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Bio-efficacy of microbial, chemical and conventional treatments against *Spodoptera litura* infesting gerbera plants

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Abstract: *Spodoptera litura* is a highly polyphagous insect found feeding on vegetables, food crops, weeds and other economic crops. In the present study the efficacy of *Beauveria bassiana*, *Metarrhizium anisopliae*, *Bacillus thuringiensis*, Ekalux, Malathion and a mixture of cow's urine and kanthari chillies (*Capsicum frutescens*) were tested against the pest of gerbera, *Spodoptera litura (Fabricius) (Lepidoptera: Noctuidae)* under laboratory conditions. Among the treatments Ekalux resulted in 100% mortality, mixture of cow's urine showed 70% mortality. Only 26% mortality was noted after the treatment of Bt. But in all other treatments mortality was zero percentage. The variations in the susceptibility of insect species to fungal invasion may result from several factors, including differences in the structure and composition of exoskeleton, the presence and activity of antifungal protein in hemolymph as well as efficiency of cellular and humeral defense reactions. So, there is an increasing demand to discover environment friendly insecticides and pesticides in general.

Keywords: *Spodoptera litura, Beauveria bassiana*, *Metarrhizium anisopliae* , *Bacillus thuringiensis,* Ekalux, Malathion, cow's urine and kanthari chillies.

Introduction

Insect pests are known to cause significant damage to crops and affect agricultural productivity. The larval stage of *Spodoptera litura* causes major damage to an array of economically valuable crops including cotton, tomato, corn and sorghum. Its host range covers at least 120 species[1]. Several outbreaks of this pest on cotton, tobacco and chillies have been reported in Tamil Nadu and the economic loss range between 25.8-100% depending upon the crop stage and its infestation level .It passes through 5-6 overlapping generations annually[2] and if not controlled timely, it may result in huge crop losses in various parts of India[3].

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For the management of this pest, insecticide use is most widely practiced. Recently, broad spectrum chemical insecticides have been the primary control agent for agricultural pests, with about 40% targeted to the control of Lepidopteran insects[4]. Present studies were planned to find out the time-related toxicity of ekalux and malathion in the category of insecticides against *Spodoptera litura.* Although effective in reducing pest population in short term, these chemicals have little long term regulatory impact on pest population and often cause unwanted environmental side effects. Over the years the widespread use of pesticides has led to pesticide resistant insects, a reduction in beneficial insect populations and harmful effects to humans and the environment [5]. Microbial pesticides are one such alternative to tackle insecticide resistant population of *S. litura* [6].

Fungal pathogen particularly, *Beauveria bassiana, Metarhizium anisopliae, Verticillium lecanii* and *Nomuraea rileyi* have been found to be promising in the control of several agricultural pests[7]. *B. bassiana* is a hyphomycete insect-pathogenic fungus in the subdivision Deuteromycotina which occurs worldwide. Over 200 species of insects in nine orders, mainly Lepidoptera and Coleoptera have since been recorded as hosts [8]. It is found naturally on some plants and in soils and is regarded as a safe biopesticide[9].

The mitosporic ascomycete (hypocreales) fungus, *Metarhizium anisopliae* has gained significant attention as a biocontrol agent due to its wide geographic distribution, high virulence and vast spectrum of infectivity to a wide range of insect pests[10]. The conidia of *M. anisopliae* usually enter into insect mainly through the integument by adhesion, penetration into haemoceol and development of fungal infection[11]. The process of penetration through the insects integument by a hypal germination from a spore involves chemical (enzymatic) and physical forces. In vitro studies indicated that the digestion of the integument follow a sequential lipase-proteasechitins process of digestion [12].Though *B. bassiana* and *M. anisopliae* has been studied against various insect pests in both green house and field, an in-depth literature survey proves that the fungi have not been studied in detail against *S. litura*. Hence, the present study aimed to explore the pesticidal activity of the fungi against the target pest.

