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RESEARCH PAPER

Efficacy of *Metarhizium anisopliae, Beauveria bassiana* and neem oil against tomato fruit borer, *Helicoverpa armigera* under field condition

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A field study was conducted at farmers field of Jorhat, Assam during 2010-11 to evaluate the efficacy of three commercial biopesticides, two based on insect pathogenic fungi *viz.*, *Beauveria bassiana* and *Metarhizium anisopliae* and one botanical-Neem oil in comparison with chemical-cypermethrin against the tomato fruit borer (*Helicoverpa armigera*). The study revealed the reduction in fruit damage was upto 92.20 per cent in cypermethrin treated plot followed by 91.12 per cent, 88.74 per cent and 87.01 per cent in the plots treated with Neem oil, *B. Bassiana* and *M. Anisopliae*, respectively due to *H. armigera* larvae over control. The study showed that neem oil was nearly as effective as cypermethrin in reducing fruit damage leading to increased yield. The highest increase in yield over control was noticed in cypermethrin treated plots (62.85%) followed by neem oil treated plots (41.83%).The entomopathogenic fungi- *Beauveria bassiana* and *Metarhizium anisopliae* could be effectively used as pest management option in production of organic tomato to reduce the pest population below economic threshold level and increased yield.

Key words : *Metarhizium anisopliae, Beauveria bassiana,* Neem oil, *Helicoverpa armigera*

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INTRODUCTION

Tomato (*Lycopersicum esculentum* Mill) is the world's 2nd most important vegetable crop known for its protective and its special nutritive value and its wide spread production. India produces nearly 7.1 million tonnes of tomato annually ranking it fifth in the world, from an area of 5.4 lakh ha, placing the country at second position globally based on its area of production.

Because of its fleshy nature tomato fruit is attacked by a number of insect pests and diseases (Pandey *et al.*, 2006 and Reddy *et al.*, 2007) resulting in the consumption of large amount of pesticides which leave their toxic residues (Kumari *et al.*, 2002). The yield loss in tomato due to tomato fruit borer (*H. armigera*) alone amounts to 22-38 per cent (Dhandapani *et al.*, 2003) in addition to loss occurred due to damage caused by polyphagous insects such as white fly and aphid. Indiscriminate pesticide use is detrimental to the environment and human health and increases insect's resistance to pesticides. Pesticide application often exceeded 50 sprays per tomato crop season in South India (Nagaraju *et al.*, 2002). The share of chemical pesticides can be very high in the total material input cost for certain vegetables. For instance, it was 55 per cent for eggplant and ranked first when compared to tomato (31%) and cabbage (49%) in Philippines (Orden *et al.*, 1994). Alternative pest management strategies are hence warranted to reduce the misuse of chemical pesticides in vegetables. Therefore, an eco-friendly alternative is the need of the hour. Biopesticides have the potential to help in the management of these pests as safe alternatives to synthetic insecticides (Schmutterer, 1995; Elshafie and Basedow, 2003; Lowery *et al.*, 1993; Basedow *et al.*, 2002).

Entomopathogenic fungi play a vital role in managing the insect pests in humid tropics. *Beauveria bassiana* and *Metarhizium anisopliae* constitute about 68 per cent of the entomopathogenic fungi based microbial pesticides (Faria and Wraight, 2007). Botanical pesticides act as a synergistic component in several IPM strategies. Among the botanical pesticides, neem (*Azadirachta indica*) is being widely used and several formulations thus, containing the active component azadirachtin are commercially available. Cypermethrin is highly effective against *H. armigera* (Raijal *et al.*, 2008).

The main aim of this study was to reduce the load of synthetic chemical pesticides and evaluate field efficacy of biopesticides *viz.*, *Metarhizium anisopliae, Beauveria bassiana*, and neem oil as an alternate component of pest management to have pesticide residue free tomato and environmentally safe approaches.

