Resistant Pest Management Newsletter

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Letter from the Editors

Dear Subscribers,

I have a few announcements that I would like to share with you. First, Vijay K. Nandula, an assistant professor at Mississippi State University, has published a book entitled Glyphosate Resistance in Crops and Weeds: History, Development, and Management. This book provides insight to glyphosate-resistant crop technology and how it has revolutionized crop production in many parts of the world. The authors have reviewed and analyzed all the latest research findings as well as the latest technologies developed to manage glyphosate – resistant (GR) crops and weeds. This book covers topics such as the developmental processes of first and subsequent generations of GR crops, strategies for effectively managing glyphosate resistance, genetics and genomics of glyphosate resistance, and much more. For ordering information please go to www.wiley.com.

Secondly, the 2011 Resistance International Conference will be held from Septemeber 5-7, 2011 at Rothamsted Research, West Common, Harpenden, Hertfordshire AL5 2JQ, UK. This conference will review new research on the origins, nature, development and prevention of resistance to insecticides, fungicides and herbicides. The themes will include: The current status of resistance to pesticides, resistance mechanisms, population biology and modeling, applications of genomics, risk assessment and regulation and transgenic crops. To register an interest in presenting or attending, please visit http://www.rothamsted.bbsrc.ac.uk/resistance2011.html or contact the organizing committee at resistance2011@bbsrc.ac.uk.

Lastly, I would like to thank all of our subscribers, contributing authors and supports for their continued support of the RPM newsletter. For any additional information or questions please feel free to email me at rpmnews@msu.edu.

Sincerely,

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Resistance Management from Around the Globe

Susceptibility of *Bactericera cockerelli* (Sulc) (Hemiptous: Triozidae) to insecticides in the State of Coahuila, Mexico

Abstract: Two field population of *Bactericera cockerelli* (Sulc) from Saltillo and Huachichil with a susceptible line to determine the resistant levels. Results show that Saltillo population was 3.2, 1.6, 4.1, 20.4 and 1.6 times more resistant to abamectin, cypermethrin, endosulfan, imidacloprid and profenophos respectively than the susceptible line. Huachichil population values were 0.3, 3.1, 5.3, 1.1 and 1.1 times more resistant to the same products.
Key words: Potato psyllid, Resistance, LC_{50}

INTRODUCTION

Potato is one of the main crops worldwide. Northern Mexico is the leading potato region in the country with yields being dramatically affected by plant health issues, among which *Bactericera cockerelli* (Sulc) psyllid is one of the main pests. This is due to the damage caused during sap sucking and the release of a toxin (Munyaneza *et al*., 2007) as well as phytoplasms transmitter (Garzón *et al*., 2004). Such phytoplasms produce the disease known as potato purple tip or tomato permanent disease (Garzón *et al*, 2005). Almeyda *et al.* (2008) detected the relationship between this psyllid and the potato blue tip

phytoplasm. Flores *et al.* (2004) mention that this disease is the main disease limiting potato production, reporting that in 2003 and 2004, the incidence of this disease increased to 100 % in Coahuila and Nuevo León southern regions, causing huge losses, since the yield dropped by 90%. 12 insecticide applications are the standard in these states during the crop season, (Vega *et al*., 2008). On the other hand, Rubio *et al.* (2006) mentioned cases where insecticides were applied up to 30 times. Very few essays have been conducted to learn about the level of resistance that this psyllid develops towards the different toxicological groups of insecticides used in the control of this pest. Therefore, the goal of this research work is to determine the resistance level of two *B. Cokerelli* populations from Saltillo and Huachichil, Coahuila, Mexico potato growing regions.

MATERIALS AND METHODS

This work was carried out at Universidad Autonoma Agraria Antonio Narro (UAAAN). Insects were sampled on greenhouse from Saltillo (Sa) and potato fields from Huachichil (Hu) and a susceptible line (SL) and were later reared in the toxicology laboratory of UAAAN. In each sampling site, 200 leaves infested with nymphs were collected. As a susceptible line a greenhouse population without selection pressure reared since 2004 was used, and all populations then were reared in a greenhouse to carry out the experiments. Bioassays were done using the leaf immersion technique used for the pear psyllid (*Psylla pyricola* Foerster), with slight modifications (IRAC, 2005). Insecticides used were Abamectin®, Cypermethrin®, Imidacloprid®, Endosulfan®, and Profenophos®. Purified water and Bionex as dispersant were used to prepare the different concentrations from 0.01 to 2000 ppm. Mortality readings were done 24 and 48 h after treatment, considering dead nymph the ones that were dehydrated or did not respond to stimulus. Once determined the LC_{50}, the resistant proportion was determined by dividing the LC_{50} values of the field population against the susceptible (Georghiou, 1962), Data was analyzed by a probit analysis with the maximum verisimilitude method and using a SAS system Windows 9.0 (2002).

RESULTS AND DISCUSSION

Table 1 shows LC_{50} of the SL which was 0.06, 82.59, 62.28, 3.65 y 1.67 ppm for abamectin, cypermethrin, endosulfan, imidacloprid and profenophos respectively. Vega *et al*. (2008) on a laboratory reared susceptible line since 2002, report LC_{50} values from 0.01 to 76.5 ppm to insecticides of similar toxicological groups; so, the susceptible line of this research, can be used a reference line to test *B. cockerelli* susceptibility.

Table 1: Lethal concentration and fiducial limits of susceptible line (LS) of fourth instar nymphs of *Bactericera cockerelli* (sulc).

Susceptible Line (SL)					
			Ppm		
Insecticide	N	df	LC_{50}	Fiducial Limits 95 %	LC_{95}
Abamectin	480	5	0.06	(0.0367-0.1169)	0.9614
Cypermethrin	480	5	82.59	(26.894-178.799)	962.083
Endosulfan	480	5	62.28	(25.971-128.667)	2110.05
Imidacloprid	480	5	3.65	(1.8561-6.8615)	81.4120
Profenophos	480	5	1.67	(0.6570-3.4305)	48.3460

n: Number of nymphs, df.: Degrees of freedom

Tables 2 and 3 also show resistance ratio of Saltillo and Huachichil populations against the susceptible line. Saltillo population was 3.2, 1.6, 4.1, 20.4 and 1.6 times more resistant to abamectin, cypermethrin, endosulfan, imidacloprid and profenophos respectively, and for Huachichil population was 0.3, 3.1, 5.3, 1.1 and 1.1 times more resistant to the same products tan the susceptible line. Resistance threshold for imidacloprid was 20.4 times higher on Saltillo population and others pesticides the resistance threshold was under of 10 times. The reason to find imidacloprid with resistance problem is probably due that multiple used in the greenhouse with 4 or 6 sprays pear season (Vega *et al.*, 2008).

Table 2: Lethal concentration and fiducial limits from Saltillo (Sa) population and it´s resistance ratio against the susceptible line (SL).

Saltillo Line (Sa)						
			Ppm			
Insecticide	n	d.f.	LC_{50}	Fiducial limits 95 %	LC_{95}	Sa_{vs} Ls
Abamectin	480	5	0.22	(0.0632-0.6271)	5.7799	3.2 X
Cypermethrin	480	5	135.74	(79.967-220.202)	2081.5	1.6 X
Endosulfan	480	5	260.44	(113.261-623.215)	8250.3	4.1 X
Imidacloprid	480	5	74.88	(47.876-127.171)	3254.6	20.4 X
Profenophos	480	5	2.79	(1.0458-7.2165)	77.023	1.6 X

n: Number of nymphs, df.: Degrees of freedom, Sa_{vs} SL: Resistance proportion.

Table 3: Lethal concentration and fiducial limits resistant proportion against of the Huachichil (Hu) population and it´s the susceptible line (Ls).

Huachichil Line(SR)						
			Ppm			
Insecticide	n	df	LC_{50}	Fiducial Limits 95 %	LC_{95}	SR_{vs} SL
Abamectin	480	5	0.02	(0.0127-0.0401)	0.3729	0.3 X
Cypermethrin	480	5	257.63	(150.876-393.278)	2130.5	3.1 X
Endosulfan	480	5	333.35	(302.571-365.868)	1250.8	5.3 X
Imidacloprid	480	5	4.17	(3.3730-6.0880)	85.02	1.1 X
Profenophos	480	5	1.85	(0.7909-3.8374)	34.858	1.1 X

n: Number of nymphs, df.: Degrees of freedom, SR_{vs} SL: Resistance proportion.

Saltillo population LC_{50} for abamectin, cypermethrin, endosulfan, imidacloprid and profenophos had values of 0.22, 135.74, 260.44, 74.88 and 2.79 ppm respectively (Table 2) and Huachichil population LC_{50} (Table 3) had values of 0.02, 257.63, 333.35, 4.17 and 1.85 ppm to the same products. Saltillo population had the LC_{50} higher values to abamectin, imidacloprid and profenophos, whereas Huachichil population to cypermethrin and endosulfan. This variation is probably due to the origin of Saltillo population (Greenhouse) when pesticides, application interval are different to products and application methods used by growers in field (Huachichil).

CONCLUSION

Imidacloprid presented resistant levels on Saltillo population (greenhouse); however, it´s use could be restricted to 2 or 3 sprays per season, alternating pesticides with lower resistance ratio as abamectin, cypermethrin and profenofos.

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Bio-efficacy of microbial and chemical Insecticides on major lepidopterous pests of Cotton and their (insect) natural enemies in Cotton Ecosystem in Tamil Nadu

ABSTRACT
Cotton (Gossypium spp.) crop is invested by various pests and among them lepidopterous pest cause a major yield loss. Three microbial (Halt,biotrol,and thuricide) and three chemical insecticides (Lambdacyhalothrin, Acephate and carbaryl) were compared for efficacy on four major lepidopterans and their naturalenemies in replicated field trials at Kancheepuram district,Tamil Nadu. Thuricide was evaluated at different combinations with lambdacyhalothrin in a second trial. The results showed that the microbials caused the mortalities of destructive bollworms and leaf cutworms but allowed the survival of their natural enemies. The chemicals on the other hand caused mortalities of both destructive and useful species. Both groups of insecticides enhanced seed cotton yields. Application of thuricide followed by lambdacyhalothrin was better than other combinations evaluated.
Key words: *microbial insecticides, Bacillus thuringiensis, cotton.*

INTRODUCTION

The cotton bollworm (*Helicoverpa armigera* Hubn), the spiny bollworms (*Earias insulana* Boisd and *E. biplaga* Wlk.), and the leaf cutworms (*Spodoptera Litura*) are major lepidopterous pests of cotton in Tamil Nadu. These insect pests are currently being controlled by the application of broad spectrum insecticides such as lambdacyhalothrin, Acephate or carbaryl four times at weekly intervals during the growing season. However, these broad spectrum materials are highly toxic to insect natural enemies (Hamilton and Attia, 1976). On the other hand, *Bacillus thuringiensis* Berl, is active against many lepidopterous species and has no adverse effects on natural enemies of target pests (Fadare and Osisanya, 1998). The lepidopterous pests natural enemies include parasites (syrphids, tachnids, braconids) and predators (coccinelids, and reduviids). A control programme based on selective materials, which would allow survival of beneficial species and cause the mortality of destructive ones is desirable. The efficacy of *B. thuringiensis* could be increased by the addition of sublethal doses of pesticides and could be used in such a programme. Here we report the comparative efficacy of three microbial insecticides, Halt, biotrol, thuricide and three chemical insecticides, lambdacyhalothrin, acephate and carbaryl on cotton bollworm, spiny bollworms, the Leaf cut worms and their natural enemies.

MATERIALS AND METHODS

The treatments comprised three microbials, Halt at 0.52kg/ha, biotrol, 0.56, thuricide, 0.50 and three chemical insecticides, lambdacyhalothrin at 0.68kg. a.i./ha, Acephate, 0.75, carbaryl, 1.50,each in 225 litres of water/ha, and different combinations of one microbial (thuricide) and one chemical insecticide (lambdacyhalothrin).The treatments were arranged in a randomized complete block design experiment with four replicates. The cotton plots of 10 m X 5 m each were established as per standard agronomic practices for cotton production. The experiment was conducted over 1 year. The treatments were applied with a 9-liter pressurized Falcon sprayer to the plants when one plant per plot was infested by any of the target pests. Post spray counts of *Sylepta* were taken from 5 plants per plot while *Helicoverpa* and *Earias* damaged bolls were counted and removed from 10 randomly selected plants of each plot. Pre and post spray samplings of populations of natural enemies (parasites and predators) were carried out with an aerial net. Parasitised larvae and pupae were taken to the laboratory for emergence of parasites. The inner two rows of each 10 m X 5 m plot were used for the estimates of seed cotton yield. Data collected
was subjected to statistical analysis. Efficacy of treatment was based on plot means of live Leaf cut worms, percentage bollworm damages, seed cotton yield and live Leaf cut worms enemies recovered from the sprayed and unsprayed plots.

RESULTS

Experiment I

The post spray application mean (Leaf cut worms) counts ranged from 2.62 to 3.14 per plant for the microbial insecticides and from 1.57 to 2.14 per plant for the chemicals. Both were however better than the 6.4 live Leaf cut worms per plant from the unsprayed control plots (P = 0.05) (Table 1). Percentage bollworm damages ranged from 12.10 to 13.14 per plant for plots sprayed with the microbials and were significantly higher (P =0.05) than the range of 5 – 7 per plant for plots sprayed with the chemical insecticides. The percentage bollworm damage from the unsprayed control treatment was 20.00 and was significantly higher from those of microbial and chemical insecticide treated plots (P = 0.05). Corresponding percentage bollworm control ranged from 35 – 36 for the microbials and 65 – 75 for the chemical insecticides (Table 1). Mean seed cotton yields ranged from 981 to 1082 kg/ha for the microbials and 901 – 1106 for the chemicals, and were not significantly different. However, the 388kg/ha seed cotton yield from the control plots was significantly lower than those from the sprayed treatment (P = 0.05).Corresponding percentage yield increases of sprayed plots over the control plots ranged from 152 – 178 for the microbials and 132 – 185 for the chemical insecticides (Table 1). The mean numbers of parasites and predators recovered from plots sprayed with microbial – and chemical insecticides were low and similar for both and not significantly different from those of the unsprayed control plots (Table 2). The numbers of braconids recovered from each plot were higher than the numbers recovered for other parasites (Table 2). Also, numbers of parasites and predators generally increased after spraying with the microbials, but stayed the same or reduced with chemical insecticides.

Table 1: Comparative efficacy of treatments on the cotton pest in the filed conditions

Treatment	Rate/ha	Leaf Curworms	Bollworm	Boll Damage	Seed Cotton	Yield
	(Kg)	count/plant	Damage (%)	Control (%)	Yield (Kg/ha)	increase (%)
Halt	0.52	2.62b	12.1b	34.68	1006a	159.2
Thuricide	0.56	3.14b	12.93b	36.14	981a	152.24
Biotrol	0.5	2.71b	13.14b	35.1	1082a	178.21
Lambdacyhalothrin	0.34	2.14b	5.02c	75.03	1106a	185.27
Acephate	0.38	1.92b	7.01c	65.12	914a	137.1
Carbaryl	0.75	1.73b	5.11c	74.4	901a	132.1
Blank Control	0	6.4a	20.25a		378b	

No significant difference between means with same letter at 5% level

Table 2: Population dynamics (in mean) of the predator and parasites recorded in the treated plots

Treatment	Parasites			Predators	
	Rate/ha	Braconids	Tachinids	Syrpids	Coccinelids	Reduvids
	(Kg)					
Halt	0.52	20 (37)	1(2)	0(3)	2(3)	1(5)
Thuricide	0.56	19 (34)	0(2)	2(4)	4(5)	3(6)
Biotrol	0.5	26 (38)	1(3)	1(5)	3(5)	4(6)
Lambdacyhalothrin	0.34	22(15)	1(3)	1(0)	3(2)	3(2)
Acephate	0.38	24(15)	1(1)	1(1)	4(3)	3(3)
Carbaryl	0.75	26(20)	0(0)	1(0)	5(3)	4(4)
Blank Control	0	22(23)	2(2)	1(2)	4(4)	2(3)

post spray counts in parenthesis

Experiment II

The results of the different combinations of microbial and chemical insecticides (thuricide/lambdacyhalothrin) are presented in Table 3. All sprayed treatments were better than the unsprayed control treatment. Corresponding percentage yield increases of 132.34 over the control was highest for T3 treated plots and lowest for T5, 91.40%.

Table. 3 Efficacy of various treatments combinations on the pest of cotton

Treatment	Leaf Cutworms	Bollworm	Boll Damage	Seed Cotton	Yield
	count/plant	Damage (%)	Control (%)	Yield (Kg/ha)	Increase (%)
T1	1.15cd	7.24bc	44.65	1321.10 (a)	104.51
T2	1.26c	7.41bc	43.51	1254.40(ab)	94.34
T3	0.58d	5.84c	58.53	15.02.00(a)	132.34
T4	2.0b	10.20b	23.45	995.00 (ab)	53.78
T5	2.5b	8.34b	37.1	1234.40(ab)	91.4
T6	4.50a	13.25a		650.00(c)	

DISCUSSION

The post spray cutworm larval counts for both microbial and chemical insecticides show that they were effective in reducing the level of live Leaf cut worms. There was no significant difference between both materials. However in bollworm damage, the chemicals performed significantly better (P = 0.05) than the microbials. The chemicals reduced the level of bollworm damage from 20 percent in the unsprayed control plots to 5.66 while the microbials reduced such a level to 13 percent which is more than two-folds that of the chemicals. The seed cotton yields of plants sprayed with microbial or chemical insecticides were very high and superior to that of the control. Both raised the yield more than 2-fold. The high yields could be attributed to the effective control of the foliage pests and bollworms which consequently enhanced the quantity and quality of the end products. The parasites and predators recovered from the microbial, chemical and control plots were similar and low in numbers in the pre-spray counts. Such numbers were marginally increased in the microbial and control plots but marginally reduced in the chemical plots in the post spray counts. Apparently the microbials allowed the survival of the beneficial species but caused the mortality of the destructive ones. On the other hand the chemicals caused the mortality of both the beneficial and destructive species. The parasites recovered from the trial plots ncluded live syrphids, tachnids and braconids and, the predators were coccinellids, forficulids, entatomids and reduviids as listed in an earlier report (Fadare and Osisanya, 1998).

