



Biorational Effects of Organic Manures, Botanicals and Biopesticides against Tomato Fruit Borer, *Helicoverpa armigera* and Its Egg Parasitoid, *Trichogramma chilonis*

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Studies were carried out to evaluate biological activity of organic manures against tomato fruit borer, *Helicoverpa armigera* (Hub.) and safety of botanicals and biopesticides against egg parasitoid, *Trichogramma chilonis* Ishii and biochemical effects of *Pseudomonas fluorescens* on tomato under pot culture conditions. The feeding and infestation of the larvae of *H. armigera* were significantly low in farm yard manure (FYM) + *Azospirillum* + silicate solubilising bacteria (SSB) + *Phosphobacteria* + neem cake applied plants followed by FYM + *Azospirillum* + SSB + *Phosphobacteria* + mahua cake applied plants. *Trichogramma* parasitization on *H. armigera* eggs was adversely affected by neem oil 3% on treated plants followed by neem seed kernel extract (NSKE 5%) + spinosad 75 g a.i./ha. Under laboratory condition among the microbial pesticide tested Spinosad (75 g a.i./ha), *HaNPV* + Spinosad + *Bt* (1.5×10^{12} POBs/ha + 75 g a.i./ha + 15000 IU/mg (2 lit/ha), Spinosad + *Bt* (75 g a.i./ha + 15000 IU/mg - 2 lit/ha) showed higher insecticidal toxicity (100 per cent mortality on 72 h) to all instars of *H. armigera* larvae. Biochemical parameters like phenol content, peroxidase and phenyl alanine ammonialyase (PAL) activity recorded higher levels in *Pseudomonas fluorescens* seed treatment @ 30 g/kg of seed and its foliar spray @ 1 g/litre in treated tomato plants.

Key words: Organic manures, botanicals, biopesticides, *H. armigera*, *T. chilonis*

Tomato, *Lycopersicon esculentum* Mill (Family: Solanaceae) is one of the most important “protective foods” because of its superior nutritional values. Tomato is the world’s largely consumed vegetable crop after potato and sweet potato and it tops the list of canned vegetables too. Of the several biotic limiting factors of tomato production, tomato fruit borer, *Helicoverpa armigera* (Hub.) is a serious pest in the flowering and fruiting stages causing severe damage up to 50 per cent in tomato. Control strategies applied by using synthetic insecticides led to the development of cross and multiple resistances in *H. armigera*. Of several options, organic manures, botanical pesticides and biopesticides are the best alternatives to manage pests below economic threshold level (ETL) and provide security to mankind from the residues of pesticides. In the use of botanical pesticides,

the major limiting factor is their faster photo-degradability of biologically active compounds under field conditions. Hence, studies were undertaken to stabilize the neem compounds with other botanicals namely pungam and sweet-flag biopesticides namely *HaNPV*, *Bacillus thuringiensis* (*Bt*), spinosad and *Pseudomonas fluorescens* application in pot culture experiment to ascertain their use in eco-friendly pest management and their safety to egg parasitoid, *Trichogramma chilonis* Ishii.

Materials and Methods

Organic Manures and Biofertilizers

Compost, farm yard manure (FYM) and cakes of neem, castor, mahua and pungam were obtained from the central farm unit of Agricultural College and Research Institute, Madurai, Tamil Nadu. The biofertilizers viz., Silicate solubilizing bacteria (SSB), *Azospirillum*, and *Phospho*

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Table 1. Effect of organic manures on *H. armigera* infestation in pot cultured tomato