Bacillus thuringiensis is a naturally occurring gram-positive bacterium commonly present in soil. Various strains of *B. thuringiensis* (*Bt*) are capable of producing crystal (Cry) proteins (delta - endotoxins) or inclusion bodies that have selective insecticidal effects against

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different groups of insects[13]. The inclusions produced by *Bt* subspecies are generally composed of several proteins (designated as delta - endotoxins or Cry proteins) each having a narrow activity spectrum. Partly because of their selectivity and short half-life, *Bt*-based microbial insecticides are generally considered to have fewer adverse impacts on the environment than synthetic chemicals[14].

Plants are important natural sources of bioactive compounds and many such plant compounds have been included in commercial botanical pesticides [15].Many plant products are safer to non-target organisms and effective against phytophagous insects. Plant products bring about wide range of behavioral and physiological effects on the insects[16].A mixture of cow's urine and chilly is tested here.

Gerbera (Family: Asteraceae) is a perennial and herbaceous flowering plant producing different colors of flower. It has massive demand in the floral industry as cut flower as well as potted plant due to its beauty and long vase life which ranked at fifth among the top ten cut flowers in the world[17].Attack of *S.litura* lead to defoliation, stunted development and reduction in the number of flowers produced. This project was aimed to investigate the effects of the above six treatments on *Spodoptera litura* infesting gerbera.

Materials and method

Insects

Third instar larval stages of *S. litura* were collected from gerbera fields adjoining Regional Agricultural Research Station (RARS), Kerala Agricultural University (Wayanad) and maintained in petri dishes.

Insecticides

Beauveria basssiana , *Metarhiziium anisopliae* , Bt, Ekalux, Malathion, mixture of cow's urine and kanthari chillies.

Bioassays

One ml of each suspension of insecticide was sprayed in each petri-dish containing leaves and larvae. In case of control, larvae were treated with distilled water.10 larvae were used for a single replication. A total of 40 larvae were used for each treatment *i.e.,* 3 replications (10+ 10 +10) and control (10). Insect mortality was assessed daily up to ten days.

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To avoid mortality due to unhygienic conditions the rearing petri dishes were cleaned and fresh leaves were provided daily.

Bioefficacy of entomopathogenic fungi against third instar larvae of *S. litura* (treatments 1, 2, 3 & 4)

5 ml of *Beauveria basssiana* was weighed and dissolved in 1litre of water and added 1ml tween 80, mixed well .This suspension was sprayed (1ml) on fresh leaves and caterpillars (3rd instar) in each petri-dish. Insect mortality was assessed daily up to ten days. Fresh leaves should be given to the larvae every day. In the same way 10g of *Beauveria bassiana*, 5 & 10 g of *Metarhiziium anisopliae* was also prepared and sprayed.

Bioefficacy of Bt against sixth instar larvae of *S. litura* :treatment 5

5ml of Bt was weighed and dissolved in 1 litre of water and added 1ml tween 80, mixed well. From this suspension, 1 ml was taken and sprayed to each petri-dish containing fresh leaves and caterpillars (5th instar). Insect mortality was assessed daily up to ten days. Fresh leaves should be given to the larvae every day.

Effect of pesticides against *S.litura*

Treatment 6 ekalux & treatment 7 malathion

2 ml of ekalux was taken by means of a micropipette and dissolved in 1 liter of water. From this, 1 ml was sprayed on fresh leaves and caterpillars (3rd instar) in each petridish. Mortality was observed for two days. Similarly 2ml of malathion was tested.

Conventional method

Treatment 8

10 g of kanthari chillies were crushed and dissolved in 100 ml of urine. This mixture was made upto 1 litre .Then 1 ml of this mixture was sprayed on fresh leaves and caterpillars. Mortality was observed after 7 days.

Result and Discussion

The toxicity of *Spodoptera litura* to ekalux at 2 ml/l concentration recorded 100% mortality. Mortality ranged from 80 – 100% in first and second day. The data are tabulated in table: 2.

