Resistant Pest Management Newsletter

A Biannual Newsletter of the **Center for Integrated Plant Systems (CIPS)** in Cooperation with the **Insecticide Resistance Action Committee (IRAC)** and the **Western Regional Coordinating Committee (WRCC-60)**

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Table of Contents

Letter from the Editors

Dear Subscribers,

Recently The Arthropod Pesticide Resistance Database (APRD) was mentioned twice in an article in *Science Magazine*. The article citation is:

Coehlo, Sara. 2009. European Pesticide Rules Promote Resistance, Researchers Warn. *Science* 323: 450.

Remember that the APRD can be found at http://www.pesticideresistance.org/DB. It is a database of reported resistance cases from 1914 to the present, citing when resistance is first discovered for a specific time and place. Pesticide resistance is a dynamic, evolutionary phenomena and a record in this database may or may not be indicative of your area. Similarly, the absence of a record in this database does not indicate absence of resistance.

An extensive piece was published about the database in the Fall 2007 (Edition 17.1) Newsletter. Please review this edition of the newsletter for additional information on the Database. If you would like an additional copy of this section of the newsletter, please email us at RPMNews@msu.edu.

Once again, thank you for your continued support and contributions.

Sincerely,
Brittany Harrison
RPM Newsletter Coordinator
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Resistance Management from Around the Globe

Colorado beetle resistance to insecticides in South Urals

Abstract
After the considerable intermission more then 10 years in South Urals (Republic Bashkortostan territory) the evaluation of susceptibility in overwintered Colorado beetle adults to some modern insecticides, are widespread applying in private potato plantations was scored. The multiresistance forming in Bashkortostan objected. Investigations funded partially by RFBR 09-04-00391.

Introduction

Private potato plantations spread on the main part of total potato plantations area in Bashkortostan. There is no crop rotation in these plantations, so there are rather favorable conditions for stabilization of Colorado beetle in local populations.

Increasing of resistance level in Colorado beetle populations in Bashkortostan predicted still in 1986 (Amirkhanov et al., 1986) seemed to be the same everywhere. Under the toxicological evaluation of susceptibility since 2000 the resistance forming to organophosphates and pyrethroids revealed (Benkovskaya et al., 2004). However, the annual susceptibility analysis in overwintered adults from 10 to 38 excerpts carried out in 2003-2006 allowed us to detect the mosaic character of resistance spreading in the Bashkortostan territory (Benkovskaya et al., 2004; Leontieva et al., 2006; Udalov, 2006).

Materials and Methods

Overwintered Colorado beetle adults were collected (200 – 400 individuals from each of 30 localities) during the mass oviposition on potato in the private plantations in different districts of Bashkortostan. Evaluation of susceptibility to the set of insecticides from classes of POI (malathion), pyrethroids (deltamethrin), neonicotinoids (acetamiprid and thiamethoxam) and phenylpyrazoles (fipronyl) was carried out by topical infliction of ethanol solutions of insecticides (1 mcl per individual) on the thorax of adults. Diagnostic concentrations of insecticides corresponding with this manner of treatment were determined in the preliminary experiments. Diagnostic concentrations were calculated as doubled LC 95, determined in 2005 for deltamethrin (0.005 %), malathion (0.5 %), acetamiprid and thiamethoxam (0.0005 %), fipronyl (0.0003 %).

Under laboratory conditions the share of susceptible and resistant to diagnostic concentrations individuals was estimated in each excerpt of 100 adults.

Results and Discussion

Data of susceptibility level in Colorado beetle adults to diagnostic concentrations of some insecticides obtained in 2005 presented in Table 1.

Table 1. Mortality of overwintered Colorado beetle adults under the treatment by insecticides diagnostic concentrations (Bashkortostan, 2005)

District	Excerpts number	Mortality, % by Abbot's correction			
		malathion	deltamethrin	acetamiprid	thiamethoxam
Ufimsky	2	1.25±1.25	37.5±2.3	69.5±9.5	94.0±4.0
Tuymazinsky	2	22.2±17.2	72.5±2.5	74.5±24.0	94.5±4.1
Salavatsky	2	11.3±6.3	64.3±5.5	74.3±15.5	94.1±5.9
Blagovarsky	4	52.8±16.1	83.6±4.03	no data	no data
Arkhangelsky	2	25.0±10.3	76.2±6.6	no data	no data

There is evident difference between the local populations been comparing by share of the susceptible and resistant individuals. This is probably the manifestation of species habitats insularization (Yablokov, 1987).

In our investigations insularization processes are illustrated mainly with the data of toxicological experiments with POI, which has been applying in Bashkortostan about 30 years. Pyrethroids are in use during 15-20 years, and neonicotinoids are in use during 4-6 years.

The results of toxicological experiments in 2006 confirmed multiresistance forming in South Urals territory (tab. 2).

Table 2: Mortality of overwintered Colorado beetle adults under the treatment by insecticides diagnostic concentrations (Bashkortostan, 2006)

Districts	Mortality, % by Abbot's correction				
	deltamethrin	malathion	thiamethoxam	acetamiprid	fipronyl
Ufimsky	43.8±4.2	no data	81.3±2.4	no data	93.8±3.8
Burzyansky	50.0±4.9	10.0±2.5	88.9±2.9	33.3±0.5	94.4±2.48
Sterlibashevsky	15.8±1.2	21.1±2.0	99.9±0.1	52.1±5.3	100
Karmaskalinsky	20.0±5.6	25.0±5.1	100	90.0±8.2	95.0±4.5
Ilishevsky	47.4±2.6	42.0±4.9	100	78.9±1.1	95.0±5.0
Aurgazinsky	16.7±2.0	11.1±2.3	94.8±1.2	72.2±6.8	100
Arkhangelsky	5.0±0.5	0.0	94.1±8.9	64.7±4.8	100
Miyakinsky	40.0±3.9	13.3±1.1	100	no data	100

Arising of resistance to new compounds was not an unexpected phenomenon. Resistance forming to neonicotinoids in some regions of Russia took place in 5 years and to fipronyl during 2 years (Roslavtseva, Mikhina, 2001; Sukhoruchenko, 2005).

Complicated subdivided structure of species population in Bashkortostan is supporting probably by perennial private potato plantations, repeating non-coordinated insecticidal treatment without preliminary estimation of effectiveness of insecticides applying and environmental conditions of potato cultivating regions.

Conclusion

The situation turned out in South Urals requests the systematically investigation of Colorado beetle local populations and evaluation of susceptibility to insecticides changing. Recommendations for insecticides using in rotation schemes can be given after the next checking of their effectiveness in the concrete regions of Bashkortostan.

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INVESTIGATION AND MONITORING OF ACARICIDE RESISTANCE TO TWO SPOTTED SPIDER MITE *TETRANYCHUS URTICAE* (KOCH) IN BHENDI

INTRODUCTION

Vegetables are comprised of a large number of plants, mostly annuals, of which different parts like leaf, stem, flower bud, fruit, root etc are eaten. They are rich in nutrients and form an essential item of a balanced diet by providing not only energy but also supplying vital protective nutrients like minerals and vitamins. India is the second largest producer of vegetables in the world next to China with an estimated production of about 78.2 million tonnes from an area of 5.73 million hectares with an average yield of 13.6 tonnes per hectare (Anonymous, 2003).

Among the vegetables, okra or bhendi, *Abelmoschus esculentus* (L.,) is the most popular vegetable in India. It is an annual vegetable crop propagated from seed in tropical and subtropical regions of the world. In India, it occupies an area of 3, 70,000 ha with the production of 35, 50,000 metric tonnes (FAO Report, 2005).

Among the non insect pests, several species of mites belonging to the genus *Tetranychus urticae* cause damage (Srinivasa and Sugeetha , 1999) and exert a loss in the yield of okra fruits ranging from 7-48 per cent (Anonymous, 1996). These spider mites are also reported as major pests on field and horticultural crops including sorghum, cotton, castor, brinjal and tomato and fruit crops like plum, peach, apple and citrus (Van de vrie, 1985).

Though, many non chemical control strategies are advocated under the IPM umbrella, still the farmers in developing countries like India mainly rely on chemical pesticides for the management of the pest due to unsatisfactory control from cultural and biocontrol methods (Jeyachandaran , 2003). Moreover *T. urticae* can easily adapt to plant varieties that have been especially selected for resistance to it (Gould, 1979).

As mite control at present relies overwhelmingly on chemicals, attention must be given to prevent the loss of such potential, which is a key factor in Integrated Mite Management programmes (IMM). Thus, every effort has to be made to prolong the effective lifespan of currently available selective acaricides especially those that fit into IMM programme. However, the ability of this mite to develop high levels of resistance

to acaricides rapidly even after only a few applications cannot be ruled out (Rauch and Nauen, 2003). Resistance to almost all the available groups of acaricides to *T. urticae* is known from different parts of the world (Devine *et al.* 2001). Therefore, focus should be oriented towards the establishment of baseline toxicity data for the frequently used acaricides and use the same for monitoring of acaricide resistance in *T. urticae*. In India, very little work has been done on the acaricide resistance in *T. urticae* on okra.

Hence keeping the above view, the present study was undertaken on the acaricide use pattern, establishment of baseline toxicity data for wettable sulphur to *T. urticae* on okra and monitoring of acaricide resistance.

MATERIALS AND METHODS

Investigations were carried out to the fixation of baseline toxicity data for the red spider mite, *Tetranychus urticae* for wettable sulphur was carried out and monitoring of resistance in red spider mite for the above acaricide was done in Coimbatore district on okra crop. The materials and methodologies adopted for the studies are described below.

Mass culturing of test insects for baseline toxicity data generation

The test insects of red spider mite required for the generation of baseline toxicity data were mass cultured in the insectary of Dept. of Agrl. Entomology, using potted plants of okra (var. Mahyco No .10 hybrid) at 28 ± 1°C with 80 ± 5 % relative humidity. The mites collected from two locations were mass cultured separately by generation wise with careful transfer of populations of one generation to the potted plants of okra and labelled systematically for carrying out the bioassay studies.

Acute toxicity studies

The required acaricide concentrations were prepared from the formulated products using distilled water.

Leaf disc bioassay for *T. urticae*

The bioassay method followed for *T.urticae* was Insecticide Resistance Action Committee, (IRAC) method No. IV.

Fresh okra leaf discs of 45 mm diameter, unexposed to acaricides were used. The required different concentrations of wettable sulphur were prepared using distilled water. Then the leaf discs were dipped in the respective acaricide concentrations for five seconds and then shade dried on a filter paper in open air (Croft and Nelson, 1973). Then the treated leaf discs were placed on the moistened cotton mat kept in a petridish (50 mm diameters).

Thirty adult females collected from the respective location and generation were transferred to the treated leaf disc using fine camel hair brush and covered with the upper lid. Leaf discs treated with distilled water served as control. Three replications were maintained. Mortality of the adult female mites was assessed at 24 and 48 hours after exposure to acaricide. A mite was scored as alive if at least one leg moved repeatedly when the mite was prodded by a brush. Necessary corrections were made for the natural mortality using Abbot's formula (1925).