All the treatments combinations of thuricide and lambdacyhalothrin were effective in reducing the level of live Leaf cut wormss population significantly. The seed cotton yields from all sprayed plots were significantly higher than that from the control. Based on the results of experiment II, treatment T3 (thuricide followed by lambdacyhalothrin) is very consistent, giving either significantly superior or marginally higher performance than other sprayed treatments and the control. In general, the findings from these studies are similar to the reports of earlier research workers. Ali Niezee and Jensen (1973) working with spray formulations of biotrol, Halt and thuricide, found that the three formulations gave as good a control of the grape leaf-folder *Desmia funeralis* (Hubn) (Pyralidae) as the chemical insecticide, carbaryl. McGarr et al. (1970), compared the effect of microbial and chemical insecticides on cotton insects and reported percent bollworm damages of 23.8, 14.2, 18.7, 20.2 and 39.7 for methyl parathion, *B. thuringiensis* (HD-I), Toxaphene +methyl parathion, carbaryl + methyl parathion and control, respectively. They also reported significant yield increases of seed cotton over the unsprayed control treatments. They concluded that the *B. thuringiensis* was more effective than the chemicals in controlling the bollworms. Based on the results of the second trial, straight applications of either microbial or chemical insecticide for the suppression of cotton pests may not be advisable. Microbial insecticides, being highly selective conserve the populations of parasites and predators as well as other beneficial species while they suppress lepidopterous populations for which they are specific. Applications of chemical insecticides for the control of cotton pests is not advisable early in the season as they may reduce yields due to an apparent adverse reactions by the plants, and such applications may result in increased bollworm, beet armyworm or cabbage looper populations. Bull et al. (1979) had reported a deliberate suppression of beneficial species populations with methyl parathion and subsequent rapid increases in outbreak of *Heliothis* sp.on cotton fields. Simultaneous application of both microbial and chemical insecticides though not significantly different from 'Thuricide' or lambdacyhalothrin alone, may not be advisable because of adverse reactions of emulsifiable concentrate insecticides (Morris and Armstrong, 1975; Morris 1975a; Patti and Carner, 1974). But with lambdacyhalothrin and *B. t*. there is no such risks as Morris (1976) has confirmed that both are compatible. However the chemical may have adverse effects on parasites and predators in the agroecosystem. Sequential application of microbial insecticides and low doses of chemical insecticides has been a major input in the implementation of integrated control of insect pests. The expected effects of such an approach are reduced pest populations and crop damages, substantially reduced chemical hazards in the environment coupled with enhanced parasite and predator and other beneficial insect activities. The trials have confirmed that microbial formulations can be as effective as the

commonly used chemical insecticides on lepidopterous pests. There was also superior performance of sequential application of thuricide followed by lambdacyhalothrin over all other combinations. The potential application of a microbial insecticide followed by chemical insecticide should be adopted in cotton pest control programme for Tamil Nadu

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Emerging looper pests of tea crop from sub-Himalayan West Bengal, India

ABSTRACT

Caterpillars of three major geometrid species, *Hyposidra talaca*, *H. infixaria* and *Buzura suppressaria* severely defoliated tea plantations of sub-Himalayan plains of Terai and the Dooars region. The feeding activity of these pests often leads to heavy crop loss of tea bushes almost throughout the year. A clear understanding of the diversity of these sympatric species is necessary in order to contemplate their management strategies.

Field observations bear out that amongst the loopers there is a dominance of *H. talaca* and *H. infixaria* at different seasons compared to the third species, *B. suppressaria*.

Although a clear morphological difference of the adult moths of three concerned species was evident along with their distinct morphometry and weights, the larval instars of the congeners of *Hyposidra* were difficult to distinguish. However, when stadial period and morphometrics were studied a significant difference was observed in their development periods which were 55 days for *H. talaca* and 48 days for *H. infixaria*. A clear distinction of the morphometrics and weight of pupa of the concerned species was also evident.

Loopers have assumed the status of severe pest of tea in the sub-Himalayan plains in recent past mainly due to invasion of the two species of *Hyposidra* that have joined *B. suppressaria* in sharing the tea leaves as their choicest host. The newer pest spp. (*Hyposidra*) otherwise known to occur on wild forest and fruit plants has of late turned as major defoliator of tea. A preliminary bioassay of these two major folivores of tea against Cypermethrin (10% EC) revealed the LC_{50} values of *H.talaca* and *H. infixaria* which were 330.41 ppm and 107.767 ppm respectively.

Key words: *Hyposidra talaca, H. infixaria, Buzura suppressaria*, seasonal occurrence, morphology, morphometry, bio assay.

INTRODUCTION

History of tea cultivation in Darjeeling slopes is about 150 years old. Later on the tea plantation was extended to the Terai and the Dooars regions of the Himalayan foothills and plains. This crop with perennial foliage is infested by about 167 insect species in the North-eastern tea growing regions of India (Mukhopadhyay and Roy, 2009) including the Darjeeling slopes and plains. Of these, six species have attained major pest status causing 11-55% crop loss in general (Gurusubramanian et al., 2008). Among the lepidopterans attacking tea, *Buzura suppressaria* Guen was reported as a major tea pest in 1900 (Das, 1965). Initial migration of *B. suppressaria* to the tea plantations occurred from forest trees (Das, 1957). Recently two polyphagous geometrid folivores, commonly nick named "black inch worms", *Hyposidra talaca* (Walker) and *Hyposidra infixaria* Walker that are reported to feed on a number of forest plants and weeds from India, Malaysia and Thiland (Browne, 1968; Mathew et al., 2005; Winotai *et al*., 2005; Das and Mukhopadhyay, 2008; www.mothsofborneo.com) have turned to tea. The looper stage of these species have joined the band of Darjeeling Terai and the Dooars causing substantial damage to the crop (Basumajumdar and Ghosh, 2004; Das and Mukhopadhyay, 2008, 2009).

In the present work seasonal occurrence of the three looper species was studied from Terai tea plantations along with their morphological distinctions and developmental traits. As no detailed study on the distinction of the morphometrics, developmental parameters and seasonal incidence of the common and emerging looper species from Terai tea plantation is available, the present work envisages to furnish essential information on these aspects.

MATERIALS AND METHODS

Field trips were undertaken monthly during 2007-2008 to study the seasonal occurrence of *B. suppressaria* and the two species of *Hyposidra*. Sampling was done from three tea plantations of Terai. Block of the size 10 sq.mt. was sampled in replicate of three from all the selected tea plantations. Sampling was done in four seasons of a year – March-May (spring), June-August (summer), September-November (autumn) and December-February (winter). Mean relative abundance of the different looper species was calculated on the basis of six observations per season. Larvae collected from field were reared in the laboratory on tea twigs in transparent plastic containers (13 liter capacity). Male and female adults were weighed and their wing spans measured for all the three species of defoliators.

To study developmental traits of the presently dominant looper species of the Terai and the Dooars plantations, adults of *H. talaca* and *H. infixaria* emerging from laboratory cultures were released in large containers for mating and egg laying. Cotton plugs soaked in dilute honey were provided as adult feed and tissue paper strips were provided for egg laying. After hatching, larvae were maintained on tea twigs individually in plastic containers (6cm/4cm dia) with their mouths tied with fine cloths. Life cycle traits were studied during December, 2008 – February, 2009. During these months maximum temperatures (ºC, mean ± SD) ranged between 23.37±1.006 and 26.12±0.78, while the minimum temperature ranged between 9.81±1.688 and 14.90±2.48. Duration of different life stages and development period (egg-adult) were calculated. Morphometric analysis at the larval stages was based on body length and body weights 3rd instar onwards with the help of a fine electronic balance (BT 124 S, d=0.1mg, Sartorius made). Maximum thoracic width, abdominal width and length of pupae were measured for morphometric analysis along with determination of their weights.

Estimation of insecticide susceptibility

Collection of insect material:

Gravid adult females of *H. talaca* and *H. infixaria* were collected from a tea plantation situated at the central Dooars. They were brought to the laboratory and kept in plastic containers for egg laying. Hatched larvae were reared on TV 26 clone tea leaves collected from the experimental tea plot maintained organically by the Dept. of Zoology, North Bengal University up to 3rd instar stage which were then exposed to different concentrations of the pyrethroid, Cypermethrin, used in spray for chemical control of loopers in the tea plantations.

Preparation of insecticidal concentrations

In the tea plantations of sub-Himalayan West Bengal synthetic pyrethroids are mainly used to chemically control the looper caterpillars. So the present Lc study was done for Cypermethrin (10%EC).

Technical grade of Cypermethrin (10 % EC, Aventis Crop Science Ltd., UK) was used to prepare 1,000 ppm stock solution in distilled water from which further dilutions were prepared subsequently.

Determination of LC values of *H. talaca* and *H. infixaria*:

Bio-assay was performed by following the standard method recommended by Insecticide Resistance Action Committee (IRAC method No. 6) by using *H. talaca* and *H. infixaria* 3rd instar larvae (F_1 generation) from the stock culture maintained in the laboratory. Graded concentrations of Cypermethrin (50, 100, 200, 300, 400, 500, 700 ppm) were prepared in distilled water from the stock solution. TV 26 clone healthy tea shoots were collected from the experimental tea plot and used as food for the caterpillars. Plastic tubes containing tea shoots were placed in plastic containers (10cm x 6 cm). Muslin cloths were tied with the help of rubber bands on top of the containers, and were kept in culture room. Ten of the pesticide exposed caterpillars were released separately into each container containing tea shoots. Three replicates were done for each pesticide concentration. Control larvae were exposed to distilled water. Observations of larval mortality were recorded in all the three replications of each concentration 24 hours after the treatment. Moribund insects were counted as dead (Gurusubramanian and Bora, 2007). Five concentrations of cypermethrin were tested against *H. talaca* and *H.infixaria* population to obtain a concentration – probit mortality curve. The mortality data was converted to corrected percent mortality by using Abbott's formula (Abbott, 1925) and subjected to probit

analysis (Finney, 1971) to obtain LC_{50} values, LC_{95} values and a regression equation.
All the statistical analyses were performed with Tukey's multiple comparison test.

RESULTS

Studies on occurrence of looper populations in Terai tea plantations during 2007and 2008 clearly indicated the dominance of the two congeners of *Hyposidra* over the earlier known major tea looper, *B. suppressaria* (Fig. 1). Highest relative abundance of 20.98% for *B. suppressaria* was recorded during March-May. *Hyposidra* spp. were found prominently even during the winter months due to lack of obligatory winter diapauses with at least eight broods per year. Generally four broods of *B. suppressaria* were recorded per year (Das, 1965). Prolonged winter diapause of *B. suppressaria* as sub-soil pupae was evident through it's almost 0% occurrence of looper stage during December-February (Fig.1). The newer pest spp. (*Hyposidra*) otherwise known to occur on wild forest and fruit plants have of late emerged as major defoliator of tea consuming leaf at a rate of almost 25 sq. cm. per day at early 5th instar stage. As the occurrence of these loopers in peak season was about 200 individuals per bush or more, the quantum of crop loss appeared to be substantial. Besides these three looper species, few looper of genus *Ascotis* and *Cleora* were also recorded from the Terai tea plantations but could not be considered to be of pest status as major pests.

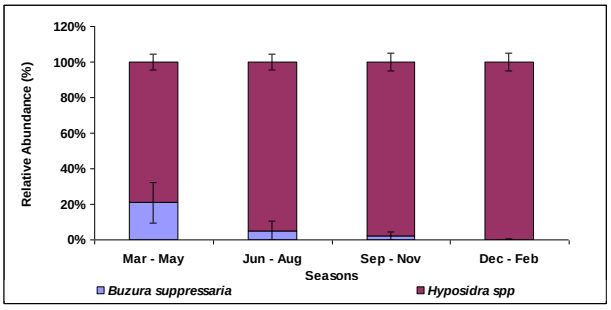

Fig.1. Seasonal occurrence of *Buzura suppressaria* and two species of *Hyposidra*, *H. talaca* and *H. infixaria.*

Adult *B. suppressaria* were morphologically very distinct from the two species of *Hyposidra* (Table I, Fig.2), so were its larval (Fig. 2) and pupal stages. Larval (looper) stages of *B. suppressaria* were clearly distinguishable with their green to brown body colour, triangular head, prominent red spiracles, large body length (6.4 cm ± 0.089) of final larval instar and higher weights (1.674gm.±0.064). Pupae were also with distinctly greater length (2.32cm ±0 .050) and weight (0.876gm ± 0.055) having a characteristic pair of anterior ridges and posterior cremaster like process. These characters were absent in the pupae of the two species of *Hyposidra*.

Table I: Adult morphology and measurements of three looper species. (Mean±SE, N=20)

		B. suppressaria	*H talaca*	*H infixaria*
Wing morphology	F	Margin even with series of yellow spots. Wings grey. Finely speckled with black and with a yellow, wavy antimedial band on the fore wing.	A distinct sub-marginal notch present on the forewing. Grayish to blackish brown with diffused and wavy dark patches.	Sub marginal notch not prominent. Margin more crenulate. Colour is as H. talaca on which sharp and dark lines present.
	M	Wing margin not fringed. Differs from female in wing span, which is much shorter.	Sub-costal dark line on forewings and sub-marginal series of dark spots absent.	Longitudinal sub-costal dark line on fore wings. Sub-marginal series of dark spots on meso- and meta-thoracic pair of wings in both sexes.
Wing span (cm) (Mean ±SE)	F	6.24 ±0.23 Aa	5.208 ± 0.089 Ab	5.00 ±0.084 Ab
	M	4.97 ±0.117 Ba	3.65 ± 0.043 Bb	3.67 ±0.057 Bb
Body weight (gm) (Mean ±SE)	F	0.712 ±0.030 Aa	0.218 ± 0.018 Ab	0.167 ± 0.007 Ac
	M	0.252 ± .013 Ba	0.072 ± 0.005 Bb	0.048 ± 0.002 Bc

Different capital alphabets in each column and different small alphabets in each row indicate significant difference when $p \leq 0.05$.


Fig2.A.i


Fig.2.A.ii


Fig.2.B.i


Fig.2.B.ii


Fig.2.C.i.


Fig.2.C.ii.

Fig. 2. A.i.& A.ii.: *B.suppressaria* adult (F) & final larval instar; B.i. & B.ii.; *H. talaca* adult (F) & final larval instar and C.i. & C.ii. *H. infixaria* adult (F) & final larval instar.
Note: Black arrows indicate characteristic features of adults and white arrows that of larvae.

A detailed life cycle along with developmental traits had been studied in details for the similar looking

congeners, *H. talaca* and *H. infixaria* during Dec. 2008 – Feb. 2009. The post embryonic development periods of the two species were similar till 4th instar stage. However, significant difference could be noted in the duration of final larval instar (5th) and pupa (Table II). *H. talaca* exhibited a development period that was longer by eight days on an average than *H. infixaria*. Shorter development period of *H. infixaria* might be resulting in more number of broods during winter months than that of *H. talaca*.

Table II. Duration (days) of different developmental stages of *H. talaca* and *H. infixaria* (Mean ± SE, N=20)

Life stages	*Hyposidra talaca*	*Hyposidra infixaria*
Egg	6±0 a	8±0 b
1st instar	4.64 ± 0.125 a	5.44 ± 0.114 a
2nd instar	3.25 ± 0.116 a	3.80 ± 0.092 a
3rd instar	3.80 ± 0.141 a	3.96 ± 0.080 a
4th instar	4.41 ± 0.150 a	4.14 ± 0.105 a
5th instar	11.58 ± 0.335 a	10.38 ± 0.165 b
Pupa	18.94 ± 0.300 a	11.14 ± 0.203 b
Total development period	55.00 ± 0.28 a	47.82 ± 0.163 b

Different small alphabets in each row indicate significant difference when p≤ 0.05.

Generally five larval instars were recorded for the two species of *Hyposidra* under the study conditions. However, a small percentage of loopers (8% of *H. talaca*) resorted to an additional (6th) instar. This might be due to the fact that the decision to pupate often depends on attainment of some minimal body weight (Slansky, 1982). In fact, the larvae which entered 6th instar as final larval stage weighed significantly less in 5th instar (40.6mg ± 0.859) entered 6th instar as final larval stage, as compared to those with heavier 5th instar as final larval stage (116mg ±8.27 for females and 93mg±4.249 for males). However, total development period didn't vary significantly among these two groups.

When body length was considered almost a similar growth pattern was recorded in the larvae (Table III). However, advanced female (F) and male (M) fifth instar larvae of both the species of *Hyposidra* differed significantly in their lengths, for *H. talaca* the measurements being 4.344 cm ± 0.080 (F) and 3.657cm ± 0.060 (M), whereas for *H. infixaria* these were 4.23cm ± 0.079 (F) and 3.64cm ± 0.084 (M).

Table III: Growth parameters of *H. talaca* and *H. infixaria* at different developmental stages. (Mean±SE, N=20)

Developmental stages		Bodylength (cm)		Bodyweight (mg)	
		H talaca	*H infixaria*	*H. talaca*	*H. infixaria*
1st instar		0.2	0.2	----	----
2nd instar		0.43 ±0.008	0.39 ± 0.006	----	----
3rd instar		0.73 ± 0.017	0.77 ± 0.018	2.92 ±0.180	2.43 ± 0.173
4th instar		1.325 ± .023	1.290 ±0.027	20.1 ± 0.894	18.00 ± 0.671
5th (0d) instar	Female	2.411 ± 0.110	2.341± 0.085	116.0 ± 8.273Aa	93.0 ± 4.249Ab
	Male	2.250 ±0.098	2.112 ±0.072	80.0 ±4.226 Ba	69.0 ± 2.460Bb

Different capital alphabets in each column and different small alphabets in each row indicate significant difference when p≤ 0.05.

A marked difference in the measurement and morphology at the pupal stage could be recorded between the two species of *Hyposidra* (Table IV).

Table IV: Comparison of pupal morphology and morphometry between *H. talaca* and *H.infixaria*. (N=20, Mean±SE)

		Htalaca	*H.infixaria*
Morphological difference		Wider. Process at the abdominal end either single or bifurcated. Single process may or may not be forked; bifurcated ones are not forked. Apex of both types of processes never recurved.	Slender. Process may be either single or bifurcated. The apex of the processes in both types forked and recurved.
Thoracic width/ pupal length	F	0.33 ± 0.004 a	0.30 ±0.003 b
	M	0.34 ± 0.004 a	0.29 ±0.003 b
Abdominal width/ pupal length	F	0.26 ± 0.004 a	0.25 ±0.003 b
	M	0.27 ± 0.004 a	0.23 ±0.005 b
Weight (mg)	F	327 ±11 Aa	240 ±8 Ab
	M	72 ± 5 Ba	48 ±2 Bb

Different capital alphabets in each column and different small alphabets in each row indicate significant difference when p≤ 0.05.