Treatment	Dose	Fruit damage (%) [*]	
		30 DAT ^{**}	45 DAT ^{**}
Compost + SSB + <i>Azospirillum</i> + Phosphobacteria + Neem cake	12.5 t ha ⁻¹ + 2 kg ha ⁻¹ + 2 kg ha ⁻¹ + 300 kg ha ⁻¹	3.51 (10.79) ^f	3.00 (9.97) ^f
Compost + SSB + <i>Azospirillum</i> + Phosphobacteria + Mahua cake	12.5 t ha ⁻¹ + 2 kg ha ⁻¹ + 2 kg ha ⁻¹ + 2 kg ha ⁻¹ + 400 kg ha ⁻¹	2.04 (8.21) ^d	2.46 (9.02) ^e
FYM + SSB + <i>Azospirillum</i> + Phosphobacteria + Mahua cake	12.5 t ha ⁻¹ + 2 kg ha ⁻¹ + 2 kg ha ⁻¹ + 2 kg ha ⁻¹ + 400 kg ha ⁻¹	1.80 (7.71) ^c	1.26 (6.44) ^b
FYM + SSB + <i>Azospirillum</i> + Phosphobacteria + Castor cake	12.5 t ha ⁻¹ + 2 kg ha ⁻¹ + 2 kg ha ⁻¹ + 2 kg ha ⁻¹ + 400 kg ha ⁻¹	2.55 (9.18) ^e	2.07 (8.27) ^d
FYM + SSB + <i>Azospirillum</i> + Phosphobacteria + Neem cake	12.5 t ha ⁻¹ + 2 kg ha ⁻¹ + 2 kg ha ⁻¹ + 2 kg ha ⁻¹ + 300 kg ha ⁻¹	1.08 (5.96) ^a	1.05 (5.88) ^a
FYM + SSB + <i>Azospirillum</i> + Phosphobacteria + Pungam cake	12.5 t ha ⁻¹ + 2 kg ha ⁻¹ + 2 kg ha ⁻¹ + 2 kg ha ⁻¹ + 400 kg ha ⁻¹	1.35 (6.67) ^b	1.77 (7.64) ^c
Compost + NPK	12.5 t ha ⁻¹ + 150:100:50 kg ha ⁻¹	5.80 (13.93) ⁱ	5.26 (13.25) ^j
FYM + NPK	12.5 t ha ⁻¹ + 150:100:50 kg ha ⁻¹	4.86 (12.73) ^h	4.20 (11.82) ^h
Compost	12.5 t ha ⁻¹	5.10 (13.05) ⁱ	4.66 (12.46) ⁱ
FYM	12.5 t ha ⁻¹	4.26 (11.91) ^g	3.54 (10.84) ^g
NPK	150:100:50 kg ha ⁻¹	7.53 (15.92) ^k	8.43 (16.87) ^l
Untreated check	-	7.62 (16.02) ^k	8.16 (16.59) ^k

^{*}Mean of three replications.

^{**}DAT – Days after transplanting.

Values in parentheses are *arc sine* transformed.

Means followed by same letter(s) are not significantly different (p= 0.05) by DMRT.

bacteria were obtained from the Department of Agricultural Microbiology, Agricultural College and Research Institute, Madurai.

Plant Materials

Neem (*Azadirachta indica* A.Juss), pungam (*Pongamia glabra* Vent.) and sweet-flag (*Acorus calamus* Linn.) extracts were prepared, formulated and used for evaluation. Seeds of neem and pungam were collected from farm premises of Agricultural College and Research Institute, Madurai. Rhizomes of sweet-flag were obtained from local market. Extracts of seed kernels and rhizomes were prepared using ethanol as solvent and formulations were made

in mixtures by the following procedure. Seed kernels/rhizomes were ground to fine powder in an electric grinder. One hundred gram of seed kernel/rhizome powder was stirred with 500 ml of ethanol for 3 hours using a magnetic stirrer and filtered through Whatman No.1 filter paper. The marc was restirred with 500 ml of ethanol in a distillation unit at 50° C under reduced pressure. The extract was formulated to 60 EC using a suitable organic solvent and an emulsifier at 30 % and 10 %, respectively (patent applied). The mixtures were prepared @ Neem + Sweet-flag + Pungam (NSP) 60 EC in 1:1:1 (v/v) and Neem + Sweet-flag (NS) 60 EC in 2:1 (v/v) from the extracts and formulated.

Table 2. Influence of botanicals and biopesticides on parasitization by *Trichogramma* on *H. armigera* eggs