RESEARCH METHODOLOGY

The present study was carried out at farmers field of Jorhat, Assam during 2010-11 :

Field preparation and site selection :

The tomato seedlings were raised in nursery bed and 25 days old seedlings were transplanted to the experimental plot. The seedlings were planted at a spacing of 100 cm × 50 cm (20000 plants/ha) as recommended for organic cultivation of tomato (var. Arjun) under the DBT project on “Biotechnology led organic farming in North Eastern Region”. The site selected for experiment was virgin land situated with $26^0 82$ N Latitude and $94^0 44$ E Longitude at a distance of 25 m from other cultivable land whereas 5-10 m buffer zone recommended for organic cultivation of tomato was also maintained. FYM @ 5 ton/ha and vermicompost @ 1 t/ha treated with *Trichoderma harzanium* (@1kg *Trichoderma harzanium* per 100 kg) as a fertility input was added along with neem cake @ 250 kg/ha at the time of final land preparation. The plot for each replication was 10 × 10 m^2 in size.

Experimental design :

A Randomized Complete Block Design with five treatments and four replications were used in the present study. Treatments included *Beauveria bassiana* (10^6 CFU/g) 10 g/l, *Metarhizium anisopliae* (10^9 CFU/ml) 10 g/l, neem oil (.03% EC) 5 ml/l, -commercial bio pesticide of Peak Chemical Industries Limited, West Bengal as recommended for organic cultivation of tomato under the DBT project on “Biotechnology Led Organic farming in North East Region” and water as control were sprayed at 30 days after sowing at 10 days interval throughout the cropping season. The synthetic chemical-cypermethrin (10 EC) 1 ml/l was taken as one of the treatment to compare with biopesticides as the farmers of the locality effectively controlled the *Helicoverpa* by using cypermethrin.

Insecticide application, observation and data analysis :

The insecticide spray was applied at 10 days interval starting at 30 days after sowing. Two, three and four sprays of insecticides were applied at vegetative, flowering to fruit setting, fruiting stage and onwards. Throughout the study, 15 plants were sampled from each treatment and each replication for observation. The number of larvae of *H. armigera* was recorded in each of the 15 plants per replication at 1 day and 7 days after spray of each treatment during vegetative, flowering and fruit formation stage. The larvae of *H. armigera* were found mostly on the top of the fruits. Counting of insects was done before 8 O’clock in the morning when the activity of the insects was still low. To determine the per cent fruit damage data was recorded from three (3) separate 1m^2 area of the same treatment and replication and the average was calculated. Data on the yield and yield parameters were also recorded in a similar way. Six pickings of fruits were carried out at an interval of 7 days. Sampling was made by counting and weighing the fruits in 25 m^2 (3 times) in every plot. The average of the three records was taken to represent the productivity per plot.

Statistical analysis :

Treatments were arranged in a Completely Randomized Block Design and the data were statistically analyzed by ANOVA. Duncan’s Multiple Range Test was used for means separation.

RESEARCH FINDINGS AND ANALYSIS

The findings of the present study as well as relevant discussion have been presented under the following heads :

Effect of treatments with control agents on *H. armigera* larvae population :

During the vegetative stage, 1 DAT there was comparatively a smaller number of *Helicoverpa* larvae observed in the plot treated with neem oil over the other treated plot and control while there was no significant difference in larval count among the plots treated with *M. anisopliae, Beauveria bassiana*, cypermethrin and control. Similarly at 7 DAT, the number of *Helicoverpa* larvae observed was lowest in plot sprayed with cypermethrin in addition to significant reduction in number in all the treated plots as compared to control. However, the number of *Helicoverpa* larvae observed in different plots treated with *M. anisopliae, Beauveria bassiana*, neem oil and cypermethrin were not significantly different (Table 1).

During the flowering stage of the crop, significantly lower number of *Helicoverpa* larvae were observed in all the treated plots compared to the control plot while there was no significant difference in the number of larvae among the plots treated with *M. anisopliae*, *Beauveria bassiana*, neem oil and cypermethrin.

During the fruiting stage, 1 DAT there was significant reduction in the number of *Helicoverpa* larvae in all the plots treated with *M. anisopliae, Beauveria bassiana*, neem oil and cypermethrin other than the control, but the number of *Helicoverpa* larvae observed in plots treated with *M. anisopliae*, *Beauveria bassiana*, neem oil and cypermethrin was not significantly different. Similarly at 7 DAT, there were lower number of *Helicoverpa* larvae observed as compared to control without any significant difference in larvae number among the plots treated with *M. anisopliae, Beauveria bassiana*, neem oil and cypermethrin (Table 1).