Monitoring of acaricides resistance

Field level monitoring of wettable sulphur resistance in *T. urticae* was assessed based on the mean mortality of mites at discriminating dose. The mite populations for monitoring were collected from three locations in each block of Coimbatore district. Total monitoring was done in ten blocks of Coimbatore district. Finally, the mean per cent mortality of mites at 24h and 48 hr were arrived for the different blocks of Coimbatore district.

RESULTS

Acute toxicity of wettable sulphur to *T. urticae*

The LC_{50} and LC_{95} values for seven generations of *T. urticae* collected from Thondamuthur, Coimbatore are presented in Table. 1. The LC_{50} of wettable sulphur assessed for F_1 population was 18.822 ppm and LC_{95} value was 192.92 ppm (Table 1). The susceptibility of F_7 generation was moderately increasing with an LC_{50} value of 10.11 9 ppm. The LC_{95} value was also found to decrease form 192.92 ppm (F_1) to 131.39 ppm (F_7) (Table 1).

Table 1: Acute toxicity of wettable sulphur to *Tetranychus urticae* by leaf dip method (Thondamuthur)

Number of generations	Regression equation	Chisquare	LC_{50}(ppm)	Fiducial limit UL	LL	LC_{95}(ppm)	Fiducial limit UL	LL
First generation	Y= 2.925+1.627x	3.389	18.822	13.724	25.814	192.92	99.578	373.79
Second generation	Y=2.902+1.671x	4.181	17.911	13.242	24.226	172.58	93.610	318.17
Third generation	Y= 3.069+1.613x	1.680	15.720	11.428	21.623	164.36	88.669	304.68
Fourth generation	Y= 3.272+1.534x	1.895	13.352	9.480	18.806	157.57	82.639	300.45
Fifth generation	Y= 3.416+1.487x	3.947	11.590	7.981	16.833	147.83	73.208	298.52
Sixth generation	Y=3.532+1.452x	2.807	10.240	6.974	15.036	139.01	65.971	292.94
Seventh generation	Y=3.515+ 1.477x	2.518	10.119	6.894	14.852	131.39	65.177	264.88

The susceptibility index (SI) of F_7 generation over F_1 was 2 and 1.26 based on LC_{50} and LC_{95} respectively. The rate of resistance decline (R) was found negative indicating that susceptibility increased with the subsequent generations. The R value was – 0.0381. Thus the number of generations required for a 10 – fold decrease LC_{50} was calculated as 26.31 (Table 2). The discriminating dose (DD) arrived for wettable sulphur based on F_7 generation was 145 ppm. The LC_{50} and LC_{95} values for nine generations of *T.urticae* collected from Annur, Coimbatore are presented in Table 3.

Table 2 Susceptibility index (SI) and rate of resistance decline of wettable sulphur for *Tetranychus urticae* (Thondamuthur)

Acaricides	Susceptibility Index		Rate of resistance decline	
	LC_{50}/LC_{50}	LC_{95}/LC_{95}	R	G
Wettable sulphur	2	1.26	-0.0381	26.31

Table 3: Acute toxicity of wettable sulphur to *Tetranychus urticae* by leaf dip method (Annur)

Number of generation	Regression equation	Chisquare	LC_{50} (ppm)	Fiducial limit UL	LL	LC_{95} (ppm)	Fiducial limit UL	LL
First generation	Y=2.507+1.765x	4.968	25.801	19.606	33.955	220.513	113.158	429.714
Second generation	Y=2.691+1.707x	3.911	22.494	16.843	30.04	206.702	105.561	404.750
Third generation	Y=2.769+1.693x	2.683	20.753	15.409	27.949	194.293	100.355	376.16
Fourth generation	Y=2.952+1.635x	1.984	17.846	12.898	24.694	180.798	93.108	351.07
Fifth generation	Y=3.062+1.594x	1.600	16.401	11.881	22.641	176.322	89.572	347.089
Sixth generation	Y=3.025+1.663x	0.886	15.369	11.186	21.117	149.752	80.574	278.324
Seventh generation	Y=3.072+1.669x	0.367	14.278	10.290	19.813	138.042	74.688	255.136
Eight generation	Y=3.115+1.679x	0.253	13.241	9.455	18.544	126.207	70.205	226.883
Ninth generation	Y=3.081+1.719x	0.395	13.056	9.374	18.183	118.172	67.763	206.081

The same trend as recorded in Thondamuthur was noticed. The susceptibility index (SI) of F_9 generation over F_1 was 1.26 and 1.86 based on LC_{50} and LC_{95} respectively. The rate of resistance decline (R) was found negative indicating that susceptibility increased with the subsequent generations. The R value was -0.0124. Thus, the number of generations required for a 10 fold decrease in LC_{50} was calculated as 80.64 (Table 4). The discriminating dose (DD) arrived for wettable sulphur based on F_9 generation was 130 ppm.

Table 4: Susceptibility index (SI) and rate of resistance decline of dicofol and wettable sulphur for *Tetranychus urticae* (Annur)

Acaricides	Susceptibility Index		Rate of resistance decline	
	LC_{50}/LC_{50}	LC_{95}/LC_{95}	R	G
Wettable sulphur	1.26	1.86	-0.0124	80.64

Monitoring the level of resistance

The results of the studies on monitoring the level of resistance of wettable sulphur to *T. urticae* in eight blocks of Coimbatore district are presented in Tables 5. When the discriminating dose of wettable sulphur (145 ppm) were applied to field collected *T. urticae* it was found that the mean per cent mortality of *T. urticae* in different blocks ranged from 79 to 83.00. Highest mean per cent mortality (83) was recorded in Periyanayaganpalayam and madukkarai block (Table 5).

Table 5: Mortality level of different field population of *Tetranychus urticae* at discriminating dose of wettable sulphur

S.No	Blocks	Per cent mortality
		Mean ± SD
1	Periyanayagan palayam	83±4.48
2	Madukkarai	83±4.48
3	SS Kulam	81±4.77
4	Karamadai	81±4.77
5	Udumalaipetti	81±4.77
6	Kinathukadavu	80±4.92
7	Pollachi North	79±5.06
8	Pollachi South	79±5.06

DISCUSSION

The result of the present investigations is the first platform to fix the susceptibility index and baseline toxicity of wettable sulphur to *T. urticae* on okra. The discriminating dose arrived for wettable sulphur based on F_7 generation in Thondamuthur and on F_9 generation in Annur was 145 ppm and 130 ppm.

The baseline toxicity data established can be used for further monitoring of resistance in *T. urticae* for wettable sulphur.

T. urticae populations worldwide had developed resistance to dicofol , amitraz, organotins, propargite , pyrethroids , fenbutatin oxide and METE (Mito Chondrial Electron Transport Inhibitors) acaricides , fenazaquin , fenpyroximates , pyridaben and tabufenpyrad (Croft and Van De Bann, 1988; Nauen *et al* ., 2001; Knight *et al* ., 1990; Herron and Rophail, 1998 ; Kim *et al*. 2004).

In India, there are no published literatures on the establishment of base line toxicity data for dicofol and wettable sulphur to *T. urticae* on okra and thus the results obtained in the present investigations would be useful for future studies.

Monitoring the level of resistance

The current resistance level in *T. urticae* to wettable sulphur was assessed on okra using the developed discriminating dose as a one time survey in eight blocks of Coimbatore district.

When the discriminating dose for wettable sulphur at the discriminating dose (145 ppm), the mean per cent mortality of *T. urticae* in different blocks ranged from 79 to 83.00 (Table 5).

Besides due to the lower selection pressure also higher mortality might have occurred. However, the present investigation has disclosed that the possibility of development of initial resistance in *T. urticae* to wettable sulphur, since more than 10 per cent *T. urticae* populations survived in the respective discriminating doses. This should be confirmed by future studies. However, the Korean population of *T. urticae* showed high levels of resistance to fenpyroximate (Resistance Ratio (RR , 182)), dicofol (RR ,82) and pyridaben (RR, 78) but little or no tolerance to abamectin (RR 6.5) fenpyropathrin (RR , 9.1) , propargite (RR, 6.5) and azocyclotin (RR, 5.1) (Cho *et al* .1995).

In India, this is the first documented report of resistance monitoring in *T. urticae* to wettable sulphur. However, the wide spread acaricide resistance has been a major obstacle in the cost effective integrated mite management programme in Korea (Kim *et al*., 2004).

In the present preliminary study on establishment of baseline toxicity data for wettable sulphur to *T. urticae* on okra for the first time in India and the field level monitoring of resistance in *T. urticae* for wettable sulphur encouraging results were obtained. Intensive research on this approach paves way for effective management of *T. urticae* on okra which will reduce the insecticide / acaricide load and preserve the biodiversity.

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MODELING THE OCCURRENCE OF *PROSTEPHANUS TRUNCATUS* (HORN) (COLEOPTERA: BOSTRICHIDAE) IN MALAWI

ABSTRACT
Historical climate data for Ngabu and Chikwawa district (Southern Malawi) were used in investigating the role of climatic factors on *P. truncatus* abundance. Temperature and relative humidity data were fitted in the Hodges *et al*. (2003) model in order to test if it could work under Malawian climatic conditions. All data was subjected to $\log_{10}$ (x+0.5) before linear regression and cumulative sum chart (CUSUM) analysis were carried out. Results indicated that none of the climatic factors significantly influenced the abundance of *P. truncatus.* It remains to be determined whether these observations hold true across other locations, or is specific only to the study area.

INTRODUCTION

Research has also shown that *P. truncatus* occurs in selected areas and not in others, and that infestation varies from one season to the other. In addition, the numbers of beetles flying around looking for food is strongly affected by climate (Borgemeister *et al*., 1997b; Nansen and Meikle, 2002). Several models have been developed to predict the occurrence of LGB (for example, Shires, 1979; Haubruge and Gaspar, 1990; Tigar *et al.*, 1994 and Giles *et al.*, 1995; Hodges *et al*., 2003). The latest model by Hodges *et al*. (2003) model is the most recent. The model predicts the population of LGB adults likely to disperse. As soon as predictions of beetle numbers have been made and they exceed a predetermined threshold, based on the known probabilities of store infestation, then farmers can be warned to take action. This action will either be in the form of advice to farmers to immediately market their maize and dried cassava or invest in pest management. As store infestation is defined as the presence of a single beetle, it would be several months before serious damage would be expected. Thus, prediction of likely *P. truncatus* infestation in an area/region would give a reasonable lead-time for pest management action. The model has been tested in Tanzania and Ghana. In these countries, the model was able to predict two bad years (Hodges, 2002). However, the predictability of the model has not been tested rigorously. The present study therefore set out to collect and analyze historical meteorological and *P. truncatus* trap catch data from southern Malawi in order to test the prediction model developed by Hodges *et al*. (2003).