Resistance in *S. litura* against organophosphates has been reported from various parts of the Asian countries[18].Organophosphates are toxic to insects by virtue of their ability to

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inactivate acetylcholinesterase or its inhibitors. Such resistance was found at a high level to malathion. Which provide evidence of high level of resistance. This could be related to the commonly reliance in the use of organophosphates against insects in these areas. Resistance in *S. litura* against organophosphates has been reported from various parts of the Asian countries, such as Pakistan India and China[19] providing evidence of high level of resistance against organophosphates insecticides.

The second most effective treatment was a mixture of cow's urine and kanthari chilies(*Capsicum frutescens*) against third instar larvae and was observed up to 7 days .The treatment resulted in 70% mortality. The data is recorded in table: 3.

As per recent studies cow urine has proved to be an effective pest controller and larvicide when used alone and also in combination with different plant preparations[20].The effect of plant extracts, neem, *J.curcas* L., *Chrysanthemum cinerariaefolium* (Trev.), Eucalyptus *globulus Labille & Vitex negundo* L mixed with cow urine in 1: 9 parts and alone cow urine @ 0.5% against *Brahmina coriacea* were observed. Cow urine alone was ineffective but the NSKE in urine resulted in no egg laying, minimum egg hatching [21].

Treatment with the bacterial species *Bacillus thuringiensis* against the 5^{th} instar larvae showed the next lowest range of mortality of 26.6% within 7 days of exposure. The remaining 73.4% were alive and showed gluttonous feeding. They were observed upto their emergence as adult moths. But none of them showed any abnormalities. They were allowed to mate and laid clusters of eggs. Observations are recorded in table: 1.

The proteins such as d-endotoxins and cytolytic proteins produced by Bt are efficient sources of insecticides for food crops and stored grains [22].The Cry toxin of Bt is one such endotoxin that acts against a wide range of insects [23].The gut regions of these are targeted by toxins causing death by starvation. Many transgenic plants have been developed that can produce insecticidal proteins that are derived from this genera. They have been successfully used in control of pest and protecting important high acre crops[24]. Cry toxins are of various types and differ in their host specificities.

But the most common mechanism of resistance is by the disruption of binding of Bt toxins to receptors in the midgut membrane. This disruption may be either due to mutation in the receptors or changes in the expression of the receptors[25]. The resistance mechanism

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associated with ABC transporter loci has also been reported[26]. In our study, Bt resulted in 26.6% mortality, which indicates *S.litura* is moderately resistant to it.

Among the experiments, *S.litura* showed high resistance against malathion (table:2), *Beauveria bassiana* and *Metarhizium anisopliae* (table:1) .No mortality was observed.They were quite active and showed voracious feeding. As like Bt the emerged moths were healthy and laid clusters of eggs.

Studies suggest that eggs of fall armyworm, *Spodoptera frugiperda*, were highly susceptible to insect pathogenic fungi; *M. anisopliae* [27]. The egg mortality increases with increase in the conidial concentration[41]. Susceptibility of the insect to entomopathogenic fungi decreases with advancement in age of larvae of the target host[28]. Chemical constituents of insect cuticle change gradually with advancement in age of larvae resulting in hardening of the cuticle and increased humoral immune to the microbial infections. It was observed that adult emergence was delayed in fungal treated pupae. Some of emerged adults were also malformed with reduced wings or reduced body size. They were unable to fly and died without mating. It has been investigated that pupae treated with fungal pathogens often result reduction in the adult emergence[29].

But in contrast, another laboratory study revealed that the two entomopathogenic fungi, *B.bassiana* and *M.anisopliae*, cannot control the *S.litura* populations and their incidence. The defensive responses to fungal infection leads to elevated levels of Phenoloxidase in haemolymph and other enzyme cascade [30].In addition, PO activity in *A. simplex* infected with *B. bassiana* was higher than uninfected controls as reported by Srygley and Jaronski[31]. *Metarrhizum* infection may results in declining haemolymph protein and phenoloxidase titres over the course of infection until the death of *Schistocerca gregaria* and *Locusta migratoria* [32]whereas *Beauverria* infection increases PO activity in grasshopper, *Melanoplus sanguinipes* and army cutworm, *S. exigua* as demonstrated by Hung and Boucias [33].In the present study *S.litura* showed high resistance against *B.bassiana* and *M.anisopliae*. This might be due to the presence of increased level of PO in their haemolymph.