Observation of the pupae and adult also showed a clear dimorphism, female pupae had a higher body length than the males (Table IV) and female adults had much wider wings (Table I).

Morphologically the early larval stages of the two species of *Hyposidra* were similar having black body with white stripes which imparted them the common name "black inch worm". However, final instar of *H. infixaria* is characterized by possessing paired lateral oblique black stripes extending from 1st abdominal segment to 3rd pair of thoracic legs which appear blackish than first two pair of legs (Fig. 2B and 2C).

The trends of development in terms of body weight between the two species showed by and large insignificant difference till 4th instar. But the two species were significantly different in their body weights at 5th instar larval, pupal and adult stages (Table III, IV and I).

Among the two looper species, *H. talaca* showed about three times higher Lc_{50} value and about two times higher Lc_{95} value than *H. infixaria* (Table V). In both the species the Lc_{95} value is much higher than the recommended field dose of Cypermethrin for tea loopers which is 500 ppm (Gurusubramanian et al. 2008).

Table V. Bioassay of *H. talaca* and *H. infixaria* against Cypermethrin 10% EC

Looper species	Lc_{50} (ppm) (lower limit - Upper limit)	Lc_{95} (ppm)	Regression equation	Chi^2	SE
H. talaca	330.41 (258.27-422.69)	2195.51	Y=2.0127x - 6.108	2.06	0.00133
H. infixaria	107.767 (77.369-150.109)	1393.19	Y=1.4893x - 2.4949	1.01	0.00241

DISCUSSION

B. suppressaria, long known from tea plantations of Assam and Darjeeling foothills, has distinct larval and adult morphology from the other two emerging species of tea loopers. Similar morphological features, lengths and body weights of the early larval stages of the two congeneric species of *Hyposidra* implied that they are close to one another. This is further supported by the observations of their common sharing of the same niche (young tea leaves) compared to the feeding on more mature tea leaves by *B. suppressaria*. So this species stands out ecologically as a more distinct one than the two looper species of the genus *Hyposidra*. Problem associated with the distinction of early insters of the two species of *Hyposidra* possibly indicated that the two species are very close. Eyles (1963) reported similar problem in morphologically and morphometrically distinguishing the nymphs of several species of *Scolopostethus* (Heteroptera: Lygaeidae) in the field. Almost a parallel example of this paradoxical situation was also noted in the immature stages of the two species of *Rhyparothesus*, *R. sparsus* and *R. bengalensis* that shared the same ecological niche wherein the first four instars showed very close similarities, even overlapping morphometrics (Mukhopadhyay, 1989).

In the present condition and time a clear abundance of the *Hyposidra* spp. as compared to *B. suppressaria* in Terai and the Dooars region of Himalayan foothills might be the result of a better adaptation of the former spp. to tea bushes. The better adaptive strategy was evident through their faster growth, shorter life cycles and multivoltinism. Moreover a continued population incidence with succession of the two *Hyposidra* species over most part of the year, especially during winter signified their adaptation to tropical weather changes and tolerance to the difference in temperature and humidity. Bioassay of *H. talaca* and *H. infixaria* also indicates their increased tolerance towards synthetic pyrethroids such as Cypermethrin. Further scientific studies are required to reveal the basis of development of such tolerance, especially the higher tolerance of the insecticide by *H. talaca*. Along with these factors, their reported polyphagy which includes many jungle plants and weeds of varied plant families (Das and Mukhopodhyay, 2008) may also help them the to develop better tolerance and survival capability as compared to other oligophagous loopers such as *B. suppressaria* that are mostly confined to the shade trees and tea.

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Research in Resistance Management

Laboratory and field evaluation of emamectin benzoate and spinetoram on cotton leafworm larvae

ABSTRACT
Emamectin benzoate and spinetoram are two important and promising new insecticides in caterpillar lepidoptera larvae/larvae control. The toxicity of these compounds against larvae of cotton leafworm (CLW), *Spodoptera littoralis* (Boisd.) was compared using topical application and feeding techniques. The persistence/residual efficacy under field conditions was also investigated. Based on the LD_{50} values against 4th instar larvae, emamectin benzoate proved to be better than spinetoram by 31516 fold. The LD_{50} values of emamectin benzoate, cypermethrin, methomyl, chlorpyrifos, pyriproxyfen, profenofos, chlorpyrifos-methyl, abamectin, spinetoram, spinosad and imidacloprid were 0.0019, 0.0039, 0.03, 1.62, 3.13, 3.38, 4.00, 9.38, 59.88, 558.25 and 37384.38 µg a.i/ g larvae, respectively. The 3rd instar larvae showed higher susceptibility toward emamectin benzoate than spinetoram by 1551 and 41 times after 2 days post-treatment using cotton and castor bean, respectively. Two days post- treatment, the persistence of emamectin benzoate decreased gradually and significantly. After spraying cotton, the mortality percentages were 98.00, 70.00, 36.67 and 0.00 % after 0, 3, 6 and 10 days, respectively. However, the mortality % of this insecticide in castor was 91.11, 93.33 and 0.00 % after 0, 3 and 6 days respectively. In spinetoram, these percentages in cotton and castor were 96.67, 3.33, 3.33 and 0.00, 2.86, 0.00 after 0, 3 and 6 days. Our investigation recommended that emamectin benzoate is one of the best bio-insecticides in controlling CLW larvae infestations in cotton fields.

Keywords: Laboratory Bioassays, Emamectin Benzoate, Spinetoram, Field Application, Cotton Leafworm, Host Plant.

INTRODUCTION

The insecticide market has been dominated by conventional classes of insecticides. Recently, a number of new insecticide classes have been discovered and commercialized. Emamectin benzoate (Proclaim) is a modified isolation of the soil microorganism, *Streptomyces avermitilis*. Emamectin affects the nervous system of arthropods by increasing chloride ion flux at the neuromuscular junction, resulting in cessation of feeding and irreversible paralysis. It affects on GABA and glutamate-gated chloride channel agonist (Dunbar et al. 1998).

The compound has activity on various lepidopteran pests. It has translaminar activity, providing thereby a relatively prolonged residual activity (Ishaaya et al. 2002).

Spinetoram, a new semi-synthetic spinosyn insecticide developed by Dow AgroSciences (Indianapolis, IN) that was accepted for expedited review under the United States Environmental

Protection Agency's Reduced Risk Pesticide Program. Spinetoram is derived from fermentation products of the soil bacterium *Saccharopolyspora spinosa* Mertz and Yao, has a high safety profile, and may have relatively long residual effects (Yee et al, 2007).

The Egyptian CLW, *S. littoralis* is one of the most notorious and destructive phytophagous insect pests in Egypt, not only on cotton but also on most field crops and vegetables (Kandil et al. 2003). These caterpillars are very polyphagous, causing important economic losses in both greenhouses and open field on a broad range of ornamental, industrial and vegetable crops. In many cases, populations have acquired resistance towards most of insecticide groups (Alford, 2000).

Our objectives are to compare the toxicity of two biorational insecticides, emamectin benzoate and spinetoram under laboratory and field conditions. Results are discussed with respect to residual toxicities of these materials and their potential use in the management of *S. littoralis* in Egypt.

MATERIALS AND METHODS

Chemicals

Emamectin Benzoate (Proclaim®, SG 5%, Syngenta Co.) and Abamectin (Romacten®, EC 1.8%, Rotam Agrochemicals Co.) were provided from the commercial pesticide market in Egypt. Spinetoram (Radiant®, SC 12%, Dow AgroSciences Co.), Spinosad (Success®, SC 24%, Dow AgroSciences Co.), Cypermethrin (polythrin®, EC 20%), Chlorpyrifos (Dursban®, EC 48%) and Chlorpyrifos-methyl (Reldan®, EC 50%), Dow AgroSciences Co. were supplied by the scientific coordinator of Dow AgroSciences Co. in Egypt. Methomyl (Lannate®, SP 90%, DuPont Agricultural Co.), Profenofos (Selecron®, EC 72%, Ciba-Geigy Co.) and Imidacloprid (Admire®, FL 24%, Bayer Crop Science Inc.) were supplied from Egypt Ministry of Agriculture. The surfactant Triton $_{X-100}$ (100% purity, BDH Chem, Ltd. Poole England) were imported from England by local chemical company.

Insects

The used strain was established from mass eggs that produced from one pair of adults of susceptible cotton leafworm that were already kept in our lab away from insecticidal contamination for more than seven years. The larvae were reared on fresh leaves of castor bean, *Ricinus communis* as described by Eldefrawi et al 1964. The strain was rearing under laboratory conditions for two generations before starting the experiments.

Laboratory bioassay tests

In the topical application, based on weight/volume, the tested insecticides were dissolved in acetone as solvent except spinosad and spinetoram which were dissolved in dichloromethane. Fourth instar larvae of CLW at an average weight of 38-40mg / larva were prepared. Half microliter of a solvent solution of each insecticide was applied to second thorax of each larvae using electrically operated ISCO Micro-applicator equipment model 131, fitted with a one ml Agla all-glass syringe having a curved, blunt hypodermic needle (20-gauge).

In the feeding bioassay, third instar larvae of CLW at an average weight of 10.3-16.0 mg / larva were prepared. Serial concentrations of emamectin benzoate and spinetoram were prepared using triton$_{x-100}$(0.05%) as detergent and tap drinkable water as solvent. Parts of cotton or castor bean leaves were dipped in the tested concentration for 5 seconds and then transferred to Petri-dishes containing filter papers and the treated leaves for half an hour to dry. The selected larvae were fed on dried treated castor bean leaves for 24 hrs. Then the larvae were allowed to feed on untreated fresh castor bean leaves for 24 hrs.

For both bioassays, 4-5 serial concentrations of insecticides were used with three replicates, using 10 larvae in each replicate. All treatments were incubated at 26±2 temperature and 12:12 L: D and 65± 5 RH till recording of the results. The mortality data were recorded after 2 days post topical application; however it was counted after 2 and 6 days after treatment in feeding bioassay. The larva was considered dead if no movement was detected when it was touched with a small brush. The toxicity of each insecticide was replicated 2 times.

Residual activity of insecticides on *S. littoralis* under field conditions

The experiments were conducted at Assiut University Farm, Assiut, Egypt. The cotton, *Gossypium hirsutum* cv. Giza 90 was cultivated on March 23, 2008. The normal agriculture practices were applied. The Experimental area was divided into plots (21 m^2). The treatment was arranged in randomized complete block design (RCBD) with three replicates. Application of insecticides was done on August, 6, 2008 using knapsack sprayer. The volume of spray solution was 476 liter/ha. Emamectin benzoate and spinetoram were applied at recommendation rate 2.86 g a.i / ha and 12.86 g a.i / ha, respectively. We used tap water in dilutions and triton $_{X-100}$ by rate 0.05 % as detergent.

With the same spraying procedures, emamectin benzoate and spinetoram were applied on randomized castor trees that were almost the same age and that already were cultivated around the Assiut Farm on November, 23, 2008.

The sprayed leaves of cotton or castor were collected after zero, 3, 6 and 10 days after treatment. They were transferred directly to the laboratory for feeding the tested larvae for 24 hours, and then replaced by untreated leaves for the same duration. The mortality was determined 48 hr post treatment. Three replicates were used in every treatment, with using 10 third instar larvae per replicate.

Statistical analysis

Mortality percentages were corrected by Abbot's formula (Abbot, 1925). The LC_{50} and slope values were determined by a computerized probit analysis program. Means ± SEM were analysed by one-way ANOVA and separated by a Tukey HSD- Post Hoc test (P= 0.01) using SPSS 16 software for Windows.

RESULTS

The data in table 1 cleared that the most effective tested insecticide was emamectin benzoate (LD_{50} = 0.0019 µg/g of larval body weight), while imidacloprid showed the lowest one (LD_{50} = 37384.38 µg/g of larval body weight) against 4th instar larvae using topical application. Emamectin benzoate was 19675989.47 fold more potent than imidacloprid. It was 4937 fold more toxic than its analogue abamectin. The toxicity order of all tested compounds was as follow; Emamectin benzoate > cypermethrin > methomyl > chlorpyrifos > pyriproxyfen > profenofos > chlorpyrifos-methyl > abamectin > spinetoram >spinosad > imidacloprid.

Table 1: Toxicity of eleven inseticides against 4th instar larvae of the susceptible strain of the cotton leafworm, *S. littoralis* using topical application.

S.	Insecticide	LD_{50} [a]	Slope	Toxicity index[b]
	Conventional insecticides			
1	Chlorpyrifos	1.62	1.07	852.63
2	Chlorpyrifos methyl	4.00	1.71	2105.26
3	Cypermethrin	0.0039	1.56	2.05
4	Methomyl	0.03	0.41	15.79
5	Profenofos	3.38	1.39	1778.95
	Bio-insecticides			
6	Abamectin	9.38	1.33	4936.84
7	Emamectin benzoate	0.0019	0.79	1.00
8	Spinetoram	59.88	0.99	31515.79
9	Spinosad	558.25	0.03	293815.79
	Neonicotinoides			
10	Imidacloprid	37384.38	0.08	19675989.47
	Juvenile hormone analogues			
11	Pyriproxyfen	3.13	0.64	1647.37

[a] ; µg a.i/ g larvae

[b] ; Toxicity Index, LD_{50} of tested insecticide / LD_{50} of emamectin benzoate

The slope values of the regression lines so far differed. The least slope was 0.03 with spinosad while the highest one was 1.71 with chlorpyrifos methyl (table1).

Using cotton in feeding bioassay against 3rd instar larvae of CLW, the LC_{50} values for emamectin benzoate after 2 and 6 days post treatment were 0.14 and 0.14 ppm, respectively. However, these values after 2 days with spinetoram were 217.17 ppm. In castor bean as food, the LC_{50} values for emamectin benzoate in the same tested period were 4.33 and 0.16 ppm but the values were 180.00 and 180.00 ppm for spinetoram. The toxicity index (TI) showed that emamectin benzoate was more toxic toward CLW when introduced in cotton food than castor bean food by about 32 fold. However in spinetoram the results could be the same, the TI is 1.14(table2).

Table 2. Toxicity of two biorational bioinsecticides against 3rd instar larvae of susceptible strain of cotton leafworm, *S. littoralis* after 2 and 6 days post treatment using feeding larval method.

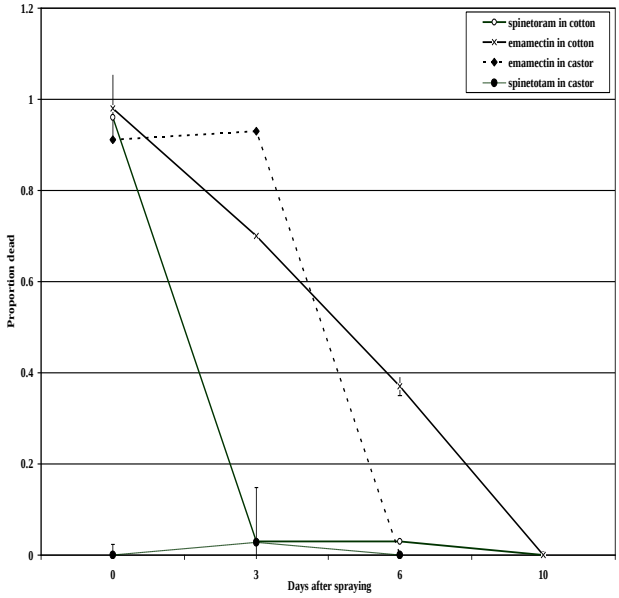
Host plant	Toxicity parameters	Emamectin benzoate		Spinetoram	
	Time	2days	6 days	2days	6 days
Cotton	LC_{50}	0.14	0.14	217.17	(-)
	Slope	1.89	1.89	2.66	(-)
Castor bean	LC_{50}	4.33	0.16	180.00	180.00
	Slope	1.00	1.74	1.93	1.93
Toxicity index[a]		30.93	1.14	3.83	(-)

[a]Toxicity Index, LC_{50} of the tested insecticide in castor / LC_{50} of the same insecticide in cotton

(-) not determined

The data in Figures 1 shows significantly decreased mean of mortality percentage were caused by emamectin benzoate post spraying. The mortality percentage values were 98.00, 70.00, 36.67, and 0.00 % for larvae that fed on cotton leaves after 0, 3, 6 and 10 days post spraying. These percentages decreased when feeding on castor leaves after 0, 3 and 6 days post spraying .These mortality % were 91.11, 93.33 and 0.00 %. Using the same procedures, the mortality results for spinetoram were 96.00, 3.33, 3.33 and 0.00 % of larvae that were treated by cotton leaves. However, with the castor leaves these percentages recorded 0.00, 2.86 and 0.00 %.

Fig 1 Residual activity of spinetoram and emamectin benzoate on cotton and castor bean against 3rd instar larvae of *S. littoral* under semifield conditions using feeding larval bioassay, the data corrected by Abbott Formula



DISCUSSION

The presented results showed that emamectin benzoate had the best toxicity profile in all tested bioassays. These results coincided with other studies (Scarpellini 2001; Argentine et al. 2002; Zhao et al. 2004; Gupta et al. 2005; Abdu-Allah 2007; Ezz El-Din et al. 2009).

The high flattening of slope indicates that the insect tested strain was relatively heterogenous in its susceptibility toward tested insecticides by topical application method. This finding may be explained by expecting that there are at least two susceptible genotypes which are usually phenotypically different because complete dominance or complete receptivity is unusual (Tsukamoto 1983).

Emamectin benzoate shows more persistence than spinetoram under field condition in our studies. Also, it shows more stability in cotton than castor bean. This may be explained by the notion that cotton may be preferred as food by larvae as compared to castor or may be the cotton plant adsorbs these tested pesticides more than castor bean. These results conformed with other investigations (Ishaaya et al. 2002; Argentine et al. 2002). Iqbal et al. 1996 found that residual activity of abamectin was less on common cabbage compared with Chinese cabbage. The reduced toxicity of abamectin on Chinese cabbage is almost certainly due to its lack of availability to non-phytophagous arthropods. This results from its adsorption into epicuticular waxes and deeper leaf tissues, and to the rapid photodegradation of the remaining surface residue as observed on the other crop plants. Baranowski 1991 stated that avermectin is a natural product which produced reservoir of long-lasting activity within the leaf.

