA with TPf 12 liquid formulation +FS with TBb8 oil formulation	92.5^{a}	9.54 (17.99)^{a}	80.29^{a}	10.85 (18.57)^{a}	73.76	48.55^{a}
T_8 -	ST+SD+SA+FS with TBb 8 oil formulation	85.5^{b}	22.55 (28.35)^{d}	53.41^{e}	9.42 (17.76)^{cd}	77.21	40.55^{b}
T_9 -	Seed treatment with Carbendazim @2g/kg of seeds)	74.8^{d}	19.42 (26.14)^{bc}	59.88^{d}	37.41 (37.11)^{c}	95.28	40.10^{b}
T_{10}-	Foliar spray with Quinolphos @25%EC	73.5^{e}	34.50 (35.97)^{e}	28.73^{f}	20.32 (25.34)^{c}	50.85	34.55^{d}
T_{11}-	Control	75.4^{d}	48.41 (44.09)^{f}	-	41.35 (40.73)^{f}	-	33.40^{d}

In field trail II also similar type of results were obtained. Treatment ST+SD+SA TPf12 + FS TBb8 bioformulation significantly reduced the disease incidence to 11.21 per cent followed by TPf12 alone (26.45%) and TBb8 alone (31.45%) compared to untreated control (51.21%) and carbendazim 24.21% (Table 4). The application of mixture of *B.bassiana* and *P.fluorescens* bioformulation drastically reduced the disease incidence than individual application of *B. bassiana* and *P. fluorescens* bioformulations in both trails.

Fruit borer incidence

In field trial I, generally more incidence of fruit borer damage was noticed. Results of field experiments from trial I revealed that fruit borer damage was significantly low 10.85% combined application ST+SA+SD TPf12 +FS TBb8 as was compared to chemical insecticide treatment (20.32%) 41.35% (Table 5). Similarly in trail II the bioformulation mixture (ST+SA+SD + TPf12 FS TBb8) had significantly less fruit borer incidence (9.54%) which was comparable with the chemical treatment 19.42% and control 37.20% (Table 5). In both the trails combined application of bioformulation recorded minimum incidence of fruit borer in tomato.

Effect of *B. bassiana* (TBb 8) and *P. fluorescens* (TPf 12) bioformulations on tomato yield under field conditions

Growth and yield attributing parameters *viz*., plant height, number of fruits/plant, weight of fruit (g/plant) were recorded significantly in both the field trials (Table 6). In the field trial I, ST+SA+SD TPf 12 +FS TBb8 treatment recorded the highest plant height (92.50cm)and fruit yield (48.55t/ha) Control where as was having plant height only 75.40 cm and fruit yield of 33.40 t/ha. Similar trend was observed in the second trial also. In second trial, showed 90.2cm plant height and 45.55t/ha tomato yield as compared to 74.2cm plant height and 30.21 t/ha fruit yield in control(Table 7).

Table 7: Effect of *P. fluorescens* (TPf 12) and *B. bassiana* (TBb 8) bioformulation on the incidence of *Fusarium* wilt and fruit borer in tomato under field conditions (Trial II)

Treatments		Plant height (cm)	Percent disease incidence (%)	Percent reduction over control (%)	Fruit borer Incidence (%)	Percent reduction Over control (%)	Fruit yield t/ha
T_1 -	ST with TPf 12 liquid formulation	79.5	26.45(18.34)^{cd}	48.34(44.05)^{bc}	30.15(25.80)^{cd}	18.95(33.30)^{g}	35.21^{e}
T_2 -	ST with TBb 8 oil formulation	78.5	31.45(36.40)^{e}	38.58(38.39)^{d}	28.23(29.40)^{bc}	24.11(32.09)^{fg}	34.12^{e}
T_3 -	ST+SD with TPf 12 liquid formulation	86.4	13.25(22.35)^{b}	74.12(59.55)^{a}	25.40(34.27)^{c}	31.72(30.25)^{d}	39.85^{c}
T_4 -	ST+SD with TBb 8 oil formulation	85.2	28.41(32.40)^{de}	44.52(41.85)^{cd}	24.00(36.55)^{cd}	35.48(29.32)^{bc}	37.20^{d}
T_5 -	ST+SD+SA with TPf 12 liquid formulation	89.4	12.15(21.56)^{ab}	76.27(61.01)^{a}	20.14(26.03)^{b}	45.86(26.65)^{b}	43.25^{b}
T_6 -	ST+SD+SA with TBb 8 oil formulation	86.2	27.41(19.49)^{d}	46.47(42.97)^{bc}	18.15(24.46)^{b}	51.20(27.20)^{b}	39.21^{c}
T_7 -	ST+SD+SA with TPf 12 liquid formulation +FS with TBb8 oil formulation	90.2	11.21(20.60)^{a}	78.10(62.20)^{a}	9.54(17.86)^{a}	74.35(60.36)^{a}	45.55^{a}
T_8 -	ST+SD+SA+FS with TBb 8 oil formulation	85.4	23.58(28.66)^{bc}	53.95(47.27)^{b}	9.00(17.06)^{a}	75.80(61.25)^{a}	40.20^{c}
T_9 -	Seed treatment with Carbendizim @2g/kg of seeds	74.1	24.21(29.61)^{c}	52.72(46.56)^{b}	27.51(36.13)^{d}	26.04(35.22)^{f}	39.11^{c}
T_{10}-	Foliar spray with Quinolphos @25%EC	73.5	37.85(41.96)^{ef}	26.08(30.71)^{e}	19.42(30.68)^{b}	47.79(37.58)^{cd}	31.25^{f}
T_{11}-	Control	74.2	51.21(49.76)^{f}	-	37.20(39.58)^{f}	-	30.21^{f}