Significance of the study was calculated using ANOVA. The effect of different treatments showered significant results.

Overall results provided ekalux as the best management tool in respect of concentration and time providing along with other treatments. It is the long- lasting control

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tactic for the farming community against this pest of economic importance. A mixture of cow's urine and kanthari chillies proved to be the second most effective insecticide after ekalux respectively. The successful management of insecticide resistance depends ultimately on a thorough knowledge of its genetic basis and the mechanisms involved. The mode of inheritance helps in resistance detection, monitoring, modeling and risk assessment. Such knowledge can provide the basis for management programs aimed at minimizing the development of resistance.

So, there is an increasing demand to discover environment friendly insecticides and pesticides in general. *S.litura* had made this situation still shoddier because of its voracious feeding habit and the extent of damage it can cause to the agricultural community. Therefore, further research in the field of "resistance mechanisms developed by *Spodoptera litura*," has been forced in this area where an effective alternative is needed.

Damage *Due to Spodoptera* Attack on Gerbera Plants

Table 1: control of *Spodoptera litura* by entomopathogenic fungi and Bt

Sl.No	Treatment	Larval instar	Concentration (g/l)	Total number of larvae introduced	Mortality		No.of animals pupated	%of mortality
					After 3	After 7		

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					days	days		
1	*Beauveria bassiana*	3rd	5	10	nil	nil	10	0
2	*Beauveria bassiana*	3rd	10	10	nil	nil	10	0
3	*Metarhizium anisopliae*	3rd	5	10	nil	nil	10	0
4	*Metarhizium anisopliae*	3rd	10	10	nil	nil	10	0
5	*Bacillus thuringiensis*	3rd	5	10	1	2	6	26%

Table : 2 control of *Spodoptera litura* by pesticides

Sl.no	Treatment	Larval instar	Concentration (ml/l)	Total number of larvae introduced	Days	Mortality	No.of animals pupated	%of mortality
1	Ekalux	3rd	2	10	Day 1	8	Nil	100%
					Day 2	2		
2	Malathion	3rd	2	10	Day 1	---	10	0%

Table 3: control of *Spodoptera litura* by conventional methods

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Sl.No	Treatment	Larval instar	Concentration (g/l)	Total number of larvae introduced	Days	Mortality	Percentage of mortality
1	Cow's urine + small green chilly(Kanthari)	3rd	(100 ml cow's urine +10 g kanthari) → make upto 1 litre	10	Day 1	---	70%
					Day 2	4	
					Day 3	No specific change, all were alive but were less active, and showed much variation in their feeding habits.	
					Day 4	1	
					Day 5	None of them were dead, but was found to be inactive and less consumption of Gerbera leaves.	
					Day 6		
					DAY 7	2	

Conclusion

The present study was conducted to test the efficacy of *Beauveria bassiana*, *Metarrhizium anisopliae*, *Bacillus thuringiensis*, Ekalux, Malathion and a mixture of cow's urine and kanthari chillies against the pest of gerbera, *Spodoptera litura (Fabricius) (Lepidoptera: Noctuidae)* in laboratory conditions. Among the treatments Ekalux resulted in 100% mortality. Next to Ekalux, conventional method showed 70% mortality. Only 26% mortality was noted after the treatment of Bt. But in all other treatments mortality was zero percentage. Hence we can conclude that the pest was highly resistant to all other treatments. Their abilities to inactivate acetylcholinesterase help to resist the action of organophosphates. The development of a broad-spectrum resistance to insecticides has complicated its chemical control. It is hoped that present study would help to investigate the mechanism triggering immune response and to shed further light on intriguing aspects of insect immunity.

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