The promising effect of the test biopesticides against major tomato insect pest which in turn increased the yield in the present investigation agreed with the finding of Mudathir and Basedow (2003). According to their findings neem formulations significantly reduced pest attack on tomato and increased yield. Neem oil used at a rate of 3 ml/l water proved to be effective in controlling white fly, *Bemisia tabaci* on egg plants and okra in Ghana (Mochiah *et al.*, 2011). The number of *Helicoverpa* population in tomato treated with neem oil over control was found to be similar with the findings of EIshafie and Abdelraheem 2012. The number of *H. armigera* larvae in the present study treated with *M. Anisopliae* and *B. bassiana*, neem oil and cypermethrin was significantly lesser than the control plots during vegetative, flowering and fruiting stage which is in confirmation with the findings of Rijal *et al.*, 2008 in chickpea. The lesser number of *Helicoverpa* larvae in the plots treated with *M. Anisopliae* and *B. bassiana* was in support of the new examples of the control of *H. armigera* larvae by *Beauveria bassiana and M. anisopliae* in other part of the world as well (Nguyen *et al.*, 2007). In addition, *B.bassiana* had successfully controlled *Chilo partellus* (Lepidoptera : crambidae) in maize too (Tefera and Pringle, 2004). The present study showed that *Beauveria bassiana and M. anisopliae* had different rates of control of *H. armigera* larvae population which is in agreement with Naher *et al.* (2004) and Deshpande *et al.* (2000) who reported that those microorganisms can effectively control *H. armigera* with different efficacy rates depending on the different environmental conditions such as temperature, rainfall, RH and sunshine (Walsted *et al.*, 1970). The larval control rate of *H. armigera* using entomopathogenic fungi was higher at one week after treatment but cypermethrin was effective in earlier days which is in agreement with the findings of Raijal *et al.* (2008).

The present study revealed that neem oil was proved to be nearly as effective as cypermethrin in controlling the insect and reduction of fruit damage. The per cent fruit damage per square meter was recorded to be the lowest (3.6%) in cypermethrin treated plot followed by 4.1 per cent, 5.2 per cent, 6.0 per cent and 46.2 per cent in neem oil, *B. bassiana*, *M. anisopliae* and control, respectively (Fig. 1). Neem based pesticides performed better than the control for controlling *Helicoverpa* larvae population and reduced fruit damage. This effect may be attributed to the repellent, antifeedent and deterrence activities of neem and its safty to the beneficial insects. This might support the less incidence of fruit damage

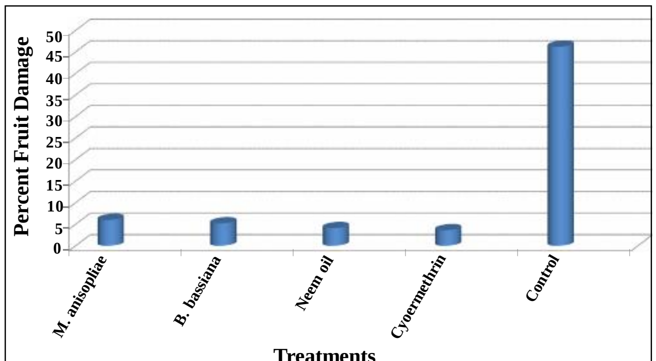

Fig. 1 : Effect of different treatments with various control agents on fruit damage by *Helicoverpa armigera with* in tomato

Table 1 : Effect of treatments with various control agents against *Helicoverpa armigera* in tomato during the vegetative, flowering and fruiting stage

Treatments	Vegetative stage		Flowering stage		Fruiting stage	
	No. of larvae/15 plants (Mean + SE)					
	1 DAT	7 DAT	1 DAT	7 DAT	1 DAT	7 DAT
Metarhizium anisopliae(109 CFU/ml) 10 gm/L	9 + 0.24	3.8 + 0.12	1.5 + 0.09	1.2 + 0.06	6.4 + 0.18	5.2 + 0.16
Beauveria bassiana (106 CFU/gm) 10 gm/L	8.6 + 0.24	4.3 + 0.13	2.3 + 0.098	1.8 + 0.09	5.7 + 0.17	3.7 + 0.14
Neem oil (.03% EC) 5 ml/L	6.6 + 0.31	4.4 + 0.13	2.7 + 0.09	1.6 + 0.09	6.2 + 0.17	5.0 + 0.14
Cypermethrin (10 EC) 1 ml/L	8.9 + 0.18	3.1 + 0.13	2.5 + 0.108	1.6 + 0.09	4.4 + 0.15	4.0 + 0.13
Control (Water spray)	10.1 + 0.32	8.8 a + 0.19	7.6 + 0.217	8.6 + 0.09	14.0 + 0.32	13.1 + 0.24
Standard error mean	0.55	0.16	0.19	0.12	0.13	0.21
C.D. (P = 0.05)	1.70	0.50	0.58	0.38	0.40	0.65
C.V. (%)	12.74	6.71	11.30	8.42	3.50	6.86