MATERIALS AND METHODS

Study Site

Chikwawa district is found in the Southern Region of Malawi (16.02° S 34.50° E). It is part of what is referred to as the Lower Shire Valley in which altitude ranges from about 120 m in the north to 40 m in the south. The area lies in the rain shadows of Mulanje and Phalombe mountains and the watershed of the Shire and Zambezi rivers. Temperature averages 30°C while rainfall averages 740 mm in a year. In addition, the area is subjected to annual flooding and these waters are used for dry season crop cultivation. A little secondary vegetation cover exists in Chikwawa (Msiska *et al.*, 1994).

Source of climate and *P. truncatus* trap catch data

Climate data for the period November 1996 to November 2002 for Chikwawa district were obtained from Department of Meteorology Headquarters in Blantyre. *Prostephanus truncatus* trap catch data (for the years 1996, 1997, 1999, 2002 and 2008) was obtained from Bvumbwe Agriculture Research Station.

Predicting *P. truncatus* abundance

The procedure and rules followed by Hodges *et al.* (2003) were adopted in the present study. Climate data were entered into a spreadsheet as 2-weekly means of daily mean temperature (T) and percentage relative humidity at mid-day (R) and the actual number of days with less than 40% relative humidity at mid-day (H). Cumulative sums of means were used in data analysis.

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PROSTEPHANUS TRUNCATUS (HORN) (COLEOPTERA: BOSTRICHIDAE) INFESTATION LEVELS ON DIFFERENT MAIZE VARIETIES IN MALAWI

ABSTRACT

Prostephanus truncatus (Coleoptera: Bostrichidae) is a very destructive pest of stored maize in Malawi, causing serious losses of up to 30% of stored maize in three to six months. Susceptibility to *P. truncatus* was evaluated for 10 hybrid maize varieties by infesting 50 g (shelled grain) of each with 32 *P. truncatus* larvae. Samples were incubated at 27±1°C and 70±5% r.h. for 9 weeks before resistance parameters were determined. Susceptibility assessments were based on number of beetle progeny emerged during the incubation period; percentage grain damaged and grain weight loss. The role of grain physical characteristics on *P. truncatus* development was also investigated. Results indicated that *P. truncatus* was able to infest and develop on all the 10 varieties. There were significant differences (P = 0.005) among the maize varieties for grain weight loss, percentage grain damaged and the number of *P. truncatus* progeny emerging during the 9 week incubation period. Weight loss incurred by the varieties ranged from 7.7 to 30.3%, percent grain damaged ranged from 33 to 66.7%, and numbers of *P. truncatus* progeny ranged from 41 to 99, respectively. Maize varieties with the thickest pericarps were highly susceptible to infestation by *P. truncatus*. Nine of the 10 maize varieties evaluated were classified as susceptible to *P. truncatus* infestation. The type of maize variety played a significant role in the determination of susceptibility to *P. truncatus* infestation. Since maize cultivation in Malawi is not restricted to hybrid varieties only, there is also need to evaluate resistance factors and susceptibility of open pollinated varieties (O.P.Vs) and other 'local' varieties.

Random sampling of maize stores in four selected villages showed that the maize was infested with *P. truncatus* and *S. zeamais*, although the latter was the most abundant. No *P. truncatus* biological control agent(s) was found in all the maize stores. Results also indicated that 64% of the maize stored was treated with ACTELLIC SUPER ® (1.6% pirimiphos – methyl + 0.3% permethrin) while the rest was not treated. *Prostephanus truncatus* and *S. zeamais* were found infesting even these Actellic-treated maize stores. These studies demonstrated that *P. truncatus* was capable of infesting all kinds of maize varieties under traditional storage procedures and that no biological control agent was playing a role in suppressing its populations. There is a need therefore to enhance current pest management measures being used by farmers. Future work should aim at determining the use and effectiveness of insecticides being used, and level of insecticide resistance in the storage pests.

INTRODUCTION

Damage due to storage insect pests results in direct food loss and reduces the future maize production for farmers who use saved grain as seeds (approximately 70% of all planted maize in Eastern and Southern Africa) (CIMMYT, 1994; Pingali and Pandey, 2001). Available literature on the control of storage insect pests shows that major emphasis has been on the use of insecticides and biological control agents. The increasing occurrence of insecticide resistance (Perez-Mendoza, 1999) and environmental concerns about the use of chemical insecticides, calls for alternative pest management methods.

Studies by Singano et al. (2007) indicated that maize in storage in Malawi is infested by the following insect pests: *Sitophilus* species, *Oryzaephilus* species, *Plodia interpunctella*, *Tribolium species*, *Ephestia species*, *S. cerealella* and *P. truncatus*. However, the occurrence and abundance of these species varies with the time of the year, storage type and period, and pest management option used (Borgemeister et al., 1997a). Having knowledge of insect pest spectrum likely to be present in an area and the common pest management option used by farmers may help in deciding how much effort

the problem warrants and provides a key to effective pest management.

Limited research has been carried out to investigate the role of plant resistance in the management maize storage insect pests (Kossou et al., 1993). According to available literature, most work on maize resistance to insect pests was focused on Sitophilus species and stemborers (Giles and Ashman, 1971; Almy and Asanga, 1988; Demissie et al., 2008). Results from such studies indicate that varieties of the same grain commodity may vary quite significantly in their inherent susceptibility to postharvest insect pests due to a number of factors. Such factors include various biochemical and physical characteristics of the grains, growing conditions and damage incurred during harvesting and storage (Li, 1988; McNee, 2003: Smale and Jayne, 2003 and Garcia-Lara et al., 2004). It is important, therefore, to identify maize varieties suitable for a given agro-ecological zone, which are tolerant to insect pests and diseases found in the area. Farmers can be encouraged to grow such varieties because use of disease- and pest-resistant maize varieties is considered to be the safest and cheapest means of pest and disease control.

In addition, the use of resistant grain varieties can enhance the effectiveness of biological and chemical control methods against stored-product insect pests (Schöller 1998). For instance, Ofuya and Credland (1996); found that the development of larvae of Bruchidius atrolineatus was prolonged in some varieties of cowpea, increasing the opportunity for parasitoids to attack the larvae, which is the pest stage of this bruchid. Further, the use of resistant plant varieties would extend produce storage period without the use of insecticides. Maize seed breeders may incorporate the characteristics that confer resistance during breeding so that disease- and insect pest-resistant varieties are developed. Hence, the present study set out to investigate *P. truncatus* infestation levels on ten maize varieties grown in Malawi.

MATERIALS AND METHODS

Insect Cultures

Prostephanus truncatus larvae and adults were obtained from infested stored cassava chips in Chikanda, Bwaila and Four Miles. All these are farming communities found within Zomba district. Shelled MH18 and 'Kanjerenjere' were used for rearing of *P. truncatus.* MH18 (a non-conventional hybrid made from top cross of Malawian hybrids and an International Maize and Wheat Improvement Centre (CIMMYT) population) is considered to be one the most susceptible maize varieties to maize weevils. Therefore, MH18 was used as the susceptibility check and 'Kanjerenjere' as the resistant check.

Then four hundred randomly selected LGB adults were added to 6 kg of shelled MH18 grain, while 200 LGB adults were added to 3.2 kg of shelled 'Kanjerenjere' grain. In addition, 316 LGB larvae were added to 2.6 kg of MH18. All these were kept in separate PVC pipes. The PVC pipes measured 11 cm in internal diameter and 100 cm high, covered with a double muslin cloth at the top but the bottom end was plugged with an inverted plastic container that had a plastic base. The PVC pipes were placed in an incubator and left undisturbed for 4-5 weeks under controlled conditions of 27±1ºC and 70±5 % r.h. Relative humidity was maintained by using a sodium hypochloride solution in an open 5 L basin. This solution was refreshed every third day. A simple Psychrometer was used to calculate relative humidity with the aid of dewpoint and relative humidity charts (World Meteorological Organisation, 1971). The stock culture was maintained throughout the study period.

Determination of grain physical characteristics

Determination of grain physical characteristics involved the following procedures. The maize grains were dried to 10.5% moisture content by drying six batches of 20 g seeds at 120ºC for 2 h. Moisture content measurements were taken using a RCHA FB2 electronic grain moisture meter. Pericarp thickness (mm) and kernel length (mm) were measured for 10 randomly selected seeds using a vernier caliper. These measurements were repeated five times. The pericarp was obtained by carefully scraping off the endosperm prior to measurement. Kernel length was taken as the length of the seed measured from the top of the seed to the hilum. Kernel weights were determined by dividing the weight of 20 g sample by the total number of grains in this 20 g sample.

Damage Levels and Development of LGB on Different Maize Varieties

Laboratory experiments

Ten varieties, including one local variety (Kanjerenjere) and a non conventional hybrid (MH18) were used to assess the effects of maize variety on larval survivorship and dry matter accumulation. These experiments were set up in a randomized completely block design with 4 replications and 10 treatments. There was one independent variable, maize variety with 10 levels: SC403, SC407, SC513, SC627, SC717, DK8033, DK8053, DK8073, MH18 and a local variety. The dependent variables were: number of *P. truncatus* progeny (both larvae and adults), grain weight loss and grain damage. For the determination of grain physical characteristics, maize variety was the independent variable while kernel weight, kernel size (length) and pericarp thickness were the dependent variables.

Prostephanus truncatus larvae were collected from the stock culture using soft forceps. To 50 g grain of each experimental variety (moisture content 10.5%) contained in 250 ml glass jars covered with a double muslin cloth, 32 randomly chosen beetle larvae (counted out into labeled vials) were introduced. Larvae were used instead of adults to remove oviposition preference effects. The glass jars were then kept for a period of 9 weeks in the incubator. Adults were allowed to re-infest their cultures.

The following parameters were used in evaluating susceptibility or resistance of the maize varieties to *P. truncatus*: number of LGB progeny emerged, percent grains damaged,

$$Percent\ grains\ damaged = 100\frac{(NU^1 - NU^2)}{N^1}$$

$$Percent\ grain\ weight\ loss = 100\frac{(UNd - DNu)}{U(Nd + Nu)}$$

Where

Nu^1 = initial number of undamaged grains,

NU^2 = final number of undamaged grains,

U = weight of undamaged grains,

D = weight of damaged grains,

Nu = number of undamaged grains, and

Nd = number of damaged grains.

It was assumed that very tiny larval stages remaining in the grain had negligible effects on grain weight. Development of LGB was determined by counting the number of both live and dead progeny (adults, pupae and larvae) at the end of the 9-week infestation period. The method in Table 1 was used to determine susceptibility or resistance of the maize varieties to *P. truncatus* infestation.

Table 1: Classification of resistance using the resistant and susceptible checks as reference points

Resistant:	Grain weight loss < 3% (less than the resistant check).
Moderately resistant:	Grain weight loss between 3.1 and 6% (similar to the resistant check).
Susceptible:	Grain weight loss between 6.1 and 10.0% (similar to susceptible check).
Highly susceptible:	Grain weight loss greater than 10.0 % (greater than the susceptible check).