The short residual effect of spinetoram in this study is compatible with the results reported by Elbarky et al.2008 who found that spinetoram has short residual time in cotton under field condition. The persistence of pesticides depends on many factors: the type of pesticide, pesticide physiochemical properties, the dose used, the host plant, and environmental factors. Many pesticides are significantly more persistent on kiwifruit than on other fruit species (Holland et al.1984). This also appears to apply to spinosyns with half-lives 10-20 days found on kiwifruit foliage compared to the several days reported on the surfaces on the other plant species (McDonald et al. 1998). The corresponding duration of residual activity is also considerably longer than has been reported with spinosad for other insect species on different crops.ie.7 days for beet armyworm, *Spodoptera exigua* on cotton (McDonald et al.1998).

In conclusion, emamectin benzoate was better than spinetoram in laboratory and field applications against larvae of CLW in all tested bioassays. Also this insecticide has better results in cotton and castor bean host plant than spinetoram. Spinetoram showed better results than its analogue spinosad about ten times in topically and feeding bioassays in lab. However no persistence of spinetoram was observed in castor bean. Emamectin benzoate keeps persistence under field condition. Further experiments are needed to explain why there is no persistence of spinetoram in castor bean under field conditions.

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Action of high-molecular weigh chitin derivatives on agglutinative activity of *Leptinotarsa decemlineata* haemolymph

ABSTRACT
Influence of chitin derivatives – chitosan and succinate of chitosan – on a complex of hemagglutinins of *L. decemlineata* larvae is investigated. It is shown that action of these polysaccharides causes synthesis of additional agglutinins with new carbohydrate specificity. Succinate of chitosan finds out more expressed immunopotentiating activity concerning L. decemlineata, inducing more quantity of agglutinins with wider spectrum of carbohydrate specificity.

INTRODUCTION

Immunomodulatory and immunopotentiating properties of chitinous derivatives are shown on various groups of animal and vegetative organisms. Induction of plants resistance by means of elisitors, including chitinous derivatives, is considered as alternative to application of pesticides. At the same time it is shown that products of a catabolism of chitin can carry out important regulatory functions also in an organism of insects. So, chitinous derivatives raise level of humoral immunity of insects, causing an expression of genes of antibacterial peptides (Furucawa, Taniani, 1999), raise survival rate of a honeybee at action of adverse factors (Saltykova, etc., 2001), participate in formation of the long-term immune answer of a typhoid fly (Gaifullina et al., 2010). The chitin monomer – NAGA - initiates at *L. decemlineata* protective reactions of phenoloxidase system, characteristic for the anti-infectious answer (Gaifullina et al., 2007). Chitin derivatives are supposed to simulate components of cellular walls of bacteria and by that as signal molecules stimulate immune processes of insects. Processes of immunorecognition at insects are carried out by a complex of glycoproteins – agglutinins – together with an integrated link of protective reactions - phenoloxidase system. Therefore at studying of mechanisms of immunomodulatory action of chitinous derivatives on L. decemlineata, in our opinion, it is expedient to consider as targets recognizing immune proteins – agglutinins.

MATERIALS AND METHODS

Insects. L. decemlineata larvae were collected from potato fields and reared in glasses with a volume of 0.5 dm^3. Insects in experimental variants one day prior to the analysis were fed with potato foliage moistened with 0.01% water suspension of chitosan (.) or 0.01% water solution of succinate of chitosan (M=330 кДа, deacetylation degree 95 %).

Hemagglutination assays. Agglutination activity was determined using twofold serial dilutions of larval haemolymph in U-bottomed wells in microtitrator plates with trypsin-treated human erythrocytes (Stynen et al., 1982). Human blood of group O^- has been given by the laboratory of Republican clinical hospital. Presence and agglutination degree was considered visually by registration of spontaneously dropping out flakes with an estimation from four pluses (full agglutination) to a minus (absence of agglutination). The greatest dilution of a haemolymph, at which else there was an agglutination, was accepted for titre of agglutinins. Saccharine specificity was determined using D(-)galactose, D(-)fructose, D(+)ramnose, D(-)mannose, L(+)arabinose, N-acetil-D-glucosamine (NaGlu), N-acetil-D-galactosamine (NaGal), lactose, maltose, saccharose, chitooligosaccharides (COS) (oligomers included fifteen monomers), heparin, gyaluronic acid (GUA), mucin and succinate of chitosan. The minimum concentration oppressing reaction of agglutination was determined using twofold serial dilutions of carbohydrates in phosphate-buffered saline after incubation with a solution of agglutinins of constant concentration. Initial concentration of mono- and disaccharides was 1M, and polysaccharides - 1%. The least concentration of carbohydrates, at which else was absent agglutination, was accepted for minimum inhibitory concentration of carbohydrates.

RESULTS AND DISCUSSION

Daily influence of chitinous compounds increased the titre of agglutinins in *haemolymph L. decemlineata* twice in a case with chitosan and four times in a case with succinate of chitosan (Table 1). D(-)galactose, D(-)fructose, D(+)ramnose, D(-)mannose, L(+)arabinose, D(+)lactose, D(+)maltose and saccharose weren't inhibit agglutination reaction in all variants. In norm agglutination was inhibited by mucin, heparin, GUA and COS that corresponds to earlier received data (Stynen et al., 1982; Gayfullina et al., 2005) (Tab. 2). Minimum inhibitory concentration of COS decreased twice at action of chitosan and succinate of chitosan. Besides, after the action on larvae of these carbohydrates the agglutination was inhibited by succinate of chitosan. Succinate of chitosan also induced in L. decemlineata haemolymph the agglutinins, capable to get combined with NaGlu and NaGal.

Table 1: Titres of agglutinins in L. decemlineata haemolymph under the chitosan and succinate of chitosan action

Variant	Titres of agglutinins								
	128	256	512	1024	2048	4096	8192	16384	32768
Control	3+	3+	2+	2+	+	+	-	-	-
Chitosan	4+	4+	4+	3+	3+	2+	+	-	-
Succinate of chitosan	4+	4+	4+	4+	3+	3+	2+	+	-

Table 2: Inhibition of the agglutination of human erythrocytes by carbohydrates

Carbohydrate	Minimum inhibitory concentration of carbohydrate		
	control	chitosan	succinate of chitosan
NaGal	-	-	0.5M
NaGlu	-	-	0.25M
COS	0.125%	0.0625%	0.0625%
GUA	0.250%	0.250%	0.250%
heparin	0.125%	0.125%	0.125%
mucin	0.0625%	0.0625%	0.0625%
succinate of chitosan	-	0.5%	0.5%

So, high-molecular weigh derivatives of chitin – chitosan and succinate of chitosan - induce in a haemolymph of *L. decemlineata* larvae synthesis of additional agglutinins, as well as in a case with development of an intestinal infection (Gayfullina et al., 2005). Chitosan and succinate of chitosan in an invariable kind, and also products of their degradation can be soaked up in intestines of insects, get to a haemolymph and induce agglutinating activity, both by increasing of agglutinin synthesis, and activation of inactive forms of glycoproteins. Agglutinins can be synthesized by haemocytes after direct contact with antigen (Glupov, 1998), and also by cells of a fatty body at regulation of haemocyte mediators and hormones (Kobayashi, 1998). Thus, chitosan and succinate of chitosan can induce both haemocyte agglutinins, performing the functions of receptors, and plasma opsonins.

Stimulation of immune factors by chitinous derivatives, most likely, is the widespread phenomenon for insects. So, at a honeybee chitosan raises an expression of genes of antimicrobic peptides, stimulates cellular reactions of a haemolymph and causes activity change of diphenoloxidase and antioxidizing enzymes, similar to the nonspecific answer of humoral system of insects to introduction of pathogen (Gaifullina et al., 2010). Such property of chitinous derivatives can be connected that they are similar on structure with lipopolysaccharides of microorganisms' cellular walls, playing a role of immunity activators. It is remarkable that chitosan and succinate of chitosan induce wider number of specific agglutinins, rather than *Bacillus thuringiensis* which induces the agglutinins specific only to COS and heparin (Gayfullina et al., 2005). At the same time, succinate of chitosan finds out more expressed immunopotentiating action, than chitosan, inducing more quantity of agglutinins with wider spectrum of carbohydrate specificity. Possibly, such distinction is connected with different solubility of these compounds. High molecular weigh chitosan isn't dissolved in water whereas succinate of chitosan thanking succinate anion is dissolved in water and is more active. Besides, succinic acid is not only an energy substratum, but also a regulator of functions of live systems (Kondrashova, 2002) that causes application possibility of succinate-containing substances for increase of nonspecific resistance of an organism to extreme influences.

It is necessary to mention that chitin olygomers change speed of *L. decemlineata* ontogenetic processes and, hence, the hormonal status (Ben'kowskaya et al., 2001). At the same time, agglutinins participate to a metamorphosis of holometabolous insects in processes of opsonization of disintegrated tissues and cellular adhesion during histogenesis (Komano et al., 1981; Natori et al., 1999) that defines morphogenetic processes of insects as a version of protective process. Thus, it is possible to assume that chitinous derivatives induce hormone-regulated synthesis of the specific agglutinins participating in immune and morphogenetic processes.

CONCLUSION

High molecular weigh chitinous derivatives – chitosan and succinate of chitosan – with various intensities induce in an organism of *L. decemlineata* larvae immunorecognition factors. Action of chitinous derivatives on insects can be caused by the mechanism of so-called "preventive" activation of immune factors. Possibly, in insects this immune-activation is accompanied with the changes of endocrine-metabolic status providing new adaptive possibilities of an organism.

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Baseline Susceptibility Studies of Rynaxypyr 20 SC against Okra Fruit Borer *Helicoverpa armigera* Hubner

ABSTRACT
Studies on the susceptibility of third instar larvae of okra fruit borer *Helicoverpa armigera* (Hubner) to rynaxypyr 20 SC was determined by topical bioassay technique using potter's tower under laboratory conditions at University of Agricultural Sciences, Raichur during 2009-2010. The results revealed that, susceptibility of *H. armigera* increased after five generations in insecticide free exposure culturing, the susceptibility index for LC_{50} was 0.66.
KEYWORDS: Rynaxypyr, Baseline susceptibility, LC_{50}, *H. armigera*, Okra.

INTRODUCTION

Helicoverpa armigera Hubner is one of the most destructive polyphagous pests causing severe damage to a variety of economically important crops like cotton, chickpea, pigeonpea, oilseeds, millets, vegetables etc. and has been reported to be widely distributed through out the world. Till recently, synthetic chemical insecticides have been the major weapon for controlling this pest in agriculture and accounting for the consumption of over 30 per cent of the total insecticides all over the world. It is estimated that more than 50 per cent of applied insecticides reach the soil and water bodies during application (Awasthi *et al*., 2002). However, their extensive and indiscriminate use has resulted in development of resistance in *H. armigera* populations to various insecticides in different states of India. In order to overcome this complex problem, there is need to monitor susceptibility status of this pest on priority basis, which is widely recognized and emphasized. Hence, in management of pests with chemical insecticides, resistance has often been a problem and one of the most important reasons why insecticides with a new mode of action have been desired. One such new group is anthranilic diamide; rynaxypyr discovered as a novel class of insecticide having a unique chemical structure and showed excellent activity against broad spectrum of lepidopterous pests. DuPont India ltd has developed a newer insecticide formulation, rynaxypyr 20 SC for use against lepidopteran insect pests. With the above background, research work was carried out to develop baseline susceptibility data of rynaxypyr 20 SC against okra fruit borer *H. armigera*.

MATERIALS AND METHODS

The susceptibility status of *H.armigera* to rynaxypyr 20SC was determined in laboratory by bioassay technique. The dilutions required for the insecticidal assays prepared from the formulation of insecticide of known purity diluted with distilled water using serial dilution technique. The third instar larvae (excluding moulting larvae) reared on artificial diet in the laboratory were used for treatment. Before the treatment the larvae were weighed on an electronic balance and confirmed for weight range between 30 to 40 mg. A batch of ten larvae was taken in separate

glass petridish (9 cm dia) for each test concentration of insecticide separately. One ml solution of each concentration was sprayed directly on test larvae kept in the petridish with the help of potter's tower at 340g/ cm sq atmospheric pressure and dried for ten minutes (Dhawan *et al.* 2007) and incubated at temperature of 26 ± 1°. Each treatment was replicated thrice. Two minutes after spraying, the individual larva was transferred to a multicavity tray containing fresh artificial diet to avoid cannibalism. Simultaneously, the larvae subjected to distilled water spray were kept as control. Mortality was assessed after 24, 36, 48 and 72 hrs after treatment. A larva was considered dead if it was unable to move in a coordinated manner when prodded with a blunt needle otherwise considered as alive.

Pure culture of *H. armigera* was used to carry preliminary range finding tests that were conducted to attain suitable series of doses which gives a range of mortality from 10 to 100 per cent. Sufficient replicates (30 larvae/treatment) were used to provide a reliable regression line known as log dose probit mortality (ldpm) or log concentration probit mortality (lcpm) line for F1 generation/ field collected population. To generate the base line data, another set of F1 generation pure culture larvae which were not exposed to insecticides were cultured continuously without any selection pressure throughout the generations up to the sixth generation (F6). Bioassays were conducted based on the dosages obtained from preliminary range finding test to construct ldpm line for susceptible population known as base line data.

Detection of susceptibility index

Susceptibility indices were calculated based on LC_{50} and LC_{95} values obtained for the F1 and F6 generations (Regupathy and Dhamu, 2001) of the laboratory populations maintained without insecticidal exposure.

$$\text{Susceptibility index (SI):} \quad \frac{LC_{50} \text{ of F1}}{LC_{50} \text{ of F6}}$$

RESULTS AND DISCUSSION

The LC_{50} values for six generations of *H. armigera* for a laboratory maintained culture are depicted in the Tables. The median lethal concentration of rynaxypyr assessed for F1 and F6 generation were 0.002 and 0.003/larvae. The susceptibility of *H. armigera* increased after six generations of insecticide free exposure culturing. Susceptibility index for LC_{50} value after 72 hours after treatment was 0.66. This lower LC_{50} values indicate the higher susceptibility of larvae to rynaxypyr.

Table 1: Larval susceptibility *Helicoverpa armigera* to rynaxypyr in a topical bioassay (F1generation)

Hours after treatment	Replication	N	χ2 at P=0.05	Regression equation (slope)	LC50 ppm	95 per cent fiducial limit	
						LL	UL
24	3	30	2.40	0.58+5.57X	0.106	0.039	0.466
36	3	30	3.20	0.46+5.54X	0.070	0.022	0.391
48	3	30	6.14	0.41+5.77X	0.013	0.004	0.058
72	3	30	8.50	0.46+6.32X	0.002	0.001	0.004

Table 2: Larval susceptibility *Helicoverpa armigera* to rynaxypyr in a topical bioassay (F6generation)

Hours after treatment	Replication	N	χ2 at P=0.05	Regression equation (slope)	LC50 ppm	95 per cent fiducial limit	
						LL	UL
24	3	30	5.34	0.59+5.66X	0.075	0.029	0.291
36	3	30	6.31	0.48+5.73X	0.031	0.011	0.129
48	3	30	7.21	0.47+6.04X	0.006	0.002	0.020
72	3	30	9.39	0.50+6.29X	0.003	0.001	0.007

The present results are in close conformity with the earlier findings of Lahm *et al*. (2005) who worked out the LC_{50} values of rynaxypyr as 0.002 ppm for *P. xylostella* and *Spodoptera frugiperda* while LC50 values for *Heliothis virescens* (F) was 0.04 ppm. LC_{50} value of rynaxypyr on *Spodoptera litura* (Fabricius) was 0.004 ppm as reported by Dhawan *et al.* (2007). Mary and Laurie (2007) revealed that in topical bioassay method the LD_{50} values ranged from 0.5 to 1.43 µg/g larval weight, where as in diet incorporation bioassay method LC_{50} values ranged from 0.2 to 0.11 µg/ml of diet and in adult vial test LC_{50} values ranged from 1.21 to 1.71 µg/vial for bollworm, fall armyworm and tobacco bud worm respectively. Whereas, Joshua *et al*. (2008) in bioassay studies of rynaxypyr against bollworm obtained LC50 values ranging from 0.038 to 0.089 µg/ml of diet.

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The influence of population density on defense system state of lobster cockroach (*Nauphoeta cinerea*) nymphs

ABSTRACT
In this research the activity of enzymes phenoloxidase and antioxidant systems of *N. cinerea* nymphs has been defined under the action of population density.

The obtained data allows speaking about stress in cases of isolation and overpopulation.

INTRODUCTION

Social life is generally associated with an increased exposure to pathogens and parasites, due to factors such as high population density, frequent physical contact and the use of perennial nest sites. However, sociality also permits the evolution of new collective defense mechanisms. Social insects have special defense mechanisms, also known as "social immunity" (Ugelvig, 2007). Cockroaches are not eusocial insects, such as termites, bees, ants. But, cockroaches adapted to high population density. It may be attributed to common nests and food sources. High population density increases exposure to pathogens and parasites and we suggest that cockroaches have social-like defense mechanisms. The aim of experiments was definition of the influence of population density on defense system state of lobster cockroach (*Nauphoeta cinerea*) nymphs.

MATERIALS AND METHODS

Last stage nymphs from laboratory population were used for experiments. Insects were put on Petri dishes 1, 20 and 50 in number. After 5 days rearing, we tested diphenoloxidase (DPO) activity in haemolymph, catalase and peroxidase activity in gut. DPO activity was determined in tris-HCl buffer (pH 7.5) with respect to substratum L-digydroxiphenilalanin and was measured on spectrophotometer Shimadsu at 475 nm by optical density change during 5 min (Raushenbach, 1997). Peroxides activity was determined at incubation in tris-HCl buffer (pH 7.5) by velocity of benzidine oxidation on spectrophotometer Shimadsu at 540 nm by optical density change during 1 min (Bojarkin, 1951). Catalase activity was determined by velocity of hydrogen peroxide destruction on spectrophotometer Shimadsu at 410 nm. Reaction was stopped by adding 4% ammonium molybdate solution (Koroljuk, 1988). Activity of these enzymes was evaluated in protein concentration, which was measured by Bradford method (Skopes, 1982).