and increased yield in the present study.

Many synthetic insecticides were effective against *H. armigera*. In persent study, highest reduction in fruit damage (92.20%) was recorded in cypermethrin treated plot and the yield was increased by 62.85 per cent over control. This result agrees with the findings of Raijal *et al.* (2008) and Neupane and Sah (1988) who also reported that cypermethrin was highly effective against *H. armigera.*

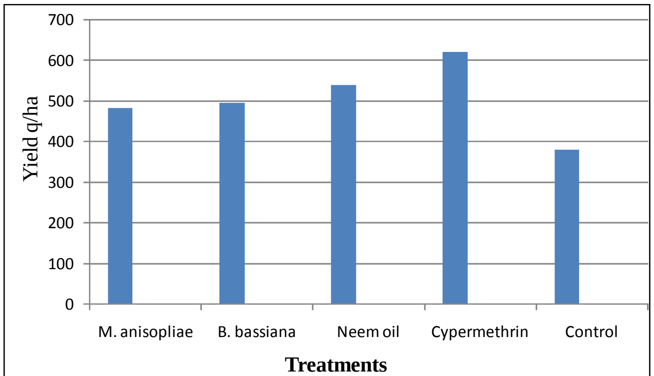

Fig. 2 : Yield of tomato with different treatments against *Helicoverpa armigera* under field conditions in Jorhat

Effect of treatment with control agents on tomato production :

The highest number of fruits (35.00) and highest marketable yield (620.00 q/ha) were recorded in synthetic insecticide treated plots followed by neem oil treated plots (540.00 q/ha) (Fig. 2). The largest tomatoes (13.70 cm^2) were obtained from neem oil treated plots closely followed by *B. bassiana* treated plots. The smallest fruits were obtained from control plots. The highest increase in yield over control was noticed in cypermethrin treated plots (62.85%) followed by neem oil treated plots (41.83%) which is also supported by highest production in cypermethrin treated chickpea plot followed by neem over control plot (Raijal *et al.*, 2008). So, to obtain synthetic pesticide free healthy fruits with attractive size and yield, application of neem oil (.03% EC) 5 ml/l is an excellent option for controlling insect pests of tomato. The yield of tomato in neem oil treated plot was closer to the cypermethrin treated plot (Synthetetic chemicals) which is similar to the findings of Elshafie and Abdelraheem (2012). This result is also in agreement with the findings of Elshafie (2001) who reported that the average yield of potato treated with neemazal and sumidin (Synthetic chemical) was 6.09, 6.35 and 3.36 t/ha for untreated control, respectively.

In Sudan, similar results were obtained with neem products in the control of the sweet potato whitefly, *Bemesia tabaci* and the leafhopper, *Jacobiasca lybica* on potato (Siddig, 1987 and 1991). Two high volume applications of 2.5 per cent aquous neem kernel extract sprayed at forthnightly intervals reduced the pest populations <50 per cent of the control and increased the yield (Saxena, 2011).

Conclusion :

The present study revealed that pesticide residue free tomato could be possible in field condition by using neem oil which was nearly as effective as cypermethrin in reduction of the fruit damage upto 91.12 per cent and increased yield upto 41.83 per cent over control. The entomopathogenic fungi- *M. anisopliae* and *B. bassiana* could be effectively used in field conditions to reduce the pest population below economic threshold level and could be an environmentally safe alternate approach for insect pest management in organic tomato production. On the other hand, a safe waiting period has to be followed according to the recommendations, when conventional pesticides used, but by using neem oil the fruits can be consumed the same day after harvest. As the fruits are perishable, this would be beneficial to farmers also.

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