On-farm experiments

Field studies of *P. truncatus* infestation in maize stores were carried out at the end of the storage season of 2007/2008. During this period, most farmers will have sold or processed their maize due to various reasons. As such, purposive sampling was employed to ensure that aims of this study were achieved. Using simple random sampling, 4 villages were selected. All household in the four villages were eligible for the study. Where 2 or more households shared a single maize store, only one household was recorded. At the time of the study, a total of 28 households had maize in storage. A pre-coded questionnaire was employed to find out the type of maize in storage, storage period (age of the sample) and pest management options. Where maize was stored in shelled form in bags, a metal grain probe (30 cm long and 1.5 cm internal diameter) was used for sampling. Metal grain probe samples of the commodity were taken at random until a 0.8 – 1.2 kg sample was obtained. The number of bags sampled was done using the following criteria recommended by FAO (1994) (table 2):

Table 2: Selection of bags for sampling

No. of bags in store	No. of bags to be sampled
Up to 10	Every bag
11-100	10 drawn at random
More than 100	Square root of the total number of bags drawn at random

Sampling was done at top layer, middle, and bottom layer. Sampling was done on different parts of the bags because most storage insect pests prefer dark places; some are thigmotactic and collect in cracks between bags. The samples were carried to the laboratory in 2.5 L glass jars with perforated covers for further laboratory analysis. This was done immediately and continued on the following days till when all the samples were examined.

Where maize was stored as cobs, the method used by Helbig (1995) was followed. In cases where maize was stored in traditional granaries, samples of 8–10 maize

cobs were taken from top surface, middle and bottom layers. The samples were examined for the presence of insect pests and for evidence of damage. Cobs infested with fungi or showing damage caused by rodents were not included in the investigation. In all the houses sampled with granaries, there was a small amount of maize remaining. As such, the householder just removed all the maize in the granary and sampling was done. In some instances, after removing the maize from the granary, the householder sieved the maize of frass and insects. In such situations, it was difficult to establish the infestation levels because a large proportion of storage insect pests were sieved out. The sampled maize cobs were kept in a cotton sack and transported to Chancellor College Research Biology Laboratory.

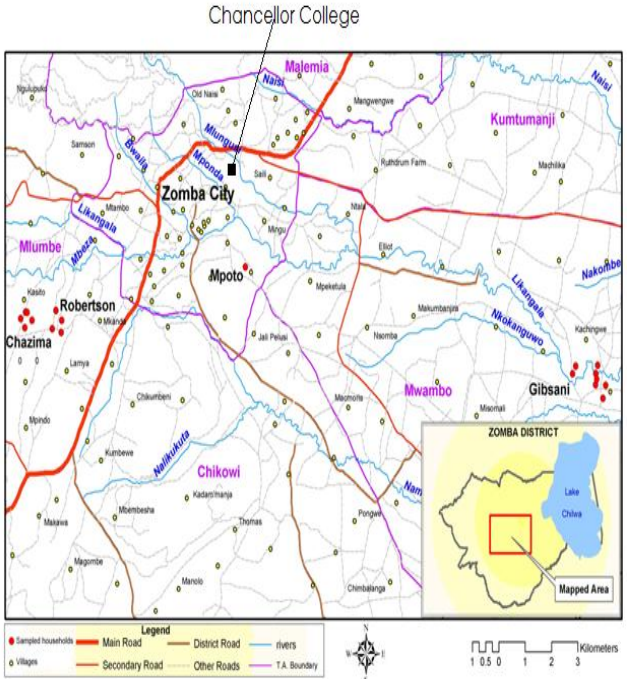

Figure 1: Zomba showing the relative locations maize stores sampled

Once in the laboratory, the maize was dehusked (where the husks were not removed) and shelled. The shelled maize was sieved using hand held sieves of aperture sizes 4.25 and 0.4 mm, respectively. The damaged and undamaged grains were separated and counted manually. A Carmel brush was used to brush off dust from individual grains. Grain weight loss was also determined.

Analysis of infestation consisted of classifying and counting the species of insects found. Insect identification was done using keys outlined by Booth *et al*. (1990). Only adult insects were recorded except for *P. truncatus* where both adults and larvae were counted. This was done by visual examination of exit holes and noting changes in colour transmitted through the seed coat using a magnifying glass (Helbig, 1995). Thus, mature, late instar larvae and pupae could be detected. The insects were sorted, defined in number and species and recorded separately for each cob. All fractions, undamaged and damaged grains, fragments, insects and boring powder were kept separately. Grain weight loss was calculated using the formulae below:

$$Percent\ grain\ weight\ loss = 100\frac{(UNd - DNu)}{U(Nd + Nu)}$$

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Research in Resistance Management

Influence of some herbicides on *in vitro* vegetative growth of *Beauveria bassiana* (Balsamo) Vuillemin

ABSTRACT
The *in vitro* effect of five commonly used herbicides *viz.*, anilophos, butachlor, fluchloralin, pendimethalin and thiobencarb on mycelial growth of *Beauveria bassiana* (BbCm KKL 1100) was evaluated each at three concentrations: the normal field application rate (I.0X), 10 fold lower rate (0.1X) and 10 fold higher rate (10.0X) on agar plate to develop suitable combinations for co-application with herbicides in integrated crop protection programme. The herbicides tested were classified in 1-4 scoring categories based on reduction in mycelial growth: 1 = harmless (<25% reduction), 2 = slightly harmful (25-35%), 3 = moderately harmful (36-50%), harmful (>50%) in toxicity tests. All the five herbicides were antagonistic to *B. bassiana* at varying intensities depending on their concentrations. At 10 fold higher the recommended field rate (10X), all the five herbicides were classified as 'harmful' causing more than 50% inhibition of mycelial growth, the range being 70.74 to 81.48 per cent. Anilophos at the recommended field rate (1.0X) was classified as 'harmful' to *B. bassiana*, while rest of the herbicides with less than 50% inhibition was rated as 'moderately harmful'. At the 0.1X rate, thiobencarb and anilophos were 'moderately harmful'; and the other three herbicides: fluchloralin, butachlor and pendimethalin caused 27.41, 30.37 and 33.33% mycelial inhibitions respectively were classified as 'slightly harmful'. The present study showed that *B. bassiana* is very sensitive to the herbicides tested, particularly at normal as well as higher field recommended dosage. Of the herbicides tested, fluchloralin, butachlor and pendimethalin which showed less adverse effects are probably compatible with *B. bassiana* in the field. However, extensive field studies complemented by parallel laboratory experiments should consider assessing the interaction between selective herbicides and *B. bassiana* isolates to evaluate their ecological impact in cropped environments.
KEY WORDS: *Beauveria bassiana*, herbicides, *in vitro* vegetative growth.

INTRODUCTION

The entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin plays a significant role in the natural control of many insect pests in diverse agro-ecosystems across the world. In peninsular India, *B. bassiana* often causes natural epizootics on a range of insect species in rice ecosystem (Rao, 1975; Nayak and Srivastava, 1979; Hazarika and Puzari, 1990; Ambethgar, 1996). Agrochemicals including herbicides are also frequently used in integrated crop protection programmes of rice cultivation. Regular application of a wide spectrum of herbicides can result in their accumulation in the fields and may affect efficiency of the fungus. Knowledge of compatibility between *B. bassiana* and commonly used herbicides is crucial to select appropriate compounds for scheduling treatments in order to minimize any deleterious effects on biocontrol efficiency and to incorporate *B. bassiana* into the integrated crop protection programme. The inhibitory effects of herbicides on the processes of fungal growth vary depending on the fungal species and strain (Vanninen and Hokkanen, 1988; Anderson

et al., 1989). Therefore, the present study was aimed to examine compatibility of a virulent *B. bassiana* isolate (BbCm KKL 1100) with some selected herbicides on mycelial growth to predict compatible combinations in integrated pest management of rice.

MATERIALS AND METHODS

Source of fungal isolate

The *Beauveria bassiana* isolate (BbCm KKL 1100) isolated from rice leaf folder, *Cnaphalocrocis medinalis* Guenee cadavers from Karaikal, Puducherry Union Territory, India (Ambethgar, 1996). The fungus was isolated on Sabouraud dextrose agar slants fortified with 1% yeast extract (SDAY) and selected based on virulence against *C. medinalis* larvae assayed by two-way screening using an initial single-dose assay with a standard concentration of $1x10^8$ conidia/ml in 0.02% Tween 80®, followed by multiple-dose mortality assay with six different conidial concentrations containing $1x10^4$, 10^5, 1^6, 10^7, 10^8 and 10^9 viable conidia/ml in 0.02% Tween 80® as surfactant.

Herbicides used

The herbicides used for rice weed management were selected for this study. The common name, trade name, active ingredient, formulation and field doses of herbicides are mentioned in Table 1. The effects of these herbicides on the radial growth of *B. bassiana* were evaluated in the laboratory. Fifteen herbicides were tested on SDAY each at three concentrations *viz.*, the normal field application rate (I.0X) (based on 500L/ha spray), 10 time lower rate (0.1X) and 10 time higher rate (10.0X) as inhibition of radial growth of the fungus (Moorhouse *et al.*, 1992).

Table 1. Effect of different herbicides on the mycelial growth of *B. bassiana*

Herbicide				Subnormal dose (0.1X)		Normal dose (1X)		Higher dose (10X)		Scoring at 0.1X
Common Name	Brand Name	Active Ingredient (%)	Field dose (ml/L^{-1})	MCD	PIC	MCD	PIC	MCD	PIC	
Anilophos	Aniloguard	30 EC	2.0	49.66	44.82	40.00	55.55	16.66	81.48	2
Butachlor	Machete	50 EC	1.5	62.66	30.37	50.33	44.07	26.33	70.74	1
Fluchloralin	Basaline	45 EC	1.0	65.33	27.41	52.66	41.48	23.33	74.07	1
Pendimethalin	Stomp	30 EC	1.5	60.00	33.33	51.33	42.96	20.33	77.41	1
Thiobencarb	Saturn	50 EC	2.0	52.00	42.22	47.66	42.96	17.66	80.37	2

MCD=Mean colony diameter (mm), PIC= Per cent inhibition over control
1 = Harmless (<25% inhibition) 3 = Moderately harmful (36-50% inhibition)
2 = Slightly harmful (25-35% inhibition) 4 = Harmful (>50% inhibition)

In vitro inoculation procedure

Twenty ml of SDAY medium was sterilized separately, cooled to 55°C and required concentration of each herbicides was incorporated separately and plated into 9cm sterile Petri dishes. A 10mm dia., 10d old agar mycelial mat of B. bassiana was inverted on the center of a SDAY plate amended with different concentration of herbicide. SDAY plates without herbicide inoculated with a mycelial disc served as control. The plates were sealed and incubated at 26±2°C in a B.O.D incubator for 14d when radial growth on control covered the Petri dish. The diameter of radial growth of the culture in excess of the plugs on 14d after inoculation was measured. The effect of the herbicides was scored as fungicidal if growth dropped totally, as otherwise it was taken as fungistatic. The experiments were replicated three times.