RESULTS AND DISCUSSIONS

Catalase and peroxidase activity were maximal in grope, where insects are reared 20 in number. Activity of this enzymes was minimal in grope, there insects are reared 50 in number (see diagram 1-2). DPO activity was maximal in grope, where insects are reared alone and minimal in grope, where insects are reared 20 in number (see diagram 3). Reactive oxygen species are important part of insect defense mechanisms. But excess of it can damage own tissues and organs. High catalase and peroxidase activity is supposed to protect insect organism against reactive oxygen species excess (Peng and Miles, 1991, Ahmad et al., 1991). High DPO activity may indicate that rate of biogenic amines which are stress indicator (Cui et al., 2000) is high.

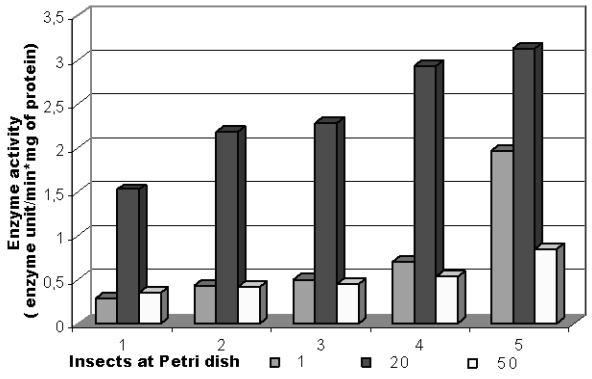

Diagram 1. ***The influence of population density on gut catalase activity of lobster cockroach nymphs.***

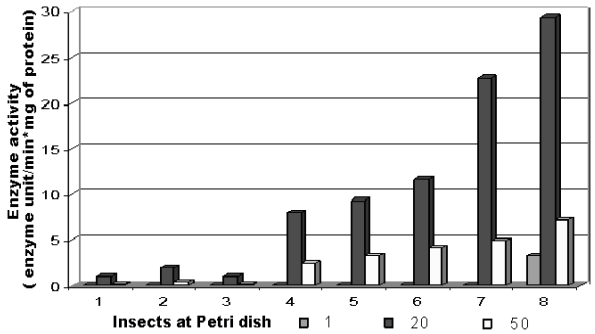

Diagram 2. ***The influence of population density on gut peroxidase activity of lobster cockroach nymphs.***

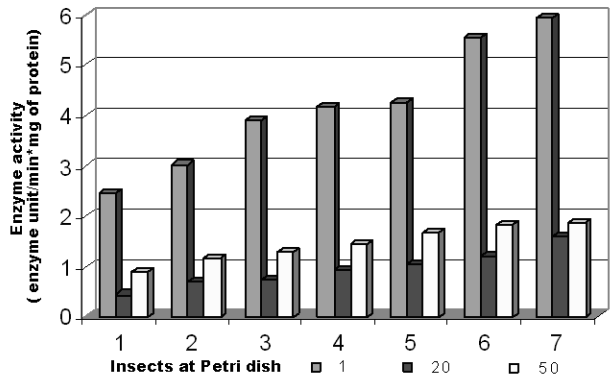

Diagram 3. ***The influence of population density on haemolymph DPO activity of lobster cockroach nymphs.***

Findings suggest that population density 20 insects at Petri dish is most optimal in our experiment. Solitary life is not typical for cockroaches. German cockroaches (*Blattella germanica*) reared in isolation showed, stronger exploration-avoidance, reduced foraging activity, reduced willingness to interact socially, and reduced ability to assess mating partner quality than conspecifics reared in groups (Lihoreau et al., 2009). Woodhead and Paulson (1983) investigated population density influence on cockroach *Diploptera punctata* larval development. Duration of development was longer in isolated animals than in animals reared in pairs and groups (Woodhead and Paulson, 1983). Nymphs that reared alone in our experiment probably are exposed to isolation stress. Some of non-eusocial insects are adapted to high population density, and need it to normal vital functions. Thus mealworm beetles (*Tenebrio molitor*) reared at high larval densities showed lower mortality when exposed to a generalist entomopathogenic fungus (Barnes and Siva-Jothy, 2000).

However, excessive population density is a reason of stress too. Overpopulation makes gypsy moth (*Lymantria dispar*) larvae vulnerable to nucleopolyhedrovirus. Time to death was faster at high densities than at lower densities (Reilly and Hajek, 2007). Nymphs that were reared 50 in number in our experiment probably are exposed to overpopulation stress.

CONCLUSION

We come to the conclusion that cockroaches immunity state is depend on population density. Cockroaches are not adapted to solitary life, and alone housing is reason of stress. It is typically for other cockroach species either. But overpopulation may be stress reason too. Population density 50 insects at Petri dish is excessively high for cockroaches. In our experiment 20 insects at Petri dish is most close of optimal population density.

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EFFICACY OF SOME ACARICIDES/INSECTICIDES AGAINST *Tetranychus urticae* Koch. ON BRINJAL

ABSTRACT

Red Spider Mite, *Tetranychus urticae* Koch is a serious pest of vegetable crops, especially on brinjal during the summer months in the Varanasi region. A field experiment was conducted during the summer months of 2007-2008 to evaluate the effectiveness of some acaricides viz. abamectine, acephate, dicofol, ethion, gronim, lannate, magister, mitex-s, polo and propargite at their recommended concentration and doses. The mortality data was recorded as pre-spray, and at 1, 3, 7 & 14th days as post -spray of acaricides/insecticides. The result reveals that dicofol showed maximum mortality followed by abamectin, propargite and ethion i.e., 91.56%, 87.44%, 81.66% and 72.94% respectively. Whereas moderate mortality showed with polo, acephate, magister and gronim i.e., 67.76%, 62.32%, 59.87% and 55.54% respectively. The lowest mortality showed with mitex-s (49.20%) followed by lannate (52.93%). The present investigation was carried out proper for management of this polyphagous mite.

Key words: Red spider mite, acaricide, brinjal**.**

INTRODUCTION

The Phytophagous mite causes serious damage to all crops including stored grain and its products. During the last two decades the mite infestation to vegetable crops has been recognized as an important limiting factor (ChannaBasavanna 1971). Many species of tetranychid mites have gained the status of major pest of many vegetable crops, mostly tetranychid mites feed on both surfaces of the leaves. Pillai and Palaniswamy (1985) reported that on cassava the red mites, *Tetranychus cinnabarinus* and *T. neocaledonicus* usually feed on the lower surface of the leaves. During the last two decades the red spider mite, *Tetranychus urticae* Koch. has been found to be more aggressive in nature as a destructive pest to most of the vegetable crops in different parts of the country as well as in Varanasi. Brinjal was found to be worst suffered by the attack of this mite in eastern part of Uttar Pradesh. The indiscriminate use of pesticides, fertilizers and irregular cultural practices has probably favored the outbreak of phytophagous mite population. Pesticidal approaches are an important component of Integrated Mite-Pest Management (IMM) for vegetable production. There are slightly different approaches to manage this pest through those insecticides, which have some acaricidal properties were selected for the evaluation. There is no extra cost on the vegetable growers, once they manage them then they can get a good quality and quantity of their produce. It also affects the other component of IMM.

MATERIALS AND METHODS

The study was carried out under field condition at vegetable grower's field, surrounding the B.H.U., Varanasi in the months of summer (March to June) 2007-2008 on brinjal crop, variety Muktakeshi. The details of the selected acaricides/ insecticides (chemicals) were given in Table 1. The commercial grade formulation of the chemicals was used at their recommended dose. The amount of proprietary ingredient required was calculated and was mixed with 1 liter of water then sprayed on the infected brinjal plants. The chemical solutions were prepared just before the application. The spraying was done with the help of foot sprayers. After spraying, the live mite population was counted on the basis of 2cm^2

leaf areas at four spots per leaf. The data was recorded from five randomly selected and tagged plants. Two leaves was plucked from top, middle and bottom from each selected plant, all the plucked leaves were kept in the polythene bag (from each plot) and brought to the laboratory for observation under a stereo binocular microscope. The observation of TSM population was taken at different intervals as pre spray, 1,3,7, and 14th days of post spray. The data was analyzed statistically.

RESULTS AND DISCUSSION

The efficacy of acaricides/insecticides viz. abamectine, acephate, dicofol, ethion, gronim, lannate, magister, mitex-s, polo, and propargite were evaluated at their recommended dose and compared with control (water) against the populations of red spider mite, *Tetranychus urticae* Koch on brinjal at the farmer's field. The result reveals that the used acaricides/insecticides showed significant variation in the mortality observed at 1,3,7, and 14th days of post treatment (Table 1).

Table 1: Efficacy of acaricides / insecticides against *Tetranychus urticae* Koch on Brinjal

Sl. No.	Acaricides/ Insecticides	Dose (ml.or gm/lt.)	Mean percent mortality (Days after spraying)				Mean
			1st	3rd	7th	14th	
1	Abamectin 1.9%(w/w)EC	7.36	94.62* (78.03)**	92.22 (74.88)	89.15 (71.66)	73.78 (59.67)	87.44 (70.09)
2	Acephate 75% S.P.	1.56	71.59 (57.17)	69.84 (56.11))	64.63 (54.15)	43.23 (41.67)	62.32 (52.12)
3	Dicofol 18.5% EC	2.70	97.64 (83.45)	95.26 (78.76)	93.88 (76.95)	79.46 (63.72)	91.56 (74.21)
4	Ethion 50%EC	1.00	83.20 (65.05)	79.57 (62.44)	75.64 (61.14)	51.33 (46.32)	72.94 (58.31)
5	Gronim (Triterpenoid based EC AZADIRACTINE 0.15% w/w) (insecticide)	5.00	66.13 (53.79)	61.44 (51.00)	57.55 (49.95)	36.53 (38.35)	55.44 (48.27)
6	Lannate/Methomyl 12.5 L	2.25	61.58 (52.30)	57.88 (50.13)	55.26 (48.56)	36.88 (38.00)	52.93 (47.24)
7	Magister (Fenazaqun) 10EC	2.00	73.94 (59.93)	69.44 (57.04)	67.87 (56.11)	28.23 (32.71)	59.87 (51.30)
8	Mitex-s (Nitrogen+sulphur) 80%WP	4.17	61.85 (52.48)	59.22 (50.89)	54.84 (48.39)	20.88 (27.90)	49.20 (45.11)
9	Polo 50% SC	2.50	75.54 (61.07)	73.33 (59.34)	68.21 (56.29)	53.96 (47.81)	67.76 (56.04)
10	Propargite 57% EC	3.15	87.99 (70.54)	85.46 (68.36)	82.86 (66.34)	70.31 (57.61)	81.66 (65.42)
11	Control	--	39.88 (39.76)	29.55 (33.58)	28.57 (32.96)	13.56 (22.38)	27.89 (32.52)
SEM +/-			4.628	2.828	5.012	3.142	
CD at 5%			9.625	6.112	9.883	6.532	

*Mean of every four replication, number of mites in 2.5 cm2 on 24 leaves-means of 96 observation.
**Figures in parenthesis is Arc Sine percentage transformation

After one day of post treatment less mortality was responded with lannate (61.58%) followed by mitex-s (61.85%), gronim (66.13%) and acephate (71.59%). The moderate mortality was responded with magister (73.94%) and polo (75.54%). Whereas the maximum mortality was responded with dicofol (97.64%) followed by abamectin (94.62%), propargite (87.99%) and ethion (83.20%).

After 3 days of post treatment less mortality was observed with lannate (57.88%) followed by mitex-s (59.22%), gronim (61.44%) and acephate (69.84%). The moderate mortality was observed with magister (69.44%) followed by polo (73.33%) and ethion (79.57%). Whereas the maximum mortality was observed with dicofol (95.26%) followed by abamectin (92.22%) and propargite (85.46%).

After 7th days of post treatment less mortality was shown with mitex-s (54.84%) followed by lannate (55.26%), gronim (58.55%), acephate (64.63%) and magister (67.87%). The moderate mortality was shown with polo (68.21%) and ethion (75.64%). Whereas maximum mortality was shown with dicofol (93.88%) followed by abamectin (90.12%) and propargite (82.86%).

After 14th days of post treatment less mortality was responded with mitex-s (20.88%) followed by magister (28.23%), lannate (36.88%), gronim 36.53% and acephate (43.23%). The moderate mortality was responded with ethion (53.33%) followed by polo (53.96%). Whereas the maximum mortality was responded with dicofol (79.46%) followed by abamectin (73.78%) and propargite (70.31%).

Overall the results indicate that dicofol responded maximum mortality followed by abamectin and propargite i.e 91.56, 87.44 and 81.66 per cent respectively. The moderate mortality was responded with ethion, polo, acephate, magister, gronim and lannate i.e. 72.94, 67.76, 62.32, 59.87, 55.44 and 52.93 per cent respectively. Whereas the least mortality was responded with mitex-s i.e. 49.20 percent. Many workers, Singh and Singh (1992), Kumar *et. al.* (2001), Kumar and Singh (2003), Kavitha *et.al*. (2007) and Rai and Singh (2008) earlier been supported the results.

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Comparative Feeding behavior and Ovipositional aspects of Cotton boll worms Helicoverpa armigera on transgenic and non-transgenic Cotton

ABSTRACT

The American boll worms, Helicoverpa armigera is the major constraint for Cotton production, and therefore, efforts are being made to develop transgenic Cotton with Bt and SBTI genes to minimize the losses due to this pest. The oviposition behavior of H. armigera on transgenic and non-transgenic plants was studied under no-choice, dual-choice, and multi-choice conditions. No differences were observed in the number of eggs laid on the inflorescences of the transgenic Cotton with crylAb or SBTI genes and with the non-transgenic plants. In dual-choice feeding tests, there were no differences in leaf damage, larval weights, and the number of larvae between transgenic and non-transgenic plants. The results suggested that transgenic plants have no influence on the oviposition and feeding preferences of H. armigera. Cotton (Gossypium spp.) plays an important role in nutritional security as an important source of high quality dietary proteins. It is damaged by over 150 insect species, of which Helicoverpa armigera (Hubner) is the most important pest, which causes major loss of yield in Cotton. Effort to minimize the H. armigera damage with, transgenic Cotton plants with Bacillus thuringiensis (Bt crylAb) and soybean trypsininhibitor (SBTI) genes have been developed recently(Sharma et ah, 2006). Genetic transformation of crop leads to slight changes in the chemical composition, which might influence host selection and colonization by the insects. Therefore, we studied the oviposition preference by females and feeding preference by the H.armigera larvae on transgenic and non-transgenic plants of Cotton.

Key words: Transgenic cotton, Bacillus thuringiensis, cotton, Bollworms

MATERIALS AND METHODS

The Cotton varieties, Bollgard MECH-12 and MCU were raised in a containment (P2level) green house at 24 to 28°C, 70 to 80% RH. The H. armigera culture was maintained under laboratory conditions of 24°C and 70% RH (Armes etal, 1992). Oviposition preference The oviposition behavior of H. armigera was studied under no-choice, dual-choice (in comparison to the non-transgenic control), and multi-choice conditions (all the test genotypes placed inside the cage). Fresh inflorescences (20 cm long) with flowers and tender leaves were collected from the greenhouse, and placed in a conical flask (150 ml) filled with water. A cotton swab was wrapped around the stem to keep the inflorescence in upright position. For no-choice tests three pairs and for dual- and multi-choice tests four pairs of two-day old moths were released inside the cage. Sucrose solution (10%) in a cotton swab was offered to the adults as a food, changed on alternate days. The number of eggs laid by the moths was recorded, and the inflorescences were replaced daily. The experiments were replicated six times in a completely randomized design. Percentage of eggs laid on each plant was calculated from the total number of eggs laid. The data was subjected to analysis of variance. A Student "T" Test was used to test significance of difference in dual-choice tests. Neonate feeding preference assay Fully expanded tender leaves of equal size from transformed and non-transformed Cotton plants were collected and placed one centimeter apart in a Petri dish arena (9 cm dia) lined with moistened filter paper. Ten neonate larvae were placed in the middle of Petri dish arena. Data on leaf feeding was recorded after 72 hours on a 1 to 9 scale (1 = <10% leaf area damaged and 9 = >80% leaf area damaged). The number of larvae on each leaf and their weights were recorded separately. Each treatment was replicated five times in a completely randomized design.

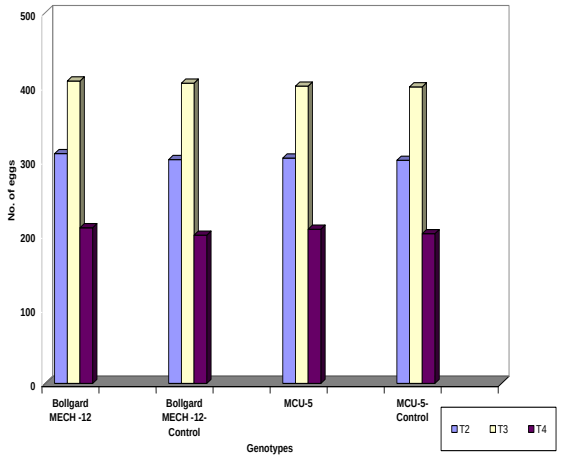
RESULTS AND DISCUSSION

There were no significant differences in the numbers of eggs laid on the inflorescences of transgenic and non-transgenic control plants under no-choice, multi-choice (Fig. 1), and dual-choice conditions (Table 1). Egg densities of the tobacco budworm (Heliothis virescens) (Parker and Luttrell,1998) and cotton bollworm (H. armigera) (Sharma and Pampapathy,

2006) have not been found to be significantly different on transgenic and non-transgenic cottons. The lack of differences in oviposition preference indicated that there are no major changes in the physico-chemical characteristics of the transgenic plants that influence oviposition behavior. This corroborates the earlier observations that the oviposition behaviour of H. armigera moths was independent of the presence of transgenes (Macintosh et ah, 1990; Orr and Landis, 1997; Ramachandran et al, 1998).