Treatment	Concentration	Average No. of eggs laid in 24h/10 females [#]	No. of parasitized eggs [#]	Per cent parasitism
NSP	0.12%	21.6 (4.64) ^{cd}	19.0 (4.41) ^e	90.2 (71.75) ^a
NSP	0.18%	20.0 (4.51) ^{cd}	16.3 (4.09) ^f	82.35 (65.15) ^d
NS	0.12%	26.0 (5.10) ^e	24.0 (4.89) ^d	90.0 (71.43) ^a
Neem oil	3%	15.0 (3.87) ^a	11.0 (3.27) ^h	77.32 (58.56) ^e
NSKE + <i>Bt</i>	5% +15000 IU / mg (2 lit/ha)	40.0 (6.36) ^g	35.0 (5.94) ^b	87.4 (69.21) ^b
NSKE + <i>HaNPV</i>	5% +1.5 x 10 ¹² POBs/ha	32.0 (5.66) ^f	29.0 (5.39) ^c	90.7 (72.24) ^a
NSKE + Spinosad	5% + 75 g a.i. / ha	17.0 (4.17) ^b	15.0 (3.87) ^g	86.2 (68.19) ^{bc}
Endosulfan	0.07%	32.0 (5.69) ^f	28.0 (5.36) ^c	88.9 (70.54) ^b
Untreated check	-	44.0 (7.03) ^h	44.0 (6.64) ^a	90.1 (71.62) ^a

*Values in parentheses are *arc sine* transformed.

Values in parentheses are square root transformed.

Mean of three replications.

Means followed by the same letter(s) are not significantly different ($p=0.05$) by DMRT.

Biopesticides

H. armigera Nucleo Polyhedro Virus (*HaNPV*) was obtained from the Biocontrol Unit of the Department of Agricultural Entomology, AC & RI, Madurai. It was used at 1.5 x 10¹² POBs/ha. Commercial formulation of *Bacillus thuringiensis* var *galleriae* (Spicturin®) was used @ 15000 IU/mg (2 lit/ha). Commercial formulation of spinosad (Success®) supplied by M/S E. I. D Parry Agro Chemicals Ltd, Chennai was used @ 75 g a.i./ha. A talc based *Pseudomonas fluorescens* (Pf 1) was obtained from the Department of Plant Pathology, Tamil Nadu Agricultural University (TNAU), Coimbatore. It was used at different concentrations for seed treatment and foliar spray to evaluate its efficacy against *H. armigera* in both laboratory, pot culture and field experiments.

Mass Culturing of *H. armigera*

Nucleus culture of *H. armigera* was obtained from the Biocontrol Laboratory, TNAU, Coimbatore for breeding and egg laying. Larvae were reared individually in multicavity trays (25 x 10 x 3 cm) using modified semi-synthetic diet developed by Shorey and Hale (1965) under 25 ±1° C and 75-80 per cent relative humidity (Sathiah *et al.*, 1998). Pupae from parental colony were kept in a 30 x 30 cm adult emergence cage. Adults are stout bodied moth typical noctuid appearance, 14-18 mm long and males are usually greenish-grey and females orange brown. Ten pairs of healthy adults were transferred to oviposition cage. A solution containing 10 per cent sucrose fortified with vitamins was provided in the cage as food for adults. Oviposition cage consisted of a mudpot kept in a round plastic tray containing wet sand.

Seed Treatment of *Pseudomonas fluorescens* (Pf 1)

Tomato seeds (variety PKM 1) were treated with *Pf 1* @ 10, 15, 20, 25, and 30 g / kg of seeds. Each treatment was replicated three times. Treated seeds were sown in pots. Seedlings were transplanted into individual pots 30 days after sowing. Leaf samples were collected 5, 10, 20, and 30 days after transplanting for biochemical analyses. Phenol, peroxidase, and phenylalanine ammonia lyase (PAL) contents were estimated as suggested by Malick and Singh (1980) and Sadasivam and Manickam (1996).

Foliar Spray of *Pf 1* against *H. armigera*

Healthy potted 45-days-old tomato plants were sprayed with *Pf 1* using a hand atomizer @ 1.0, 2.5, 5.0, 7.5 and 10 g/litre. Each treatment was replicated thrice. Leaf samples were collected individually from pre-spraying and 5th, 10th, 20th, and 30th days after spraying (DAS). Leaf samples were subjected to biochemical analyses. Phenol, peroxidase, and phenylalanine ammonia lyase (PAL) contents were estimated as suggested by Malick and Singh (1980) and Sadasivam and Manickam (1996).

Results

Effect of Organic Manures on *H. armigera*

On 30DAT, per cent damage by *H. armigera* on pot cultured tomato plants was low in FYM +SSB + *Azospirillum* + *Phosphobacteria* + neem cake applied plants (1.08%), and followed by FYM +SSB + *Azospirillum* + *phosphobacteria* + pungam cake applied plants (1.35%) compared to untreated check (7.62%). At 45DAT, application of FYM +SSB + *Azospirillum* + *phosphobacteria* + neem cake recorded the lowest level of 1.05 per cent followed by FYM +SSB + *Azospirillum* + *Phosphobacteria* + mahua cake (1.26%), which was significantly on par with FYM +SSB + *Azospirillum* + *Phosphobacteria* + pungam cake applied plants (Table 1).