Data computation and analysis

The fungus-herbicides compatibility data were analyzed according to Hassan's classification scheme (Hassan, 1989). The replicated fungus radial growth data were averaged and were expressed as percentage of growth inhibition in comparison to corresponding control following Hokkanen and Kotiluoto (1992).

$$X = \frac{Y - Z}{Y} \quad X \ 100$$

Where: X, Y, Z stand for percentage of growth inhibition, radial growth of fungus in control and radial growth of fungus in poisoned medium, respectively. The herbicides were further classified in evaluation categories of 1-4 scoring: 1 = harmless (<25% reduction), 2 = slightly harmful (25-35%), 3 = moderately harmful (36-50%), harmful (>50%) in toxicity tests (Hassan, 1989). All data were analyzed using the Statistical Analysis System (SAS Institute, Inc., 1982) programme.

RESULTS AND DISCUSSION

Agrochemicals may synergize or antagonize disease in insects, and they may be regarded as epizootiological relevant factors (Benz, 1971; Jacobson *et al.*, 2001). In the present study, all the tested herbicides hampered mycelial development of *B. bassiana* in SDAY medium either partially or completely at all the three concentrations *viz.*, the normal field rate (1.0X), 10 times subnormal rate (0.1X) and 10 times higher rate (10.0X). However, there were significant differences of inhibition rate of fungus growth by the herbicides (Table 1). Butachlor, fluchloralin and pendimethalin were found to be least inhibitory (harmless) to the fungus growth and supported more than 50 per cent colony development at normal dose. Anilophos and thiobencarb suppressed the growth of the fungus (55.55

%) with scoring index of 2. Fungal entomopathogens are generally very sensitive to some herbicides (Gardner and Storey, 1985; Keller, 1986; Mietkiewski *et al.,* 1989; Erland, 1991; Wardle and Parkinson, 1992) and increasing use of such chemicals would deplete these fungal populations in a crop before they infect arthropods and interfere in their natural epizootics (Wardle and Parkinson, 1992).

In vitro studies of Poprawski and Majchrowicz (1995) have shown delayed mycelial growth in *B. bassiana* with several herbicides. Observation of Todorova *et al*. (1998) showed that simultaneous treatment of Colorado potato beetle with *B. bassiana* and the herbicide diquat synergized the insect mortality while other herbicide, glutosinate-ammonium was harmful and not compatible with *B. bassiana*. Herbicides usually alter host-plant defenses, increase their exudation and reduce pathogen growth (Poprawski and Majchrowicz, 1995). Prior studies have indicated that when herbicides are applied in soil, the native fungal entomopathogens were inhibited *in situ*, and impeded natural epizootics (Poprawski and Majchrowicz, 1995). Alachlor reduced the number of colony forming unit of *B. bassiana* in soil by 51 per cent and halved the infection rate in target pests (Gardner and Storey, 1985). All the compounds, which were innocuous *in vitro*, need not be safe in the field and vice-versa (Poprawski and Majchrowicz, 1995). The results of the tests in agar culture had limited predictive values for field use. Therefore, all the compounds harmful in screening tests in agar culture should be tested with the fungus formulations on insects in fields as well. Prior works caution that if fungal formulations are applied together with herbicides, it may lead to pose detrimental effects on the infectivity of the fungus (Mietkiewski *et al.,* 1989). The present study showed that *B. bassiana* is very sensitive to the herbicides tested, particularly at normal as well as higher field recommended dosage. However, of the herbicides tested, fluchloralin, butachlor and pendimethalin which showed less adverse effects are probably compatible with *B. bassiana* in fields. However, extensive field studies complemented by parallel laboratory experiments should consider assessing the interaction between selective herbicides and *B. bassiana* isolates to evaluate their ecological impact in cropped environments. New techniques through biotechnology or genetic engineering may be helpful for strain improvement of fungal pathogens for tolerance to agrochemicals.

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A review on Bacillus thuringiensis characterization, Cry gene classification, and identification

Modern agriculture uses a wide variety of insecticides to control the insect damage. Most of them are chemically synthesized. The non-judicious and indiscriminate use of insecticides has led to a cascade of problems including resurgence of minor pests, development of resistance, enhancement of overhead costs, elimination of parasites, predators and other beneficial insects and accumulation of pesticide residues in plant products which ultimately find their way to higher members of the food chain. In this context, an integrated approach combining all the available strategies seems to be gaining momentum and the use of various eco-friendly bio-control agents is becoming an important component of the integrated pest management (IPM).

Of the many bio-control agents available, B. thuringiensis insecticides have proved effective and selective enough to eliminate outbreaks of Lepidopteran pests. B. thuringiensis is a fascinating bacterium, producing a number of insecticidal toxins which have spawned a whole industry. For more than 60 years, B. thuringiensis formulation has been sprayed as bio-insecticides against more than 300 insect species. Bacillus thuringiensis, a gram positive soil bacillus, was first isolated from diseased silkworm larvae (Ishiwata, 1901) and subsequently named as B. sotto (Aoki and Chigasaki 1915). A decade later another strain was found in Germany, killing grain moth larvae in stored grain (Berliner, 1915). Since, the latter strain was discovered in the province of Thuringen, it was named B. thuringiensis, a name now given to a large family of bacteria producing insecticidal parasporal crystals (Knowles, 1994). When stressed, B. thuringiensis makes a dormant spore and one or more large crystalline inclusions.

In the interesting article "keeping an eye on B. thuringiensis", by Dixon (1994) extols the virtue of B. thuringiensis as a safe biopesticide and does not advocate removal of B. thuringiensis from the market. Summarizing an extensive survey of the vast literature, Glare and Callaghan (2000) says "the information available at this time does not suggest there is danger from the use of B. thuringiensis".

MODE OF ACTION OF *B. thuringiensis*

Every year, insect pests cause between 15 and 25 per cent loss in agricultural production worldwide and the principal strategy to control the pest has been to use chemical insecticides (Pimentel, 1991). But the use of chemical insecticides is costly and indiscriminate use of non-specific products often toxic to mammals, birds and fish, has resulted in contamination of the environment and destruction of non-target species and development of pest resistance (Vincent *et al.*, 1999). This menace can be overcome by using degradable products of biological origin. *B. thuringiensis* has been the candidate biocide of choice for many decades because of several advantages over the chemical insecticide *viz.*, economical, safer, narrow spectrum of host range and easily biodegradable.

The mechanism of action of *B. thuringiensis* crystal proteins involve solubilization of the crystal in the insect midgut, proteolytic processing of the protoxin by midgut proteases under alkaline condition, which then binds to the midgut receptors. The insertion of the toxin into the apical membrane creates pores, results in loss of transmembrane potential that leads to osmotic cell lysis (Knowles, 1994). Thus, sublethal concentration of toxin leads to change in larval behavior including avoidance of feed, ultimately resulting in death due to starvation (Aronson and Shai, 2001).

Ecological studies on *B. thuringiensis* have indicated its presence in wide range of environmental niches including soil, stored grains, insect cadavers, phylloplane, rice straw compost and mammalian fecaes (Ruud *et al.*, 2001). There are various methods to isolate bacteria from different habitats including shaken flask technique, leaf lift technique and leaf scrub technique coupled with sodium acetate selection (Smith and Couch, 1991 and Travers *et al.*, 1987).

CHARACTERIZATION

For understanding the role of *B. thuringiensis* in the environment, its characterization is very important (Bravo *et al*., 1998). Individual *B. thuringiensis* strains vary in the number and type of toxins they produce. Over 170 specific δ-endotoxin genes are known (Glare and Callaghan, 2000). Although, usually referred to in the singular as Bt, *B. thuringiensis* currently is recognized as complex species, almost all of which produce crystalline inclusion during sporulation.

1. Characterization by classical methods

The methods for characterization include flagellar serotyping, crystal morphology, biochemical reactions and bioassays (de Barjac and Bonnefoi, 1962, 1968). Flagellar agglutination has been used most widely in the last decade because of its simplicity and reliability in classifying *B. thuringiensis* strains (de Barjac and Franchan, 1990). Such methods however are very laborious, making them inappropriate for screening large collection of *B. thuringiensis* isolates. However, these methods continue to be used for characterization and species differentiation in combination with more recently developed molecular methods (Hansen *et al.*, 1998). Martin and Travers (1989) developed a series of rapid tests *viz.*, starch hydrolysis, urease production, mannose, sucrose and salicin fermentation, esculin utilization and lecithinase production. They identified 16 different biochemical types of *B. thuringiensis* from a total of 1115 samples and approximately 27,000 isolates. These methods of characterization have proved difficult, because of variation in growth responses and the observation that biochemical characterization does not always correlate with serotyping results. Classification of subspecies or varieties based on serotyping using H-serovars (flagellar serotyping) has resulted in identification of 69 serotypes and 13 sub antigenic groups, giving 82 serovars (Lecadet *et al.*, 1998).

2. Characterization by DNA based methods

Recent advances in molecular biology have allowed the development of specific DNA based methods that are capable of interspecific and intraspecific differentiation. Such methods can also be used to identify the presence or absence of specific endotoxin genes. At the molecular level, *B. thuringiensis* can be characterized by analysing the plasmid profile, polymerase chain reaction (PCR) products and protein profile (Carozzi *et al.*, 1991) and in combination with restriction fragment length polymorphism (RFLP) has been used for identification of new variants of toxin gene (Kuo and Chak, 1996). Serovars of *B. thuringiensis* can be distinguished and identified on the basis of RAPD finger prints. Individual strains within the same serotype can also be distinguished (Brousseau *et al.*, 1993).

Daffonchio *et al.* (1998) used the internal transcribed spacers (ITS) between 16s and 23s ribosomal RNA genes as molecular markers to attempt to discriminate species of *Bacillus*. TeGiffel *et al.* (1997) developed DNA probes based on the V1 region of the 16s rRNA for the identification of *B. cereus* and *B. thuringiensis*. Physical maps of chromosomes has also been used to compare *B. cereus* and *B. thuringiensis* using pulse field gel electrophoresis and restriction enzyme digestions (Carlson and Kolsto, 1993).

INSECTICIDAL POTENTIAL OF cry

Cry proteins differ in their insecticidal spectrum. Differences in the amino acid sequence are related to differences in the toxicity and the host range of the toxins (Whiteley and Schnepf, 1986). Generally, proteins sharing a primary rank are toxic to the same orders of insects or other invertebrates. Proteins sharing the same secondary rank (the cryIA proteins, for example) are toxic to the same families. Finally, proteins sharing the same tertiary rank (such as cryIA(a) proteins) typically, are toxic to the same species. The cry9A(a) toxin showed an insecticidal spectrum different from cryIA(c) and cryIC(a) toxins.

MacIntosh *et al.* (1990) examined the effect of purified cry3A from *B. thuringiensis tenebrionis* and cryIAb and CryIAc from *B. thuringiensis* kurstaki on a number of pests. Only lepidoptera were sensitive to cryIAa and cryIAc while only Colorado potato beetle was sensitive to cry3A. cry2A has an unusual dual toxicity to

lepidopteran and dipteran insects, although considerable variation in response to purified toxins exhibited within those orders, purified cry2A from *B. thuringiensis kurstaki* was toxic only to lepidoptera and diptera when tested against 35 insect species (Coleoptera, Collembola, Diptera, Hemiptera, Hymenoptera, Isoptera, Lepidoptera, Neuroptera and Orthoptera) (Sims, 1997).