ST- Seed treatment (10 ml/kg) SD- Seedling dip (1000 ml/ha of seedlings) SA- Soil application (1000 ml/ha) FS – Foliar spray (1000 ml/ha) Values are mean of three replications Values in parentheses are arscine transformed values Means followed by a common letter are not significantly different at 5% level by DMRT

Discussion

Efficacy of *B. bassiana* (TBb8) against *H. armigera* under *in vitro* and glasshouse conditions

B. bassiana is the entomopathogenic fungus most widely distributed in the world, and it infects insects in tropical, temperate, humid and desert areas Zimmermann 2007 [32]. It was found that *B. bassiana* (TBb8) was highly effective on third instar larvae of *H. armigera* with a larval mortality of 88.21 per cent under *in vitro* and glasshouse conditions, respectively. This finding is in accordance with Sivasundaram 2006 [24], where *B. bassiana* (B2) was tested against rice leaffolder and also found to be effective against BPH. Similarly in pigeonpea against *H. armigera* Saravanan, 2007 [19]; Senthilraja, 2012 [20], in groundnut against leafminor (Senthilraja *et al.* 2010 [22] also found that Bb10 isolate effective for control the pest insect under *in vitro* and glasshouse conditions. Several indigenous isolates of *B. bassiana* have been reported to be pathogenic to *H. armigera* Sandhu *et al.* 2001 [15].

Efficacy of *B. bassiana* and *P. fluorescens* bioformulations against wilt and *H.armigera* under field codition.

B. bassiana and *P. fluorescens* strains that showed higher activity of cuticle degrading enzymes, highest per cent mortality and enhanced plant growth were selected and used against *H.armigera* in tomato under glass house and field conditions with two different combinations (oil and liquid). Bioformulation containing TBb8 and TPf12 besides increasing the yield effectively reduced the incidence of *H. armigera* untreated control in glass house and field conditions. Management of insect pests by different *Beauveria* and *Pseudomonas* strains either as suspension or through different formulations have been reported by many workers Saravanakumar *et al.* 2007 [19]; Sivasundaram *et al.* 2008 [25]; Lednev *et al.* 2008 [13]; Senthilraja *et al.* 2010b [22]. In addition to pest management, strain mixtures enhanced the plant growth in terms of increased seedling emergence and grain yield Dunne *et al.* 1998 [8]; Saravanakumar *et al.* 2007 [18]; Thilagavathi *et al.* 2007 [29]; and Senthilraja *et al.* 2010a [23]. Application of TBb8 + TPf12 bioformulations (oil and liquid) recorded less incidence of *H. armigera* both under glass house and field conditions. The data of the present study indicate that the mixture of *Beauveria* and *Pseudomonas* bacterial strains influence the growth and development of insects at all stages including larval, pupal and adult. *B. bassiana* and *P. fluorescens* greatly affected the feeding behavior of the larvae in the bioformulation (oil and liquid) treated plants resulting in increased the larval mortality. *H. zea*, the corn earworm larvae reared on the diet contaminated by the bacteria *P. maltophila* adversely affected the larval growth resulting in more than 60 per cent reduction in adult emergence Bong and Sikorowski, 1991[5]. Yaman *et al.* 1999 [32] reported that

P. fluorescens suspension spray resulted in 20 per cent weevil mortality in chestnut.

B. mojavensis is an excellent endophytic bacterium which controls diseases and insect pests Sturz *et al.* 2000 [27]. Bioengineered endophyte *Clavibacter xyli* delivers delta endotoxin against European corn borer in corn Fahey *et al.* 1991 [9]. Aksoy *et al.* 2008 [1] reported that *P. putida* biotype B2 as a potential biological control agent of *Tetranychus urticae.* Similarly, *Pseudomonas* treated rice leaves altered the feeding behavior of leaf folder and reduced larval and pupal weight, increased the larval mortality and incidence of malformed adults under *in vitro* condition Saravanakumar *et al.* 2007 [17]. In general, application of mixture of *B. bassiana* and *P. fluorescens* bioformulation oil and liquid) reduced the fruit borer (*H. armigera*) incidence in tomato both under glasshouse and field conditions (in both locations) which in turn resulted in enhanced number of fruit and yield. The mechanism may be direct action (enzymes, toxins, etc.) and indirect actions (ISR, and growth hormones, etc.) of the bioagents.

Conclusion

In conclusion, Biocontrol agents may be used for the management of different pests as an alternative solution to chemical pesticides. For the control of tomato fruit borers, Beauveria bassiana is recommended. However, to determine the formulation that can produce the best results, further investigations are required.

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