Trichogramma* Parasitization on Eggs of *H. armigera

The lowest number of eggs was laid in Neem oil 3% (15.00) followed by NSKE 5% + Spinosad (17.00) (Table 2). Lowest percentage of parasitism by *Trichogramma* on eggs of *Helicoverpa* was recorded in Neem oil 3% (77.32%) followed by NSKE + Spinosad (86.20%) compared to untreated check (90 %).

Biochemical Changes in Tomato Plants due to Seed Treatment of *Pf 1*

On 5 days after seed treatment (DAST), phenol content (744.0 µg/g), peroxidase (0.045 n.mol/min/g) and PAL (748.5 n.mol/min/g) in *Pf 1* (30g/kg of seed) compared to untreated check 216.0 µg/g, 0.012 n.mol/min/g and 388.2 n.mol/min/g of phenol, peroxidase and PAL, respectively (Table 3). On 10 DAST phenol, peroxidase and PAL activities considerably increased in all treatments. Phenol content ranged from 225 to 753 µg/g, peroxidase 0.0135 to 0.0441 n.mol/min/g and PAL 402.0 to 756.6 n.mol/min/g. On 20 DAST, *Pf 1* (30g/kg of seed) showed the highest amount of phenol, peroxidase and PAL contents of 756.0 µg/g, 0.048 n.mol/min/g, 786.3 n.mol/min/g respectively compared to untreated check with phenol (228.0 µg/g), peroxidase (0.014 n.mol/min/g) and PAL (414.0 n.mol/min/g). On 30 DAST phenol, peroxidase and PAL activity were significantly reduced in all treatments. Highest amount of phenol (752.1 µg/g), peroxidase (0.044 n.mol/min/g) and PAL (780.6 n.mol/min/g) compared to untreated check.

Biochemical Changes in Tomato Plants due to Foliar Spray of *Pf 1*

Table 4 shows that the phenol content was in the range of 250.2 µg/g to 258.6/g, peroxidase content ranged from 0.015 to 0.018 n.mol/min/g and PAL ranged from 437.1 to 445.5 n.mol/min/g before spraying. On 5 DAS, sudden increase of phenol, peroxidase and PAL was recorded with corresponding values of 636 µg/g, 0.048 n.mol/min/g and 1516 n.mol/min/g respectively in *Pf1* (10 g/lit of water) followed by 7.5 g/lit of water 518.1 µg/g, 0.045 n.mol/min/g, and 1016.2

n.mol/min/g of phenol, peroxidase and PAL respectively. On 10DAS, phenol content ranged from 253.5 to 648.03 µg/g, peroxidase (0.0183 to 0.052 n.mol/min/g) and PAL (436.2 to 1538.4 n.mol/min/g). On 20DAS, phenol content was 702.6 µg/g, peroxidase (0.0582 n.mol/min/g) and PAL (1596.3 n.mol/min/g) in *Pf*1 (10 g/lit of water) compared to untreated check where phenol content peroxidase and PAL values were 255.0 µg/g, 0.024 n.mol/min/g and 438.0 n.mol/min/g respectively. On 30-DAS, phenol, peroxidase and PAL content were significantly reduced in all treatments compared to 5, 10, 20 DAS. Higher range of phenol (696 µg/g), peroxidase (0.0552 n.mol/min/g) and PAL (1590.3 n.mol/min/g) were estimated compared to untreated check 255.0 µg/g, 0.024 n.mol/min/g, and 432.3 n.mol/min/g of phenol, peroxidase and PAL respectively.