Luo *et al.* (1999) analysed the binding and pore formation properties of four *B. thuringiensis* cryI toxins by using brush border membrane vesicles from *Sporoptera exigua* and *Spodoptera furgiperda* and the results were compared with the results of toxicity bioassays. cryIFa was highly toxic and cryIA(c) was non-toxic to *S. exigua* and *S. furgiperda* larvae, while cryICa was highly toxic to *S. exigua* and weakly toxic to *S. furgiperda*. In contrast, cryIBb was active against *S. furgiperda* but only marginally active against *S. exigua*.

Aronelle *et al.* (1995) conducted toxicity assay of cry11B against *Culex pipiens*, *Aedes aegypti*, *Aedes stephensi*. They observed that although cry11B share similarity with cry11A, it is more toxic to all the three species: *i.e.*, about seven times more for *A. aegypti* and *Aedes stephensi* and 37 times more for *Culex pipens*.

VARIABILITY AND DIVERSITY OF cry

The diversity of insecticidal crystal proteins (ICP) is of paramount importance in the creation of new *B. thuringiensis* based bioinsecticides by using both genetic and recombinant DNA technique. High diversity of *B. thuringiensis* strains with respect to presence of different *cry* genes has been observed.

Ben-Dov *et al.* (1997) analysed *cry* profiles of PCR products identified by universal primer from standard and 126 field collected strains. Among 126 field collected strains, 39 per cent of the strains contained *cry*A, combination of *cry*1 and *cry*2, combination of *cry*1, *cry*2, *cry*7 or *cry*8 was observed in 25 per cent of the strains. Combination of *cry*4 and *cry*7 was found in 5 per cent, *cry*7 and *cry*8 were found in 3 per cent and least of *cry*2 was found only in 1 per cent of the isolate.

Theunis *et al.* (1998) observed the discrepancies between the profile of δ-endotoxin genes detected by PCR and the proteins detected by ELISA or western blotting. In most of the cases, diversity of genes detected was greater than that of proteins. They observed *cry*2A gene in 15 isolates by PCR, but on western blot *cry*2A protein was detected in only ten of the isolates. The detection of many more genes than protein may be due to lack of expression of some of the amplified products.

2.9 ORGANIZATION OF INSECTICIDAL CRYSTAL GENES OF *B. thuringiensis*

Several studies have been made to identify the location of genes encoding cry protein. While most insecticidal crystal protein genes are typically located on plasmids larger than 30 Mda, others have been reported on chromosome (Baum and Malvar, 1995). *B. thuringiensis* subsp. *kurstaki* harbours 12 resident plasmids containing *cry*1A(a), *cry*1A(c), *cry*2A(a) and a silent *cry*2B(b) gene on 110 Mda plasmid and *cry*IA(b) gene on self transmissible 44 Mda plasmid (Widner and Whiteley, 1989). Many of the *cry*I genes are situated on plasmids as monocistronic units equipped with transcriptional start sites immediately 5' to the gene and a ρ-independent transcription terminator immediately downstream of the coding region (Baum and Malvar, 1995). Both *cry*2A(a) and *cry*2A(c) genes are present as the third gene in operons containing two upstream open reading frames (Widner and Whiteley, 1989; WuD *et al.*, 1991). The mosquitocidal *cry*4B gene is positioned as the middle gene in an operon that includes the genes encoding 19 and 20 kDa proteins (Adams *et al.*, 1989; Donovan *et al.*, 1988; Dervyn *et al.*, 1995; Ben-Dove *et al.*, 1996).

IDENTIFICATION OF NOVEL *cry* GENES

1. DNA – based method

Through PCR

Analysis of δ-endotoxin genes by bioassay has proved to be an exhaustive, time consuming process as it is necessary to screen all target insect isolates. Different methods have been developed in an effort to reduce the number of bioassays of which, PCR analysis is considered to be the best choice, since it allows rapid determination of the presence or absence of a sequence, is highly sensitive, relatively fast and can easily be used on a routine basis (Ceron *et al.*, 1994). This technique can be used to amplify specific DNA fragment and thus to determine the presence or absence of target gene (Manuel and Juarez-Perez, 2003).

Multiplex PCR is becoming an increasingly important method to identify the existence of *cry* type genes. However, the major drawback of this method is that it cannot identify the existence of a novel *cry* gene from a *B. thuringiensis* strain whose nucleotide sequence is unknown. A two step strategy named exclusive PCR or E-PCR has been developed (Juarez *et al.*, 1997) to overcome the main limitation of PCR, which only detects already known sequences only. E-PCR is based on the exclusion from the family band of PCR products corresponding to genes previously detected by type primers. By using E-PCR Juarez *et al.* (1997) detected a novel *cry*I gene that was found to be related to *cry*IB

through alignment studies of both DNA and protein sequences.

An extended multiplex PCR method was established to rapidly identify and classify *B. thuringiensis* strains containing *cry* genes toxic to species of lepidoptera, coleoptera and diptera (Ben-Dov *et al.*, 1997). Five pairs of universal primers were designed to probe the highly conserved sequences and classify most genes.

Masson *et al.* (1998) determined the *cry* gene content of *B. thuringiensis* subsp. aizawai HD-133 by combination of high pressure liquid chromotography (HPLC) and exclusive PCR. A total of six *cry* genes were detected in genome DNA purified from HD-133, four from *cry*1 family (*cry*1A(a), *cry*1A(b), *cry*1C and *cry*1D) as well as gene from *cry*2, *cry*2B and the *cry*II families known in the following groups 2 *cry*I, 3 *cry*2, 4 *cry*3, 2 *cry*4, 2 *cry*7 and 3 *cry*8 genes.

Bravo *et al.* (1998) analysed a Mexican *B. thuringiensis* strain collection based on multiplex PCR with novel general primers that could detect *cry*2, *cry*3, *cry*5, *cry*7, *cry*8, *cry*9, *cry*11, *cry*12, *cry*13, *cry*14, *cry*21 and cyt genes. Of the 496 strains, 49.5 per cent had *cry*3 genes, 7.9 per cent contained *cry*11 and cyt genes, *cry*7, *cry*8, *cry*9 genes were found in 0.6, 2.4 and 2.6 per cent of the strains, respectively. No strains with *cry*5, *cry*12, *cry*13, *cry*14 genes were found and 14 per cent of the strains did not give any PCR product.

The efficacy of PCR for *cry* gene identification relies on the alternation of conserved and variable nucleotide region (Manuel and Juarez, 2003). Most of the *B. thuringiensis* protoxin crystal genes share conserved nucleotide blocks in a number that varies from five for naturally truncated genes such as *cry*11 or *cry*3A, to eight for the largest genes with encoded proteins of 1000 residues or more (Schnepf *et al.*, 1998).

Hybridization

Even though, the homology between two *cry* sequences within the same group can be as low as 45 per cent, homology of some specific regions within the sequences can be as high as 95 per cent. Because of this homology between *cry* gene sequences, the development of specific probes was used earlier to detect and characterize putative new *cry* genes using hybridization techniques (Crickmore *et al.*, 1998).

Rakesh *et al.* (1989) identified entomotoxic protein gene from B. *thuringiensis* subsp. *kurstaki* using 20-mer btx probe. They observed a strongly hybridizing 3.1 kb *Hind*III fragment in plasmid DNA. Homology to btx probes has been located on 6.6, 5.3 and 4.5 kb *Hind*III fragments in several strains of *B. thuringiensis.*

`2. Other analytical methods

A different analytical technique to identify new *B. thuringiensis* toxins was used by Chestukhina *et al.* (1994). Rather than analyzing the gene content of a strain, they studied in detail the cry composition of the parasporal body from several *B. thuringiensis* strains. By purifying and micro-sequencing the major peptides resulting from trypsin digestion of the solubilized parasporal body, they were able to find that *B. thuringiensis* subsp. *galleriae* VKPM B-1757 and *wuhanensis* VKPM B-1226 parasporal bodies were composed of six and seven proteins, respectively. Comparison of the protein sequences with those of known cry proteins showed that most of them were already described. However, for each strain, two protein sequences lacked complete homology with the known cry proteins suggesting possible new toxins (Chestukina *et al*., 1994).

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Superiority of Fenpyroximate 5% SC (SEDNA) over Other Available Acaricides against Chilli Yellow Mite, *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae)

ABSTRACT
A comparative field trial was conducted to evaluate the bio-efficacy of a new class of acaricide with advanced Suspension Concentrate formulation having a novel mode of action, mitochondrial electron transport inhibitor, Fenpyroximate 5% SC (SEDNA) against chilli yellow mite (*Polyphagotarsonemus latus*) in compararison with other available acaricides viz., Fenazaquin 10 EC, Propergite 57 EC, Fenpropathrin 10 EC, Dicofol 18.5 EC and Bifenthrin 10 EC. The results indicated that Fenpyroximate 5% SC (SEDNA) @ 500 ml formulation per hectare was found to be the best acaricide among all other acaricides available in controlling chilli yellow mite.
KEY WORDS: Chilli, Yellow mite, Fenpyroximate 5% SC

INTRODUCTION

Chilli, *Capsicum annuum* **L.** belongs to the family of Solanaceae. It is said to have originated in the Latin American region. India is the largest producer and exporter of chilli in the world. Its production level hovered around 1.15 million tonnes which accounted to 26% of global production (Anonymous, 2008).

Chilli is attacked by many sucking pests throughout its growth period. Among them yellow mite, *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) is one of the most serious pests. Yellow mites cause the leaf curl (down word curl) and in case of severity, it causes broom stick like appearance in the apical shoot portion. The mite mostly prefers the young leaves and in the primary stage of infestation, leaf become leathery and looks dark green in colour. In West Bengal, India condition, it starts to attack normally during July – October and climbs the peak in the month of September. As the apical bud is affected by the attack, growth of the plant is arrested and thus bears less flowers and fruits. Ultimately production is hampered tremendously. Several acaricides have been tried to manage the pest. Fenpyroximate, a new acaricides molecule has recently been introduced in managing the phytophagous mites. Fenpyroximate is a contact and stomach acaricide belonging to the group of compounds 'pyrazoles'(Ware, 2001) and act as Mitochondrial electron transport inhibitor (METI). In the present study, field experiments were conducted to evaluate the bio-efficacy of Fenpyroximate against the yellow mite on chilli.