Figures followed by the same letter in a row are not significantly different at p=0.05 level

Fig. 1 Oviposition preference of *H.armigera* towards trangenic and non-transgenic cotton crop



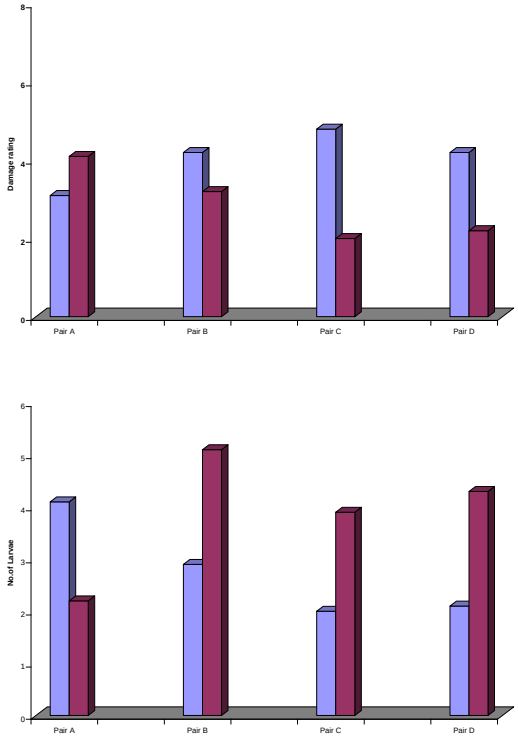
T2 & T3 generations were tested under no-choice conditions
T4 generations was tested under multi-choice conditions

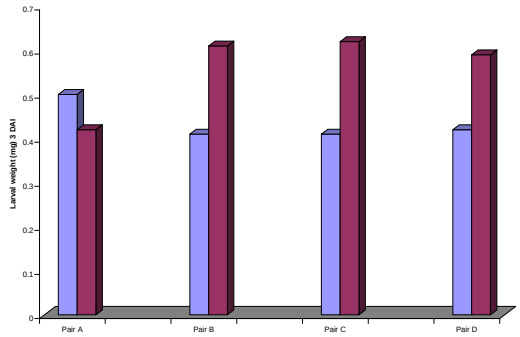
Table 1: Ovipositon preference of *H.armigera* females towards transgenic and non-transgenic cotton under dual-choice conditions.

	Genotype	No of eggs/twig	
		Transgenic	Non Transgenic
T_2 Generation			
SBTI	Bollgard MECH -12	245.2[a]	214.1[a]
Bt	MCU-5	200.8[a]	201.0
T_3 Generation			
SBTI	Bollgard MECH -12	114.1[a]	127.2[b]
Bt	MCU-5	121.6[a]	134.6[a]
T_4 Generation			
SBTI	Bollgard MECH -12	165.1[a]	158.4[a]
Bt	MCU-5	165.2[a]	154.5[a]

There are no significant differences in leaf damage, larval weights, and the number of larvae that settled on leaves of transgenic and non-transgenic plants (Fig. 2). Gould et al. (1991) observed that H.virescens larvae were able to detect and avoid high levels of B. thuringiensis toxins in diet. Increased movement and dispersal of H. virescens larvae has also been observed on transgenic cotton lines (Benedict et al, 1993; Parker and Luttrell, 1999). Lack of feeding preference by H.armigera larvae on transgenic and non-transgenic Cotton plants may be because of low levels of expression of toxin proteins in transgenic Cotton, which do not result in perceptible changes in insect behaviour and development.

Fig 2: Feeding preference of neonate larvae of H.armigera towards leaves of transgenic & non-transgenic cotton crop





Pair A : Bt MECH-12 and its non-transgenic control
Pair B: SBTI MCU and its its non-transgenic control
Pair C: Bt MCU & SBTI MCU
Pair D: Non transgenic control of MECH-12 & MCU

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BASELINE TOXICITY OF EMAMECTIN AND SPINOSAD TO *PLUTELLA XYLOSTELLA* (LEPIDOPTERA: NOCTUIDAE) FOR RESISTANCE MONITORING

ABSTRACT
Studies were conducted to assess the acute toxicity of emamectin 5 SG and spinosad 2.5 SC on Diamondback moth, *Plutella xylostella* collected from three different locations in Tamil Nadu where cabbage and cauliflower crops are grown extensively. The median lethal doses (LC_{50}) values were found to be 0.066 and 2.12 ppm for emamectin and spinosad respectively. Based on LC_{95}, discriminating doses were fixed at 2 ppm and 10 ppm for emamectin and spinosad. Revalidating the discriminating doses on field collected populations found that there was no resistance among the populations of *P. xylostella* from the three locations – Coimabtore, Ooty and Oddanchatram.
Key Words: acute toxicity, emamectin, spinosad, *P. xylostella*, resistance

INTRODUCTION

The diamond back moth (DBM), *Plutella xylostella* (Linn.) is the most notorious pest of crucifers. Their control is depended primarily and extensively on the use of insecticides. DBM shows greater propensity towards quick development of resistance to new molecules within a few years of its introduction. In India, the first report of *P.xylostella* resistance to pesticides (DDT and parathion) was made by Verma and Sandhu (1968) in Ludhiana. Subsequently, the resistance to other compounds of insecticides had also been reported widely in India *viz.* ethyl parathion (Deshmukh and Saramma, 1973), fenitrothion and malathion (Chawla and Kalra, 1976), cypermethrin, fenvalerate, decamethrin and quinalphos (Saxena *et al.*, 1989; Chawla and Joia, 1991, 1992) monocrotophos, malathion, endosulfan, dichlorvos in Haryana (Kalra *et al.* (1997) cypermethrin, fenvalerate, endosulfan and quinalphos in two regions near Varanasi (Raju and Singh (1995), permethrin and fenvalerate (Krishnakumar *et al*., 1986), endosulfan, chlorpyriphos, methyl parathion, quinalphos, methomyl, monocrotophos, carbaryl, alphamethrin, fenvalerate, cypermethrin, deltamethrin, cartap hydrochloride, *B.thuringiensis* (Sannaveerappanavar, 1995) *B.t.kurstaki* (Biobit R), lufenuron and flufenaxuron (Pereira, et. Al., 2006a,b), chlorpyriphos, dichlorvos, monocrotophos, profenofos, triazophos), methomyl, deltamethrin, fenvalerate, cartap hydrochloride (flufenoxuron, teflubenzuron) ,) and *B. t*. products (Sannaveerappanavar, . and Virktamath, 2006 a;) Mohan and Gujar, 2000) in Karnataka, cypermethrin and fenvalerate in Andhra Pradesh (Nirmal and Singh, 2001), cartap hydrochloride in Tamil Nadu (Mohan and Gujar, 2003). Rabindra *et al.* (1995) evaluated the relative susceptibility of two populations to fenvalerate, monocrotophos and *B. thuringiensis* in Tamil Nadu. Chandrasekaran and Regupathy (1996a) and Renuka and Regupathy (1996), during their survey in Tamil Nadu reported high degree of resistance to quinalphos, monocrotophos and fenvalerate.

In the past, it has not been possible to know the exact situation in respect of insecticide resistance in DBM due to unavailability of truly susceptible strains in India and lack of base-line values (Saxena *et al.*, 1989). Chawla and Kalra (1976) emphasized the need for establishing base-line data for insecticide susceptibility using standard bioassay techniques for the correct appraisal of the problems of resistance in DBM. The host specific monitoring methods with discriminating-dose technique identified will enable everyone to watch for the same signs that resistance problem may be occurring and the severity of the problem (Ayyasamy and Regupathy. 2004). Discriminating doses for carbosulfan, cartap hydrochloride, fenvalerate, monocrotophos and quinalphos were fixed for different methods of bioassay viz., vial, leaf dip, larval dip and spray tower. (Chandrasekaran, 1994; Chandrasekaran and Regupathy, 1996 b, c). The larval dip assay, though easier and economical does not facilitate treatment in batches as the larvae get clumped up. Therefore, vial assay is suggested for adoption. The leaf dip (IRAC method No.7) was used to determine the variability in baseline susceptible response to Bt in DBM field populations. Appropriate discrimination dose of Biobit 50 WP (32000 IU/mg) was determined for third instar (Chandrasekaran and Regupathy, 1996 b). Sannaveerappanavar and Virktamath, 2006 b) generated base-line values for 25 insecticides of different classes viz. chlorinated hydrocarbon (endosulfan) , organophosphates (acephate , chlorpyriphos , diazinon , dichlorvos , malathion , methyl parathion , monocrotophos , phosalone ,profenofos , quinalphos , triazophos), carbamates (carbaryl , methomyl) , synthetic pyrethroids (deltamethrin , fenvalerate , ,cypermethrin, alphamethrin),amines (cartap hydrochloride) acylurea compounds(flufenoxuron ,teflubenzuron) , thiourea (diafenthiuron) and *B. t*. products (Biobit , Centari and Dipel).

Recently, microbial insecticides extracted from actinomycetes like *Saccharopolyspora spinosa* (Spinosad), *Streptomyces arermectilis* (avermectin) were found to possess high toxicity, even at low doses and less toxic to natural enemies in agro ecosystem. Emamectin benzoate is a semisynthetic second generation avermectin 4" – deoxy – 4" epimethylamino – avermectin B, benzoate salt, effective against lepidopteran larvae (Jansson *et al.* 1997). They act as gamma amino butyric acid (GABA) against and is referred as GABA – gated chloride channel inhibitor.

Spinosad contains a mixture of two very active principles spinosyn A and spinosyn D, effective against lepidopteran pests (Sparks *et al.* 2001). Spinosad acts at the nicotinic acetyl choline receptors of insect nervous system, with GABA receptor site being secondary site of attack.

As these insecticides are used extensively now, monitoring of resistance development to these new molecules will be useful in the management strategy. Hence baseline susceptibility data were generated to monitor resistance in future.

MATERIALS AND METHODS

Lab experiments were carried out by collecting various stages of larvae from three different locations (Coimbatore, Ooty and Oddanchatram) in Tamil Nadu and were maintained separately in the insecticide resistance laboratory, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, India. Mass culturing of *P. xylostella* was carried out in the laboratory using Liu and Sun (1984) method with little modification. Mustard seedlings were used for egg collection in oviposition cage. The larvae were fed on fresh cabbage leaves grown in pots without exposure to any insecticides in a rearing cage. Raising of mustard seedlings and rearing of *P. xylostella* was done under lab conditions at 12:12 (L:D) and at 28 $\pm$ 2^{o}C.Third instar larvae (0.5 $\pm$ 0.1 cm; 1.75 $\pm$ 0.25 mg) were collected on tenth day of egg laying for bioassay. Leaf disc bioassay was followed, since it simulates the behaviour of insecticides in field condition.

The dilutions required were prepared from the formulated product of insecticide using distilled water. The insecticide formulation used for bioassay were emamectin (proclaim 5 SG, M/s Syngenta India, Limited) spinosad (Success 2.5 SC, M/s DeNocil India Limited, Chennai).

For bioassay, cabbage plants were maintained in pots without exposure to insecticides. Leaf discs of 6 cm diameter were cut covering either side of the midrib. These leaf discs were dipped in test concentrations for about a minute. They were then removed, excess fluid drained and dried for about one hour at room temperature. Leaf discs were placed slantingly on sides of the container, so that larvae can move on either side. Three, third instar larvae were released per disc for each concentration and replicated 10 times (30 larvae per treatment). Observations and mortality of the larvae were observed after 24 hrs. The dosages were arrived after preliminary range finding studies for constructing log-concentration – probability mortality (LCPM) lines using Finney's method (Finney 1971).

For generation of baseline data, the insects were reared without exposure to insecticides and cultured continuously without selection pressure throughout F_3 and F_5 generation. Bioassays were conducted to construct LCPM lines for a susceptible population. Based on lethal concentration obtained for emamectin and spinosad on the population of *Plutella xylostella* from three test locations *viz*., Coimbatore, Ooty and Oddanchatram, tentative discriminating doses were fixed based on LC_{95} values. These discriminating doses were applied on field collected larvae for monitoring of resistance.

Susceptibility index

Susceptibility indices were calculated based on LC_{50} obtained for the third and fifth generation for each culture, maintained without insecticide exposure.

$$\text{Susceptibility index} = \frac{LC_{50} \text{ of } F_1}{LC_{50} \text{ of } F_3 / F_5}$$

Monitoring of insecticide resistance

Insecticidal dilutions based on discriminating dose for each insecticide were applied on field collected larvae collected from different locations and mortality observed after 24 hours of treatment. The resistance percentage was calculated.

RESULTS AND DISCUSSION

The acute toxicity for emamectin and spinosad was found to be very high and hence low LC_{50} was obtained for all three locations. The LC_{50} for emamectin was found to vary between 0.065 – 0.69 ppm (Table 1) for the populations of Coimbatore, Ooty and Oddanchatram population. Emamectin benzoate is reported to be highly potent to wide range of lepidopteran species (Dybas *et al.* 1989). High lethal toxicities of avermectins to other insects such as *Cydia pomonella* (0.08 ppm) (Cox *et al.* 1995a). *Liriomyza trifolli* (0.35 ppm) (Cox *et al.* 1995 b), *Trichoplusia ni* (0.014 mg l^{-1}) (Thomas *et al.* 1994) has been reported.

The LC_{50} of spinosad 2.5 SC was found to vary between 1.77 – 2.24 ppm (Table 1). Acute toxicities of spinosad for finding the baseline toxicities had been attempted by Arora *et al.* (2003) and estimated the LC_{50} values to be 0.00033 per cent (3.3 ppm). LC_{50} obtained in the present study is well below to that reported by other workers for *P. xylostella*, which indicates the susceptibility of insect to spinosad. The susceptibility index was found to be 1.12 (Table 1).

Table 1: Acute toxicity of emamectin and spinosad to *P. xylostella*

Particulars	Population	Generation	LC_{50}	95% fiducial Limits	LC_{95}	95% fiducial limits	Regression equation	X^2 (n-2)	SI
Emamectin	Coimbatore	F_1	0.065	0.04-0.12	4.28	4.02-4.54	Y=3.36 + 0.09x	5.13	-
		F_3	0.064	0.03-0.15	2.81	2.44-3.18	Y = 3.10 + 1.00 x	2.55	1.03
		F_5	0.053	0.02-0.05	1.71	1.37-2.05	Y = 3.12 + 1.09x	3.11	1.09
	Ooty	F_1	0.066	0.036-0.124	5.75	5.48-6.02	Y = 3.46 + 0.85x	5.07	-
		F_3	0.066	0.03 – 0.14	3.56	3.22 – 3.89	Y = 3.27 + 0.95x	3.79	1.00
		F_5	0.58	0.027 – 0.123	2.86	2.53-3.19	Y = 3.29 + 0.97x	5.24	1.14
	Oddanchatram	F_1	0.69	0.55-0.87	2.46	2.36-2.56	Y = 3.47 + 2.98x	0.04	-
		F_3	0.69	0.95-0.50	2.35	2.21-2.46	Y = 3.74 + 3.08x	0.03	1.00
		F_5	0.66	0.40 – 0.89	2.15	2.02-2.28	Y = 4.04 + 3.20x	0.04	1.01
Spinosad	Coimbatore	F_1	1.77	1.19-2.64	26.03	25.86-26.19	Y= 0.43 + 1.41x	1.73	-
		F_3	1.72	1.00 – 2.95	25.42	23.18-25.65	Y = 0.32 + 1.45x	0.78	1.03
		F_5	1.61	1.08-2.40	24.96	24.75-25.13	Y = 0.58 + 1.8x	1.92	1.09
	Ooty	F_1	2.12	1.35 – 2.92	14.35	14.21 – 14.48	Y= 1.58 + 1.98x	1.74	-
		F_3	2.08	1.39 - 3.11	14.48	14.3-14.65	Y = 1.47 + 1.95x	1.05	1.02
		F_5	1.90	1.36-2.69	13.01	12.9-13.2	Y = 1.44 + 1.6x	4.0	1.12
	Oddanchatram	F_1	2.24	1.62-3.10	13.59	13.55-13.73	Y=2.02 + 2.09x	1.40	-
		F_3	2.06	1.49 - 2.85	1.61	13.47-13.76	Y = 1.62 + 1.99x	1.29	1.09

As *P. xylostella* from three locations are highly susceptible and there is no significant change in susceptibility among the generations. F_5 generation was found to be highly susceptible. Since the fiducial limits of LC_{50} and LC_{95} were found overlapping, there was no significant increase in mortality.

Based on LC_{90}, the discriminating doses were fixed tentatively for emamectin and spinosad at 2 ppm and 12 ppm respectively. The discriminating doses are fixed at $LC_{90} – LC_{95}$. The discriminating doses were revalidated by applying in field collected populations. All the three populations (Coimbatore, Ooty and Oddanchatram) were recorded for emamectin at 5.81 per cent to 15.56 per cent (Table 2.) The resistance for spinosad was found to very from marginal to low (34 per cent to 15.73 per cent). Zhang *et al.* (2001) reported low level of resistance to avermectins and spinosad where 81.7 per cent and 100 per cent mortality was recorded. Chayopes *et al.* (2004) recorded 17.2 fold and 3.72 fold resistance for spinosad and emamectin respectively.

Table 2: Resistance observed in field collected populations for spinosad and emamectin

Location	Emamectin 5 SG	Spinosad 2.5 SC
Coimbatore	5.81 ± 2.54	20 ± 4.50
Ooty	5.88 + 6.59	15.73 + 3.88
Oddanchatram	15.56 + 3.84	34.0 + 5.17

Though the use of newer insecticide may delay the development of resistance in *P. xylostella*, the scope of resistance development to these insecticides cannot be totally eliminated. The insecticide resistance must be continuously monitored and must form as integral part of chemical control so that detection of resistance could be as early as possible (Regupathy 1996). Baseline susceptibility data will be useful to monitor the resistance of *P. xylostella* to these compounds in future.