Discussion

In the present investigation, it was found that FYM + SSB + *Azospirillum* + *Phosphobacteria* + Neem cake applied plants recorded lower percentage of *H. armigera* infestation on 30 DAT and 45 DAT. The effect noticed may either be due to lack of nutrients or due to the presence of toxic substances. Biochemical factors such as physiological inhibitors and nutritional deficiencies may be associated with resistance of plants to insects. *Rhizobium* and *Phosphobacteria* had significant effect in reducing larval feeding of *H. armigera* on pigeon pea pods (Ramakrishnan *et al.*, 1987). Organic sources viz., FYM, compost, neem cake, pungam, mahua, castor cakes were significantly superior and recorded lower infestation than mineral fertilizers (NPK) on cotton boll worms (Chaudary and Kashyap, 1987) and chilli pod borer (Rao *et al.* 1998). Dayakar *et al.* (1995) recorded the lowest pod borer population on pigeon pea when FYM was applied. Mallik and Lal (1989) reported that deoiled neem cake application @ 5kg/plot reduced the incidence of fruit borer *E. vitella* on bhendi. Gour (1984) reasoned that higher polyphenol content in organic manure treated plants would have resulted possibly in low pest build up. Present results are in line with the above findings.

Table 4. Biochemical changes in tomato due to foliar spray of *Pseudomonas fluorescens*

Dose of <i>Pf</i> 1 (g L ⁻¹)	Pre spraying			5 DAS*			10 DAS*			20 DAS*		
	Phenol	Peroxidase	PAL	Phenol	Peroxidase	PAL	Phenol	Peroxidase	PAL	Phenol	Peroxidase	PAL
1.0	255.3	0.015	437.1	340.5	0.018	528.5	352.0	0.0195	554.7	365.1	0.027	567.0
2.5	258.6	0.018	439.2	396.3	0.024	612.0	404.1	0.0291	618.3	448.5	0.042	684.3
5.0	256.2	0.0172	440.2	428.6	0.033	828.1	432.0	0.033	828.6	458.4	0.045	848.7
7.5	257.2	0.018	435.0	518.1	0.045	1016.2	528.1	0.045	1024.5	546.0	0.048	1046.4
10.0	258.0	0.0184	445.5	636.0	0.048	1516.2	648.03	0.052	1538.4	702.6	0.0582	1596.3
Untreated check	250.2	0.018	144.66	252.0	0.018	435.0	253.5	0.0183	436.2	255.0	0.024	438.0

• Mean of three replications.

• Phenol (µg/g).

• Peroxidase (n.mol/min/g).

• PAL – Phenyl alanine ammonialyase (n.mol/min/g).

• DAS – Days after spraying

The safety aspects of botanicals and biopesticides to non-target organisms had been already studied by several workers (Raguraman and Singh, 1997; Rosaiah, 2001). *HaNPV* was not pathogenic to *T. chilonis* (Balasubramanian *et al.*, 2001). Spicturin[®] and Delfin[®] were safe to *T. chilonis* and *T. australicum* in terms of adult emergence (88-90%) and per cent parasitization (88-90%) from treated cards. Subbulakshmi (2001) reported that Spinosad at 0.05, 0.10 and 0.15 per cent was safer to *T. chilonis* recording more than 50 per cent parasitization. In the present study, among the mixtures of botanicals and biopesticides evaluated neem oil 3% recorded 77.32 per cent of parasitism compared to check (90%). The present findings are in conformity with findings of Raguraman and Singh (1999) who reported the contact toxicity of neem oil 4% to adults *T. chilonis*, which resulted up to fifty per cent mortality and reduced the percentage of parasitization.

P. floescens influences the growth and development of insects at all stages of their growth. *P. maltophi* affects the growth of larval stage of *H. zea*, leading to reduced adult emergence, (Bong and Sikowski, 1991). In the present study, among the various doses of *Pseudomonas floescens* used as foliar spray and seed treatment the doses of 10 g/lit of water and 30 g/kg of seed recorded higher amount of biochemical compounds like phenol, peroxidase, and PAL which increased gradually upto 20 days after spraying. After 20th day decreasing trend of these compounds was observed. The present findings are comparable with findings of Thangavelu *et al.* (2003).

It is concluded that the Nature holds the key for many problems of insect pest management. Organic manures to tomato, in general, improved the plant capacity to naturally resist the attack by *H. armigera*, the pest which had developed many fold resistance to commonly recommended synthetic insecticides. In addition to organic manures in the soil, other naturally occurring insecticidal principles of plant origin insecticides especially neem with pungam and sweet-flag extracts or its formulation and in combination with

Bt, *HaNPV*, and spinosad can offer desired control of *H. armigera* at field level. However, a marginal safety period is suggested while using botanicals along with release of *Trichogramma* parasitoid to avoid even minor ill effects.

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Manuscript number	:	43/07
Date of receipt	:	December 18, 2007
Date of acceptance	:	June 25, 2009