MATERIAL AND METHODS

The trial was conducted on the farmer's field at Taherpur village of Nadia District, West Bengal during July – August, 2006. The trial was laid out in a Randomized Block Design consisting of Fenpyroximate 5% SC @ 500 ml/ha, Propergite 57 EC @ 1250 ml/ha, Fenpropathrin 10 EC @ 1000 ml/ha, Bifenthrin 10 EC @ 800 ml/ha, Fenazaquin 10 EC @ 1000 ml/ha, Dicofol 18.5 EC @ 1000 ml/ha and one untreated control. The trial plots were replicated thrice having 5m x 5m plot of each. Water volume was used @ 500 L per ha. Observation on the mite population was taken at before spray and 2 days, 7 days, 15 days after application. Observation on mite population was recorded by counting of alive mites (irrespective of adult or larval stage) from 5 randomly selected leaves (upper leaves) in each plant from 10 randomly selected plants per plot which mean 50 leaves in each plot. All the data were subjected to statistical analysis.

RESULTS AND DISCUSSION

The pre-treatment mite population indicated the uniform distribution of mite population in the experimental plots ranging from 6 – 8 mites per leaf and the pest load was statistically not significant. On the 2nd day after application, it was observed that in Fenpyroximate, Fenazaquin and Dicofol treated plants mite population reduced drastically whereas in Bifenthrin and Fenpropathrin treated plants mite population remained mostly as such. Fenpyroximate 5% SC @ 500 ml/ha recorded very negligible mite population and was statistically at par with Fenazquin @ 1000 ml/ha, showing only 0.96 and 1.85 mite per leaf respectively. The next best treatment was Dicofol @ 1000 ml/ha where mite population was 3.11 per leaf and it was at par with Propergite @ 1250 ml/ha. Within these two days, mite population in untreated control increased from 7.97 to 8.65 per leaf.

In 7 days after spraying it was recorded that in both the doses of Fenpyroximate 5% SC @ 500 ml/ha also became the best treatment showing only 1.26 mite / leaf which was negligible. The next best treatment was Fenazaquin which recorded 2.32 mite / leaf. In Dicofol treated plants, it was found that mite population did not increased considerably but remained almost same as on 2 days after spray. In Bifenthrin, Propergite, Fenpropathrin and control plot, mite population increased remarkably within these 5 days.

Fenpyroximate 5% SC @ 500 ml/ha was the best treatment even on 15 days after spray. It recorded very minimum mite population, 2.55 mite / leaf which was statistically at par with Fenazaquin @ 1000 ml/ha but statistically superior over all other treatments. This result confirmed that Fenpyroximate @ 500 ml/ha had long duration control against chilli yellow mite over all other acaricides.

The findings reflected that except Fenpyroximate 5% SC @ 500 ml/ha and Fenazaquin @ 1000 ml/ha, Dicofol @ 1000 ml/ha could protect the crop against

mite up to 7 days. Propergite @ 1250 ml/ha could manage the mite population to some extent just for 2-3 days and all other treatments like Fenpropathrin @ 1000 / ha and Bifenthrin @ 800 ml/ha were not found to be effective against yellow mite of chilli at all.

The result revealed that Fenpyroximate 5% SC @ 500 ml/ha was the best treatment in controlling chilli yellow mite over a long period of time (over 15 days) and Fenazaquin @ 1000 ml/ha was the next best treatment. In the recommended dose, efficacy of Dicofol was acceptable for up to 7 days and there after the population started increasing. Propergite in the recommended dose could suppress mite population for 2 – 3 days only and there after the mite population started building up tremendously. The other treatments like Bifenthrin and Fenpropathrin were not effective at all against the chilli yellow mite. So, Fenpyroximate 5% SC @ 500 ml/ha gave longest protection duration from yellow mite of chilli. It might be due to its ovicidal effect and molting inhibition action even in low residue level.

Table 1: Effect of Fenpyroximate 5% SC against chilli yellow mite

Treatments	Dosage	Mite population per leaf			
		Before spray	2 DAS	7 DAS	15 DAS
1) Fenpyroximate 5 SC	500 ml/ha	7.91	0.96	1.26	2.55
2) Propergite 57 EC	1250 ml/ha	6.82	4.20	11.92	21.20
3) Fenproperthin 10 EC	1000 ml/ha	7.09	5.90	18.69	38.43
4) Bifenthrin 10 EC	800 ml/ha	6.21	6.47	16.45	36.67
5) Fenazaquin 10 EC	1000 ml/ha	6.29	1.85	2.32	4.56
6) Dicofol 18.5 EC	1000 ml/ha	5.64	3.11	3.24	17.10
7) Control	----------	7.97	8.65	27.98	45.13
CD		NS	1.74	7.7	11.38
SEM ±		----	0.69	2.49	3.69

DAS – Days After Spray

Ultimately, Fenpyroximate 5% SC (SEDNA) can be an ideal solution for controlling the chilli yellow mite which to some extent became resistant to the conventional acaricides. It corroborated with the findings of Srinvasan *et al.*, (2003), Ismitha and Giraddi (2006). Somchaudhuri et al., (2008) has reported that Fenpyroximate @ 40 g ai/ha was the most effective acaricide in controlling the yellow mite population in Jute. Ismitha and Giraddi (2006) also reported that Fenpyroxymate was quite safe to both predatory mite and coccinellid species. They reported that Fenpyroximate was quite effective for Chilli yellow mite control for till 21 days after spray.

The result obtained under the present study in respect of Fenpyroximate 5% SC against Chilli yellow mite was in agreement to those reported above.

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Resistance stability to spinosad and abamectin in the cotton leafworm, *Spodoptera littoralis* (Bosid.)

ABSTRACT

The ability of resistant strains to take to susceptibility gives advantages to the insecticide to be used compatibly with IPM programs, thus alleviating the appearance of insecticide resistance. Two spinosad resistant strains (SDRS, RR=99-fold and SFRS, RR= 57-fold) and one resistant to abamectin (ARS, RR=16- fold) of cotton leafworm were reared without exposure to select agents for four generations under laboratory conditions. The resistant ratios in F1, F4 were 52, 4; 42, 13 and 15, 8-fold for SDRS, SFRS and ARS, respectively. The results showed that the stability of resistance is greater in ARS than spinosad strains. The reversibility % in SDRS increased for SFRS in F1 and F4 by 18 %. Spinosad resistant strains reversed to susceptibility after four generations with stopping used spinosad. However abamectin resistant strain under the same time still quiet resistance.

Keywords: insecticide resistance, stability, spinosad, abamectin, cotton leafworm

INTRODUCTION

Until now, insecticides were still the main method used for controlling insect pests. Intensive and unwise applications of insecticides for controlling cotton leafworm in Egypt led to cotton leafworms becoming resistant to most insecticide groups (Alford, 2000; Abo Elghar et al 2005). Consequently, more problems in environmental pollution and difficulty controlling insects have happened. Stopping applications of insecticide for periods of time is one important strategy of insecticide resistance management (IRM) (Scott 1990; Metcalf, 1994).

Spinosad and abamectin, two nervous system poisons, derived from actinomycetes bacterium species (Copping and Menn 2000; Putter et al 1981). They have strong insecticidal activity against different insect orders. Spinosad alters the function of nicotinic acetylcholine receptors and GABA-gated chloride channels (Salgado et al 1997). Abamectin affects the GABA-gated channel as well (Duce and Scott 1985).

The Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisd.) is one of the most notorious and destructive phytophagous insect pests in Egypt, not only to cotton, but also to other field crops and vegetables (Kandil et al 2003). Their caterpillars are very polyphagous, causing important economic losses in both greenhouses and open field on a broad range of ornamental, industrial and vegetable crops. The Ministry of agriculture (MOA) cancelled all conventional insecticides from spraying on egg masses to conserve their enemies and uses IGR_S mainly for the newly hatched larvae during this period (Temerak 2002). For the time being, Spinosad is the recommended rapid product by the MOA to face egg masses and conserve the natural enemies.

Field populations and/or selection laboratory strain of some insects showed tolerance or resistance to spinosad and abamectin (Moulton et al. 2000; Young et al 2000; Sayyed and Wright 2004; Scott et al 1991; Rugg 1996 ; Liett et al 2005).

In Egypt, however, the field populations of cotton leafworm collected from different regions showed susceptible toward spinosad and abamectin in laboratory tests (Abdu-Allah 2007). A selection of Fayoum and Gharbia cotton leafworm larvae for 20 generations by spinosad using leaf dipping technique showed resistance for 26.56 and 34.64 fold, respectively (Mahmoud 2005). Abdu-Allah 2007 noted that field populations of cotton leafworm showed resistance 86.85 fold toward spinosad after 23 laboratory selection generations using leaf dip bioassay and 108.13 fold in 25 selection generations using larval dip technique. Selection of the field populations of cotton leaf worm by abamectin for 25 generations produced resistance 18.98 fold.

As with any new insecticide we need to answer some questions such as how rapidly could resistance develop and to what level of resistance (Shono and Scott 2003). Also we should answer the question of the stability of resistance in the absence of insecticide selection pressure. This is especially important since there is little information at this point and this information is very important in designing the appropriate resistance management strategy for any insect (Smirle et al 1998).

The aim of this investigation to answer the following questions:

1- Are spinosad resistant strains of cotton leafworm easily reverted to susceptibility with rearing free spinosad?
2- Are their differences in reversibility of two spinosad resistant strains selected by different ways?
3- Is the reversibility ranking of abamectin resistant strain alike in spinosad strains?

MATERIALS AND METHODS

Insects:

For every strain used in this investigation, eggs were allowed to hatch at room temperature, at which larvae were reared on fresh castor bean, *Ricinus communis*, leaves as described by Eldefrawi et al 1964.

Susceptible strain (SS):

The susceptible strain was brought as eggs and newly hatched larvae from Alexandria university laboratory,

kept away from insecticidal contamination for more than five years ago before starting the research.

Reversion strains:
The source of these strains was collected from cotton fields as big catches from different Lower and Upper governorates of Egypt. The supplied insects were reared without exposure to insecticide in the laboratory of Plant Protection at Assiut University for one generation. Then they were divided to three batches and selected by spinosad or abamectin in laboratory conditions for many successive generations. The characters of these strains are presented in Table 1 for reversion strains. Eggs of each strain were allowed to hatch at room temperature, at which larvae were reared on fresh leaves of castor bean, *Ricinus communis* as described by Eldefrawi et al, 1964 with insecticide free pressure for four generations.

Bioassay tests of relaxed strains:
At least 30 breeding pairs were maintained per generation. Larval susceptibility (log dose-mortality) was investigated by the following bioassay to determine the resistance level.

Larval- dip bioassay:
Fourth instar larvae of *S. littoralis* at an average weight of 38-40 mg / larva were prepared. Serial water aqueous solution of concentrations of the tested insecticide prepared+ triton x_{100}(0.05%) were used for bioassay tests. From three to four replicates were used to each concentration using 10 larvae in every replicate. The ten larvae were dipped in the tested solution for 5 seconds and then transferred to Petri-dishes containing filter papers to dry. The larvae for each replicate were similarly dipped in solution of distilled water plus surfactant as a control treatment.

After that the dipped larvae were supplied with fresh castor leaves and incubated at 26± 2 temperature and 12:12 L:D and 65± 5 RH until recording the results. The mortality was counted 48 hrs after treatment. 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.