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Effect Evaluation Tween Surfactant that Improved Performance of Abamectin on Cucumber Leafminer *Liriomyza sativae* Blanchard (Dip: Agromyzidae)

Abstract
Vegetable leafminers are one of the most important pests of greenhouse cucumber in Iran. In early years the population of this pest in greenhouse has been increased because of the irregular application of insecticides. In this study susceptibility of larvae and adults of cucumber leafminer to Abamectin (Vertimac® 1.8% EC) insecticide alone and in combination with Tween (80) surfactant was evaluated under laboratory conditions. The glass vial and leaf dipping methods were used for bioassay with adults and larvae. Bioassay tests were done on first and last-instars larvae and adults with laboratory conditions 25±1, 65±5% R.H., and 16:8 photoperiod of L:D. Data was analyzed using probit analysis procedure and POLOPC software. The results for abamectin revealed that LC_{50} on first, last instars larvae and adults were 1.5, 1.8, 14.3 ppm respectively. According to results, the rate of toxicity insecticide in larval stages was more than adult Analyses indicated that mortality resulting from the mixture was greater than that of abamectin at the concentration of LC_{50}. Hence, a noticeable synergistic effect was observed. The mixture application of two compounds could be recommended. Keywords: Abamectin, LC_{50}, *Liriomyza sativae*, Surfactant, Tween

INTRODUCTION

The Leafmining flies (LMF), *Liriomyza trifolii* (Burgess), and *L. sativae* Blanchard are important quarantined pests for a wide variety of vegetables and flower crops in different countries such as Iran (Bani Ameri, 2003). The adult flies puncture the leaves of the plant for feeding and ovipositor, resulting in stippling. The larvae reduce the photosynthetic activity of the leaves by mining and ultimately the leaves dry out and the plant is defoliated (Parrella *et al.*, 1985). This species (*L. trifolii*) of leafminer fly is tolerant to many insecticides and capable of developing a resistance to products that were originally effective. It is therefore becoming more difficult to control LMF worldwide.

Due to the low damage threshold on some crops, notably leafy vegetables and ornamentals, the most widely used method of leaf miner control is through the application of insecticides (Cox *et al.*, 1995). Some insecticides retain effectiveness for only 2 yr after introduction (Leibee 1981). In some countries such as Indonesia, the range of insecticides is routinely applied to control leafminers (Rauf *et al.*, 2000). Insecticide applications can reduce parasitism by indigenous parasitoid wasps and also decrease the number of the predatory *Coenosia humilis*. These effects reduce the controlling of leafminers. However two insecticides have been used successfully for leafminer control since the mid-1980s: Cyromazin, (N-cyclopropyl-1,3,5-triazine-2,4,6-triamine), an insect growth regulator (IGR), is registered on many vegetable (Trigard®); Abamectin, a GABA agonist, is also registered on many vegetable (AgriMek®) . An alternative control strategy involving the applications of Abamectin led to a reduction in leafminers without harmful effects on parasitoids and predators. Abamectin (1.8%) applications provide one potential component of an effective *Liriomyza* control strategy for Indonesian potato farmers. It has been suggested that the addition of certain penetrating surfactants may increases translaminar movement and insecticidal activity on pests that mine within leaves and feed on lower leaf surfaces (Larson, 1997).

YiLiang *et al.,* (2004) evaluated that efficacy of the mixture of Fenpropathrin and Abamectin to *Liriomyza sativae* in fields. They showed the efficacy of the mixture increased with the increase in the concentration of the active ingredients. Guerrero (1999) found that in potato the wage of oil raised the potency of abamectin, allowing the dosage to be reduced to 1/4 the recommended level. These findings are agreed with reports on two other pests: a plant mite, *Tetranychus urticae* (Schuster and Everett, 1983), and the cabbage diamond moth, *Plutella xylostella* (Abro *et al.*, 1988). Mujica *et al.*, (2000) showed that the mixture of oil and abamectin sprays to get a 1% oil spray concentration increased the effectiveness of the insecticide to the extent that the active ingredient of the insecticide could be reduced by one-half to three-fourths of the normal dosage (0.15%).

In the studies reported of the effect mixtures Abamectin and Tween surfactant that studies demonstrated that such mixture may have synergistic and increased the effectiveness of the insecticide to the extent that the active ingredient of the insecticide could be reduced of the normal dosage. In this study the effects combination on abamectin (biotic insecticide) and tween surfactant was evaluated.

MATERIALS AND METHODS

Host Plant Culturing

Cowpea was seeded in 10-cm-diampots (four–six seeds per pot) with holes in the bottom, with soil consisting of equal parts peat moss, vermiculite and sand. Plants were grown at 25±2^{oC}, with a photoperiod of 14:10 h (L: D), until two true leaves were fully expanded, the negin variety for bioassay tests.

Leaf miner rearing

The larvae were collected from infested cucumber from the greenhouses in pakdasht (adjacent of Tehran) on September 2007. They were transferred to the growth chamber for rearing at 25± 2^{oC}, 60-65% RH, and maintained until the emergence of pupa, then transferred in cages that included bean pots.

Insecticide

The insecticide that was used in our experiments was Abamectin (Agrimec® 0.15 EC, AgriEvo Crop Protection),a fermentation metabolite of the actinomycete *Streptomyces avermitilis*, a soil inhabiting microorganism, and surfactant Tween (80).

Bioassays

The bioassay experiments were carried out according to Cox *et al.* (1995) method and at larval stage of the pest. Additionally, in commercial practice, insecticide applications are directed against the larval stage. Sixty-four young (10-14-d-old) cucumber plants were caged (in lumite-screened cages) and exposed to several hundred tree-four-d-old flies for an oviposition access period (OAP) of four–six h. The short time period for OAP allowed for a synchronous egg hatch and age of the larvae that were present at treatment. After OAP, plants were removed and held in the laboratory condition (25± 2^{oC} and ambient light) for 96 h to allow eggs to hatch and small mines to develop. The number of small mines (<five mm) present were counted under 10× magnification. Cucumber plants were divided into groups containing equal numbers of mines, 75-200 per dose. The leaves and part of the stem were treated by submersion for 15 s into the appropriate serial dilution of insecticide in distilled water. Five to seven doses were used in each bioassay. In the bioassays the leaf dipping technique was employed against first and last instars larva. Mortality of larva observed continuously and recorded at 24h intervals for Abamectin. Based on prior experience, bioassays were conducted with abamectin at 9 ppm, which was slightly above the LC_{90} of 0.19 ppm reported by Cox *et al.* (1995).

Data was analyzed using probit analysis procedure and POLO PC software. Another experiment of this concentration abamectine and mixture with Tween (80) surfactant were treated first larvae infested leaves with dipping method. After 24 hours, mortality percentages were counted. The effectiveness of abamectin applied alone or mixed with Tween surfactant on leafminer fly (LMF), *L. sativae* was evaluated on cucumber plants under laboratory conditions.

RESULTS AND DISCUSSION

The results revealed that the values of LC_{50} for pure abamectin for the first and last instars larvae and adult were 0.26, 0.28 and 0.81 ppm (Table1).

Table 1: Compression the LC_{50} value of abamectin on the different stage larvae of *L. sativae*

Insecticide Abamectin	Slope [±SE]	LC_{10} ppm	LC_{50} ppm	LC_{90} ppm	Chi-square	D
Larvae 1	1.06 [±0.25]	0.26	1.51	8.86	0.34	3
Larvae 2	1.19 [±0.24]	0.28	1.81	14.4	0.34	3
Adult	1.02 [±0.31]	0.81	14.3	251	0.84	3

As result shows that the efficacy of abamectin of high mortality in first instars larvae and comparing the results showed that the mortality first instars were increased (Table1 and figure 1).

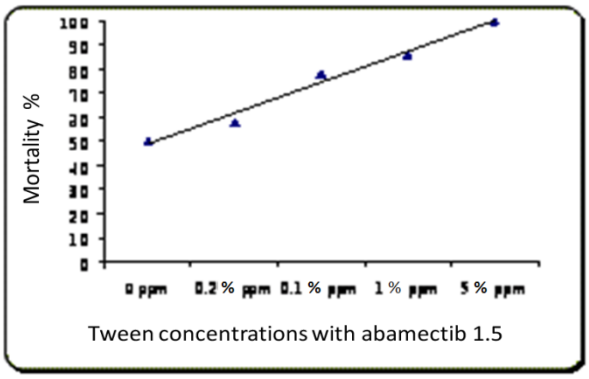

Figure 2. Effect mixture Tween surfactant and Abamectin on cucumber leafminer *Liriomyza sativae*

Abamectin alone, applied at the commercially recommended dosage, showed a satisfactory level of control at larval stage of the pest, confirming reports of that in previous studies (Ochoa and Carballo, 1993; Buxton and McDonald, 1994; Sotomayor, 1998). Lower dosages were less effective, indicating that the recommended dosage is close to abamectin's action threshold. In many cases, larvae died during the process of hatching, before any damage to the mesophyll tissue could be detected. Apparently, abamectin has no effect on the early stages of embryo development, which has also been reported by Leibee (1988).

In the current study, LC_{50} values for abamectin of the collected strain were 1.5 ppm. This is supported by the findings Mujica *et al.,* (2000) who reported the LC_{50} value of abamectin as 1.1 ppm while it has no adoption with Vanderveire *et al.,* (2002) studies. In his studies the value LC_{50} of abamectin was 6.7 for the first instar larvae. Also, based on Prijono *et al.,* (2004) researcher the defined value LC_{50} of abamectin was 1.8 for the first instar of leafminer that is adopted with this search. Based on findings Ferguson *et al.,* (2004) the LC_{50} values of abamectin on various populations of the leafminer were 0.3, 3.7, 5.2 ppm respectively.

Additionally the results indicated that the LC_{50} value for abamectin and tween surfactant for first instars larva were 1 ppm. Furthermore, the result showed that the efficacy of abamectin and tween surfactant of high mortality in first instars larvae and comparing the results showed that the mortality of first instars were increased (figure 2). As implied in figure-2 there is significant differences between the abamectin and tween surfactant slope.

This is supported by the findings YiLiang *et al.,* (2004) evaluated that efficacy of the mixture of fenpropathrin and abamectin to *Liriomyza sativae* in fields. Also, Guerrero (1999) found that in potato the wage of oil raised the potency of abamectin, allowing the dosage to be reduced to 1/4 the recommended level. These findings agree with reports on two other pests: a plant mite, *Tetranychus urticae* (Schuster and Everett, 1983), and the cabbage diamond moth, *Plutella xylostella* (Abro *et al.,* 1988). Mujica *et al.,* (2000) showed that the mixture of oil and abamectin sprays to get a 1% oil spray concentration increased the effectiveness of the insecticide. Baghdadi *et al.,* (2008) showed that residual efficacy was significantly reduced when abamectin was applied in an oil spray environment.

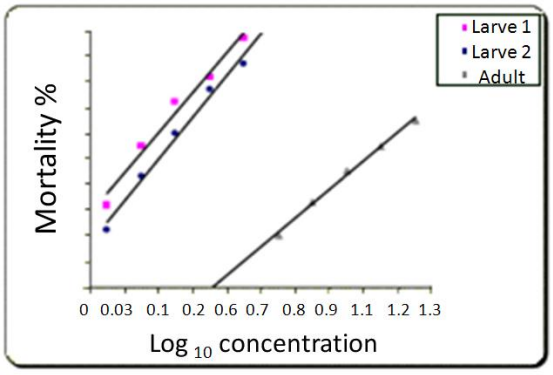

Figure 1: Effect of abamectin on the different stage larvae of *L. sativae*

In contrast, the addition of a tween surfactant with biotic insecticide resulted in significantly greater larval mortality consistent. Due to lower hazard of botanical pesticide for human and environment in comparison to conventional synthetic pesticides, it seems that these combinations can be used for the control of leafminer fly. The application of these natural products is environmentally friendly, as they

are non-toxic and they have fewer residues in the biosphere and does not produce resistance in pests.

CONCLUSIONS

This research confirmed that when used on cucumber plants, abamectin is effective against the larvae of the leafminer fly (*L. sativae*) at the commercially recommended dosages. The mixture of tween surfactant and insecticide improved the potency of the insecticide, also allowing the dosage to be reduced.

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Worker Ant Foraging Response on and near Mounds of Red Imported Fire Ants, *Solenopsis invicta* Buren

Abstract
Red imported fire ants, *Solenopsis invicta* Buren**,** are an invasive species that has infested over 300 million acres in the southern United States. Management of this insect pest often relies on the use of food lures and granular bait insecticides. This trial was conducted to determine the response of the red imported fire ant workers when a food lure was placed on the center of the mound and then 1 foot, 3 feet, and 6 feet from the mound. Results demonstrate that red imported fire ants do forage on top of their colonies. These data should help convince insecticide manufacturers to allow placement of the granular baits directly on top of undisturbed mounds in addition to scattering the bait around the mound.

Introduction

The red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), is a major exotic invasive pest ant species in the southern U. S. and in other countries where it has been accidentally introduced from its South American homeland (Oi and Drees 2009). Management often relies on results of ant monitoring using food lures and the use of granular bait formulated insecticide products (Drees 1998, Drees et al. 2006). Placement of food lures such as slices of hot dogs, or ant bait to treat individual mounds may affect response by foraging red imported fire ant workers. Directions for most ant bait products require placement around, not on top of ant mounds. This concept relies on the theory that red imported fire ants do not forage for food directly on top of their mounds. This trial was conducted to assess response to food lure bait in relation to the center of the nest.

Materials and Methods

Mound sites were selected for this study in Dallas and Brazos Counties, Texas. In Dallas Co. on the grounds of the Texas A&M Research and Extension Center, six colonies were monitored on June 5, July 11, August 3, September 11, and October 25, 2007. Slices of hot dogs (Bar-S), roughly 1/8 inch thick, were placed on top of each mound, and along a transect at intervals of 1, 3 and 6 feet away from the center of the mound. The number of foraging worker ants on the hot dog slice was identified and estimated in the field after 0, 15, 30 and 60 minutes. Results were averaged and graphed for each date and then summarized for the five dates. Environmental conditions were documented, including time, relative humidity, air and soil temperature and wind speed.

In Brazos Co., at the Pecan Genetics Lab, USDA, Hwy 50, methodology used was similar to that used in Dallas Co. However, only two fire ant mounds were monitored on each date, and ants were estimated at food lure stations after 0, 5, 15, 30 and 60 minutes. Most assessments were performed sequentially rather than simultaneously as in Dallas Co.

Resulting mean ant numbers on food lures over time were analyzed using analysis of variance (ANOVA) and means were separated using Tukey's HSD at $P < 0.05$.

Results

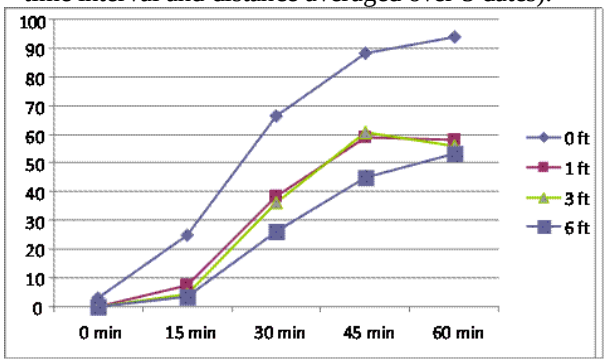
Temperatures and other environmental conditions during the assessments in Dallas County were all in the range for ant foraging (Drees et al. 2007) (**Table 1**). At all monitoring dates, the number of foraging worker ants attracted to the food lure was highest for hot dog slices placed directly on the center of the ant mounds and decreased at the 6 ft. distance from the mound. After 45 minutes, there were no significant differences between mean ant numbers at food lures along the transects. Ant numbers increased over the monitoring time period in all dates except July 11, when numbers declined after 45 minutes (**Figure 1**). During the June 11 assessment, at the 6 ft. placement of the hot dog slice, another ant species, the little black ant (*Monomorium minimum* (Buckley)), rather than the red imported fire ant occupied the hot dog slice after 30 (50 ants), 45 (70 ants) and 60 (120 ants)

minutes of placement. No other ant species were observed during these dates on any of the food lures.

Table 1: Environmental conditions during 2007 foraging ant assessments in at the Texas AgriLife Research and Extension Center, Dallas Co., TX.

	5-Jun	11-Jul	3-Aug	11-Sep	25-Aug
Conditions	sunny	sunny	overcast	overcast	clear
Time	8-9 am	8:30-9:30	7:30-8:30	1:30 - 11:30	11-Oct
Rh	80%	100%	94%	30%	10%
Air temp.	74°F	76	82	74	70
Soil temp.	76	74	74	70	70
Wind speed	0-2 mph	5	0	15-20	25

Figure 1: Mean foraging red imported fire ant workers associated with a hot dog slice food lure placed 0, 1, 3 and 6 ft from the center of the mound over time, at the Texas AgriLife Research and Extension Center in Dallas, 17360 Coit Road, Dallas Co., TX, observed on June 5, July 11, Aug. 3, Sept. 11 and Oct. 25, 2007 (n = 30; 6 ant mounds at each time interval and distance averaged over 5 dates).

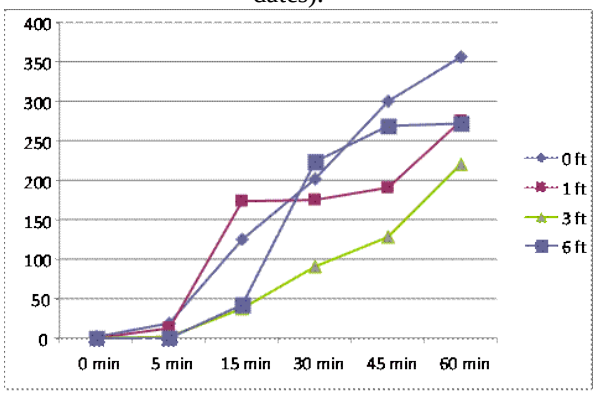


Temperatures and other conditions during foraging assessments in Brazos County were also suitable for ant foraging (**Table 2**). Ant numbers associated with food increased over 60 minutes and there was not a significant difference between the mean number of foraging worker ants attracted to the hot dog slice placed directly on top of the ant mounds or at any point along the transect over any of the observation times (**Figure 2**).

Table 2: Environmental conditions during red imported fire ant foraging worker assessments at the Pecan Genetics Lab, USDA, Hwy 50, Brazos Co., Texas, 2007.

	16-Jul		8-Aug		18-Sep		8-Nov	
	Mound 1	Mound 2	Mound 1	Mound 2	Mound 1	Mound 2	Mound 1	Mound 2
Conditions	Bright, sunny	Bright, sunny	Overcast	Part cloudy	Clear, sunny	Clear, sunny	Cloudy	Cloudy
Time	9:00-10:05 a.m.	9:50-10:50	9:00-10:00	9:45-10:45	8:50-9:50	9:10-10:10	9:00-10:00	9:10-10:10
Rh	79%	79%	79%	76%	77%	77%	71%	71%
Air temp.	84°F	88	86	86	86	86	74	74
Soil temp.	81°F	82	81	84	-	-	70	70
Wind speed	light breeze	light breeze	light breeze	light breeze	calm	calm	light breeze	light breeze

Figure 2: Mean foraging red imported fire ant workers associated with a hot dog slice food lure placed 0, 1, 3 and 6 ft from the center of the mound over time, at the Pecan Genetics Lab, USDA, Hwy 50, Brazos Co., Texas, observed on July 16, Aug. 8, Sept. 18 and Nov. 8, 2007 (n = 8; 2 ant mounds at each time interval and distance averaged over 4 dates).



Discussion

Results of these efforts document that placement of a food lure such as a hot dog slice in proximity to red imported fire ant mounds, time after placement, and presence of other ant species can affect results of foraging worker ant numbers attracted. In general, results confirm the monitoring methods already suggested for red imported fire ants: place food lures either randomly or in a grid or circular pattern during moderate temperatures (65˚ to 95°F) and estimate foraging worker ant numbers after 45 to 60 minutes. This time interval seems to be optimum to document random foraging patterns of red imported fire ant and avoids recruitment of additional foraging ants to the food lure if left out too long.

Results clearly demonstrate that red imported fire ants forage on top of their mounds or nests. These data should convince insecticide manufacturers of bait-formulated insecticides to consider revising their user directions to allow placement of the granular baits directly on top of undisturbed mounds in addition to scattering the bait around the mound. The possibility of developing bait stations deployed on top of or even inside ant mounds may hold promise as a target-specific fire ant treatment, particularly for insect growth regulator (IGR) active ingredients (Drees et al. 1992).

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Abstracts in Resistance Management

MECHANISM VERSUS MAGNITUDE IN UNDERSTANDING THE FITNESS COSTS OF INSECTICIDE RESISTANCE AND THEIR IMPLICATIONS FOR POLICIES TO OPTIMALLY MANAGE RESISTANT PESTS

Extended Abstract

Research in economic entomology has shown that the problem of insecticide resistance is akin to classic problems in resource economics (Laxminarayan and Simpson 2002). In particular, whether or not there is some genetic fitness tradeoff or "cost" associated with mutations conferring insecticide resistance in agricultural pests or disease vectors has been recognized as an important factor in determining whether or not susceptibility in the pest population can "re-charge" when insecticide use is suspended. The rate of re-charge, embodied through the magnitude of fitness costs, should therefore be a key determinant in policies which seek to optimally (i.e. economically) manage insecticide resistance (Grimsrud and Huffaker 2004; Qiao, Wilen et al. 2008).

However, we show that the mechanisms of fitness costs—and not just their magnitude—can be critical in determining optimal policies to manage insecticide resistance. Economic research in this area has usually assumed that fitness costs are present in terms of higher mortality rates in adult insects. This is a specific type of fitness cost, and it is well known among entomologists that there are multiple mechanisms aside from adult mortality which can impose a fitness cost on insecticide-resistant individuals (Gassman, Carriére et al. 2009).

We study a simple model of malaria control using insecticide spraying, allowing for the accumulation of resistance in the *Anopheles* vector population. In the model, we differentiate between fitness costs which

are incurred in the form of adult mortality versus those that are related to fecundity or are incurred at early developmental stages of the *Anopheles* lifecycle.

We calculate optimal economic policies to control malaria in a way that manages the problem of insecticide resistance, paying specific attention to how these policies change based on the distribution of fitness costs among the different biological mechanisms.

We find that economic policies seeking to manage insecticide resistance can incur unnecessary costs if based on incorrect knowledge about the mechanisms of fitness costs. For example, if a seemingly optimal policy is based on an assumption that fitness costs are entirely associated with adult mortality, but in actuality they are split roughly 50% between adult mortality and fecundity mechanisms, then we find that net economic costs are up to 200% higher than what they would be if based on the correct biological assumption.

This basic finding, aside from providing a strong motive to improve our understanding about the evolutionary dynamics of insecticide resistance in malaria vectors, has important implications for the management of pest resistance in general. In particular, our findings suggest that entomological studies identifying such fitness tradeoffs should be systematically documented in the Arthropod Pesticide Resistance Database (within which 578 cases of insecticide resistance in *Anopheles* were documented as of September 20, 2010): Such a system could contribute directly to a systems-based analysis of how to optimally manage pesticide resistance in a variety of settings.

Key words: resource economics, fitness costs, malaria, Anopheles

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Induction of systemic resistance by chitinase in tomato against Meloidogyne incognita by Pseudomonas fluorescens

ABSTRACT
The chitinase activity of four efficient strains of the bacterial biocontrol agent, Pseudomonas fluorescens were tested in tomato plants against root knot nematode, Meloidogyne incognita under field condition in Coimbatore, Tamil Nadu, India. Increased level of chitinase was induced in tomato roots treated with liquid formulation of P. fluorescens, indicates the systemic production of chitinase in tomato by the bacterial strains. The consortial formulation of P. fluorescens, Pf 128 with Bacillus subtilis, Bbv 57 recorded the highest chitinase activity in tomato plants compared with other isolates tested. The results indicated that P. fluorescens is capable of inducing systemic resistance against M. incognita in tomato by the accumulation of the defense enzyme like chitinase.
Key words. Tomato, biocontrol agent, enzymes, Meloidogyne incognita, systemic resistance.

Tomato (Lycopersicon esculentum Mill.,) is one of the most important vegetables in the world. It is seriously affected by root knot nematode, *M. incognita*. The economic loss in India due to this nematode was 27.21 per cent (Jain *et al.,* 2007). *P. fluorescens* is reported to induce systemic resistance against the plant pathogens. (Leeman *et al.,* 1995). The defense enzymes reinforce the cell wall structure and causes biochemical and physiological changes in the plant system (Chen *et al.,* 2000) which is directly inhibitory to nematodes (Paul and Kumar, 2003). The present study dealt with the induction of *P. fluorescens* mediated defense enzyme, chitinase in tomato against root knot nematode, *M. incognita*.

The treatment included four strains of *P. fluorescens viz.,* Pf 128, Pfbv 22, Pf1 and Pf 223 that had been proved efficient in suppressing root knot nematode, *M. incognita* in tomato. Two field experiments were conducted in tomato cv. 5005 infested with *M. incognita* in Coimbatore, India during 2008-09. Forty five days old tomato seedlings were used for the study. The experiment was laid out in a randomized block design (RBD) with 10 treatments and 3 replications. The treatments were

T_1 - Pf 128 @ 500ml/ha
T_2 - Pf bv 22 @ 500ml/ha
T_3 - Pf 1 @ 500ml/ha
T_4 - Pf 223 @ 500ml/ha
T_5 - Pf 128 + Bbv 57 @ 500ml/ha
T_6 - Pfbv 22 + Bbv 57 @ 500ml/ha
T_7 - Pf 223 + Bbv 57 @ 500ml/ha
T_8 - Pf 1 + Bbv 57 @ 500ml/ha
T_9 - Carbofuran 3G @ 1kg ai/ha
T_{10} - Untreated control

P. fluorescens strains *viz.,* Pf 128, Pfbv 22, Pf 223 and Pf 1 and *B. subtilis* strain Bbv 57 were sub cultured and prepared in liquid formulation containing cfu of 2.5 x 10^9/ ml and applied in the rhizosphere region through drip irrigation at the rate of 500 ml/ha. Carbofuran was applied at the rate of 1 kg ai/ha. Biochemical analyses were carried out at the time interval of 1, 7, 13, 30 and 90 days after application of biocontrol agents. Fresh root samples (1 g) randomly collected along the root system were homogenized in 1 ml of 0.1 M phosphate buffer pH 7.0 at 4 ^{o} C for 15 minutes and the supernatant served as enzyme source.

The calorimetric assay of chitinase was carried out according to Boller and Mauch (1988). The enzyme activity was expressed as nmole GluNAc equivalents/min/g fresh weight.

The results indicated that consortium Pf 128 + Bbv 57 treated tomato plants produced more chitinase (2.37 min^{-1} μg^{-1} root) on 7th DAA (Table 1) followed by consortium Pfbv 22 +Bbv 57 (2.18 min^{-1} μg^{-1} root). Untreated and Carbofuran treated plants showed a steady phase in chitinase production during the period of study. Induction of systemic resistance by *P. fluorescens* was correlated with the accumulation of chitinases (Murhofer *et al.,* 1994). Chitinase imparts resistance to the plants which results in the reduction of nematode population (Van Loon *et al.,* 1998).

Table 1: Effect of liquid formulation of *P. fluorescens* isolates on chitinase activity in roots of tomato cv. 5005 infested with *M. incognita* during different growth phases of the crop under field condition*

Treatments	Chitinase (change in glucose min^{-1} μg^{-1})									
	1 DAA		7 DAA		13 DAA		30 DAA		90 DAA	
	Root	% increase over control	Root	% increase over control	Root	% increase over control	Root	% increase over control	Root	% increase over control
Pf 128	1.61	30.11	1.66	35.23	1.54	23.20	0.79	78.79	0.035	52.17
Pfbvr 22	1.56	25.81	1.59	29.54	1.53	22.40	0.76	72.73	0.032	40.58
Pf 1	1.52	22.18	1.57	27.37	1.47	17.33	0.75	69.70	0.031	34.78
Pf 223	1.95	56.99	1.55	25.75	1.44	15.47	0.73	65.15	0.029	27.54
Pf 128+Bbvr 57	2.19	76.48	2.37	92.95	2.16	72.80	0.90	103.79	0.045	94.20
Pfbvr22+Bbvr 57	1.90	53.36	2.18	77.51	2.05	64.27	0.85	92.42	0.039	68.12
Pf 1+Bbvr 57	1.83	47.58	1.91	55.56	1.83	46.40	0.82	87.12	0.038	66.67
Pf 223 +Bbvr 57	1.57	26.75	1.70	38.48	1.64	31.20	0.79	80.30	0.038	63.77
Carbofuran.	1.29	3.76	1.29	4.61	1.29	3.47	0.45	1.44	0.025	8.70
Control	1.24	0.27	1.23		1.25	0.00	0.44		0.023	
CD (0.05)	0.04		0.06		0.11		0.02		0.041	

*Pooled analysis of data gathered from two field experiments
DAA - Days after application

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STUDY OF CULTURAL AND BIO-PESTICIDAL MANAGEMENT OF BRINJAL SHOOT AND FRUIT BORER (*Leucinodes orbonalis* Guen.)

Vegetables are one of the most important components of Indian Horticulture. In India a meal without vegetable is supposed to be incomplete. Though vegetable contribution in general is more profitable (4 - 8 times than traditional agriculture) due to their short crop duration and ready marketability, farmers are not able to harvest actual potential of the crop. One of the reasons for this is vulnerability to pest attack which causes reduced yield, unhealthy growth and produce or complete destruction of the crop.

Brinjal shoot and fruit borer, *Leucinodes orbonalis* Guenee (Lepidoptera: Pyralidae) is the most serious pest of brinjal and is the problem of brinjal growers yield ranging from 50 to 60 per cent. Damage by this pest up to 70 per cent is not uncommon and vitamin C content may be reduced by 68 per cent. Scientists are looking into the relative importance of brinjal fruit and shoot borer, *Leucinodes orbonalis* Guen. Infesting brinjal, the present work entitled, "Study of cultural and bio-pesticidal management of brinjal shoot and fruit borer (L*eucinodes orbonalis* guen.)", was carried out during the year 2003-2004. The field trials were conducted at the Agricultural Research Farm of Banaras Hindu University, Varanasi, Uttar Pradesh, India during Kharif, 2003 and 2004.

The salient finding of various experiments such as effect of intercropping and bio-pesticides on the

incidence of brinjal shoot and fruit borer, *Leucinodes orbonalis* Guen., effect of date of transplanting against brinjal shoot and fruit borer and evaluation of IPM module against brinjal shoot and fruit borer.
The intercropped with coriander and fennel showed that the minimum shoot damage (21.57 %) was observed in T_5 (Brinjal + Coriander conjugation with Azadirachtin 0.03 % @ 2.5 ml/l) followed by T_2 (Brinjal + Fennel and spray of Azadirachtin 0.03 % @ 2.5 ml/l) with 24.17 % damage.

The intercropped with coriander and funnel revealed that the minimum fruit damage (19.79 %) was observed in T_5 (Brinjal + Coriander and spray of Azadirachtin 0.03 % @ 2.5 ml/l) followed by T_2 (Brinjal + Fennel and spray of Azadirachtin 0.03 % @ 2.5 ml/l) with 21.79 % damage.

The crop combination revealed that the minimum fruit weight loss (23.33 %) was observed in $_{T5}$ (Brinjal + Coriander and spray of Azadirachtin 0.03 % @ 2.5 ml/l) followed by $_{T2}$ (Brinjal + Fennel and spray of Azadirachtin 0.03 % @ 2.5 ml/l) with 22.59 % damage.

The different transplanting dates revealed both significant as well as non-significant differences in respect of shoot damage caused by *Leucinodes orbonalis* Guen.

The minimum shoot damage (24.93%) was recorded in T6 followed by T5 with 36.01% shoot damage, which differed significantly from each other in unprotected plot.

The minimum fruit damage (41.16 %) was recorded in T6 followed by T1 with 42.15 % fruit damage, which were differed significantly from each other in unprotected plot. The similar trends in observations were found in protected plot but T3 (transplanted on 25th July) differed significantly from T5 (transplanted on 23th August) while at par with T2 (transplanted on 10th July), T4 (transplanted on 8th August), T1 (transplanted on 25th June (Table-15).

The minimum loss in weight (18.53 %) was recorded in T4 followed by T5 with 19.02 % fruit weight loss, which were at par with each other in unprotected plot.

The IPM module revealed on test variety Punjab Sadabahar that the minimum shoot damage (14.59 %) was observed in T2 (T_1 + soil treatment by carbofuran 3G 1.5 kg/ha at 30 DAT) followed by T4 (Endosulfan 35 EC @ 3 ml/lit + NSKE (5 %) at 30 days after transplanting at 15 days interval) with 14.71% shoot damage, which were differed non-significantly from each other.

The most effective treatment was T2 (T_1 + soil treatment by carbofuran 3G 1.5 kg/ha at 30 DAT) which had 14.59 % shoot damage and had significant difference from other treatments except T4 (Endosulfan 35 EC @ 3 ml/lit + NSKE (5 %) at 30 days after transplanting at 15 days interval).

The fruit damage observed in all the treatments differed significantly over the control. All the treatments differed significantly over control in recording the fruit weight loss and in test variety Pant Rituraj that the shoot damage in treatments was varied from 24.16 % to 50.22 %. The lowest shoot damage (24.16 %) was recorded in T2 (Endosulfan 35 EC @ 3 ml/lit + NSKE (5 %) at 30 days after transplanting at 15 days interval) followed by T4 with 26.85. The most effective treatment was T2 with 24.16 % shoot damage followed by T4 having 26.85 % shoot damage.

The treatments T1 to T5 were superior over control. All the treatments differed significantly from control. T2 and T4 showed significant difference from rest of the treatments and T2 (T_1 + soil treatment by carbofuran 3G 1.5 kg/ha at 30 DAT) had lowest fruit damage (19.75 %) followed to T4 (Endosulfan 35 EC @ 3 ml/lit + NSKE (5 %) at 30 days after transplanting at 15 days interval) with 22.80% as significantly differed.The treatments from T1 to T6 were found superior over control (water spray). All the treatments differed significantly from control (water spray). T2 and T5 treatments showed significant difference from rest of the treatments.The study showed that the infestation level can be minimized by multiple cultural method as intercropped brinjal with coriander and fennel in conjugation with biopesticides such as, neem seed kernel extract (5 %) and neem formulation (Azadirachtin), alteration the date of transplanting and also adoption of IPM module for management of brinjal shoot and fruit borer, *Leucinodes orbonalis* Guen.

Keywords: intercropped with coriander and fennel, different transplanting dates, IPM module

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Eco-friendly management of Sugarcane Woolly Aphid, *Ceratovacuna lanigera* Zehntner

Sugarcane woolly aphid, *Ceratovacuna lanigera* Zehntner (Homoptera: Aphididae) was observed to be a new emerging pest of sugarcane from Tropical (Maharashtra, Karnataka, Andhra Pradesh, Tamil Nadu, Gujarat and Kerala), as well as sub-tropical India (Assam, Nagaland, Tripura, West Bengal, Uttar Pradesh, Bihar, Uttarakhand and Haryana). Earlier this pest was restricted to mainly north-eastern hill region in the form of a minor pest of sugarcane until 2001-02 crop season, when unprecedented outbreak of sugar cane woolly aphid occurred at many places in India. Injury to the host plant results in the withdrawal of large quantities of sap from the dorsal surface of the leaf. The honeydew excreted from the *C. lanigera* falls on the upper surface of the lower leaves on the which black sooty mould, *Capnodium* sp. develops forming a thick black coating, which severely impairs the process of photosynthesis. As a result, vigour, plant growth, tonnage and quality of the cane are deteriorated. In severe cases tops of the infested canes are rendered useless as fodder. A cost effective and environment-friendly way to combat woolly aphid menace has been found in the form of pyralid predator, *Dipha aphidivora*. This predator has been utilized to contain the burgeoning population of sugarcane woolly aphid efficiently. *Dipha* an voracious feeder with ability to devour the pest population under favorable condition was mass multiplied under the shade nets and was released @ 1000 larvae/ha in severely infested fields with sugarcane woolly aphid having variety CoS 767 (Ratoon crop) near Triveni Engineering & Industries Ltd. (Sugar Unit), Khatauli in District Muzaffarnagar, U.P. Two releases were made at 15 days intervals in the evening hours during the month of September. The predator established well in the field and started predating woolly aphid actively. Population of woolly aphid was reduced to 63.10 per cent within 45 days of release. The predator fed both, the nymphs and adults of sugarcane woolly aphid voraciously and proved to be very effective against the woolly aphid. *Dipha* feeds on woolly aphid by forming web. Aphids coming under webbing were killed. The second and third instars of *Dipha* were found more voracious than the first and second instar larvae. Application of pesticides was avoided for conservation and protection of predator after the release of predators in the woolly aphid infested field.

Key words- Pyralid predator, Sugarcane woolly aphid, *Dipha aphidivora*, Conservation

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