Leaf -dip bioassay:
The same procedures mentioned above were used except the 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 24 hrs. The mortality was counted. Each experiment was replicated twice.

Statistical analysis:
Mortality percentages were corrected by Abbot's formula if needed (Abbot, 1925). The LC_{50} and slope values were determined by a computerized probit analysis program. The resistance values (RF) were calculated by dividing the LC_{50} of relaxed generation / LC_{50} of susceptible strain. Stability percentages= LC_{50} of relaxed generation / LC_{50} of previous relaxed generation. Reversibility percentages =100- % stability.

Chemicals:
Spinosad (Success , SC 24 %, Dow AgroSciences Co.), was supplied by Prof. Dr. Sobhy A. Temerak, a professor in Plant Protection Dept., Faculty of Agriculture, Assiut University, Egypt and the scientific coordinator of Dow Agrosciences Co. in Egypt. Abamectin (Romacten, EC 1.8 %, Rotam Agrochemicals Co.), was provided from commercial pesticide shop in Egypt. Triton X100, Iso-octylphenoxy polyethoxy ethanol polyethoxy(100 % purity, BDH Chem, Ltd. Poole England) was bought, it used as surfactant.

RESULTS
In Spinosad dipping resistant strain (SDRS) table 2, the larval-dip LC_{50} value towered spinosad for the fourth instar larvae of cotton leaf worm were sharply decreased from 16081 in parent SDRS strain to 8422 mg AIL^{-1} in F1 strain. Statistically, there is no significant difference between two values. Then in F2, F3 and F4, the value gradually decreased. There is no significant difference between them. There are significant differences between the F1 value and F3 and F4 values. The values in F3 and F4 were 1729 and 615 mg AIL^{-1}. The resistance factor values fell down from 99 to 52, 24, 11, and 4 fold in F1, F2, F3 and F4, respectively. The values of regression were 1.79, 1.52, 1.53, 0.94 and 1.17 within parent, F1, F2, F3 and F4, respectively.

Table 2: LC_{50}, RF and regression equation values of spinosad on SDRS relaxed generations using larval-dip metho (toxicity tests were done on the fourth instars larvae 38-45 mg/ la

Generation	LC_{50} , mg A.I. L^{-1} (95% C. L.)	Resistance Factor(RF)*	Regression Equation
F1	8422 a (5588-12566)	52	Y=-0.97+1.52 X
F2	3885 ab (1726-5722)	24	Y=-0.49+1.53 X
F3	1729 b (404-3295)	11	Y= 1.96+0.94X
F4	615 bc (273-1038)	4	Y= 1.73+1.17 X

Values within a column followed by different letter are significantly differe ($p < 0.05$, LSD) based on the overlap of 95 % CL. Data rounded to nearest w

Data in table 3 shows that the feeding-dip LC_{50} values in, SFRS relaxed generations, F1, F2, F3 and F4 toward spinosad were determined to be 4270, 3329, 2812, and 1277 mg AIL^{-1}. There is no significant difference between any of these values and parent (LC_{50} 5804 mg AIL^{-1}). Within SFRS relaxed generations, the resistance factors in F1, F2, F3 and F4 were determined to be 42, 33, 28, and 13 fold, respectively. The slope values in parent SFRS, F1, F2, F3, and F4 were 2.42, 1.89, 2.02, 3.11, and 4.94, respectively.

Table 3: LC_{50}, RF and regression equation values of spinosad on SFRS relaxed generations using leaf-dip method (toxicity tests were done on the fourth instars larvae 38-45 mg/ larvae).

Generation	LC_{50} , mg A.I. L^{-1} (95% C.L.)	Resistance Factor(RF)	Regression Equation
F1	4270 a (955-7301)	42	Y=-1.86+1.89X
F2	3329 a (2251-4438)	33	Y=-2.12+2.02X
F3	2812 a (1797-3806)	28	Y= -5.73+3.11X
F4	1277 a (1066-1557)	13	Y= -10.34+4.94X

Values within a column followed by different letter are significantly different ($p < 0.05$, LSD) based on the overlap of 95 % CL. Data rounded to nearest whole numbe

Table 4: LC_{50}, RF and regression equation values of abamectin on ARS relaxed generations using larval-dip method (toxicity tests were done on the fourth instars larvae 38-45 mg/ larvae).

Generation	LC_{50} , mg a.i. L^{-1} mg A.I. L^{-1} (95% C. L.)	Resistance Factor(RF)	Regression Equation
F1	1243 a (982-1572)	15	Y=-5.46+3.38 X
F3	757 a (574-1150)	9	Y= -2.83+2.72X
F4	707 a (443-74450)	8	Y= -0.16+1.81 X

Values within a column followed by different letter are significantly different ($p < 0.05$) ($p < 0.05$, LSD) based on the overlap of 95 % CL. Data rounded to nearest whole number

Within the abamectin resistance strain relaxed generations, the larval-dip LC50 values toward abamectin for the fourth instar larvae of cotton leafworm in F1, F3 and F4 were 1243, 757, and 707 mg AIL^{-1}, respectively. No significant difference between any of these values and that in parent SFRS, the value = 1339 mg AIL^{-1}. The resistance level in F1, F3 and F4 determined to be 15, 9, 8 fold, respectively. Also within ARS, the slope values were 3.38, 2.72 and 1.81 in F1, F3 and F4, respectively.

The resistance stability and reversibility percentages in SDRS, SFRS are presented in figure 1. This data further demonstrated that the stability percentages in SDRS in F1, F2, F3, and F4 were 52, 24, 11, and 4 %, respectively. In contrast, those in SFRS were 74, 57, 48, and 22 % in F1, F2, F3, and F4, respectively. The reversibility % in SDRS and SFRS in F1, F2, F3, and F4 were 48, 76, 89, 96 % and 26, 43, 52 and 78 %, respectively. Figure 2 shows the stability and reversibility percentages in ARS relaxed generations. The stability values decreased from 100 % in ARS to 93, 57 and 53 % in F1, F3 and F4, respectively. In contrast the reversibility of those increased from 0 in ARS to 7, 43 and 47 %, respectively.

Figure2: % Stability(ST) and % reversabilty(RV) of resistance in abamectin resistant strain(ARS) toward abamectin in four relaxed generations under labortory conditions.

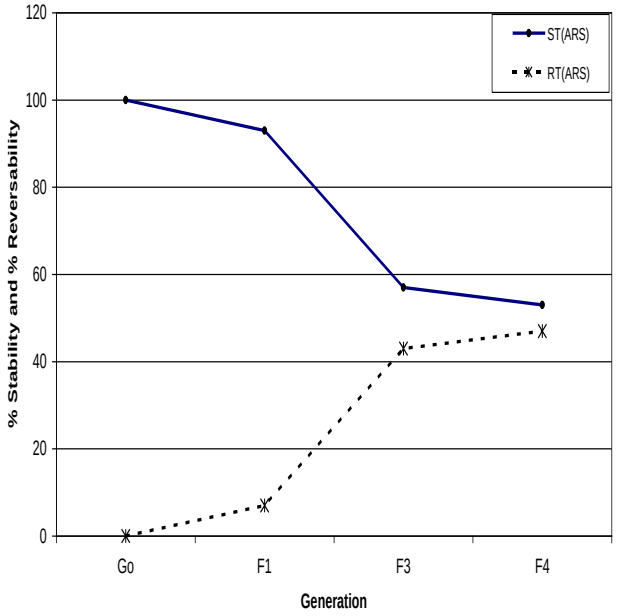
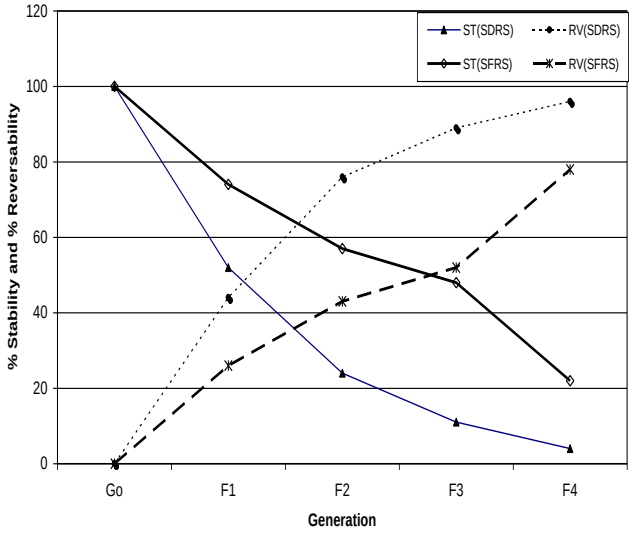


Figure1: %Stability(ST) and % reversabilty(RV) of resistance in spinosad dipping resistant strain(SDRS) and spinosad feeding resistant strain(SFRS) toward spinosad in four relaxed generations under labortory conditions.



DISCUSSION

The SDRS was rebuilt in 24 selection successive generations by using larval dipping method. The strain reached to high level of resistance, the RF around 100 fold compared the susceptible strain (Table 1). The rate of resistance is very terrible to the spinosad. On the other hand, this strain was not selected to spinosad for one generation. The resistance decreased to 52 fold. This means about 50% of the stability of resistance was lost in one generation with free spinosad. Moreover, the RV reached about 96% after four relaxed generations. This means the resistance herein is unstable.

Table 1: Resistant strains characters used in present investigation (selection pressures were done on the fourth instars larvae 30-50 mg/ larvae). Data rounded to nearest whole number

Strains	Selected Insecticide	Selection Mehod	LC_{50} , mg A.I. L^{-1} (95% C. L.)	Resistance Factor(RF)	Regression Equation
Spinosad Dipping Resistant Strain(SDRS)	Spinosad	Larval dip	16081 (10976-24778)	99	Y= -2.53+1.79 X
Spinosad Feeding Resistant Strain(SFRS)	Spinosad	Leaf dip	5804 (3958-7890)	57	Y= -4.11+2.42 X
Abamectin Resistant Strain(ARS)	Abamectin	Larval dip	1339 (1141-1732)	16	Y= -9.07+4.5 X
Susceptible strain(SS)	Spinosad	Larval dip	162.03 (39.99-275.29)	1	Y= 1.86+1.42 X
		Leaf dip	101.87 30.51-194.17	1	Y= 2.45+1.27 X
	Abamectin	Larval dip	84.46 (34.15-203.67)	1	Y= 2.59+1.25 X

Our results supported by the results of Ferguson, 2004, who found that the spinosad resistant strain of leafminer, *Liriomyza trifolii* (RR > 188 fold) reverted to the susceptible strain after reared absence of spinosad pressure fore five generations. Osman et al 1991 found that rearing of two resistant strains of pink bollworm, *Pectinophora gossypiella* under conditions free insecticides resulted in reversion of resistance in four to five generations. Sawicki et al 1980 reported that even highly resistant clones of the green peach aphid, *Myzus persicae* lost resistance when selection pressure was relaxed. Abo Elghar et al 2005 stated that resistant field strain of cotton leafworm was reared for 6- 8 generations free of insecticide pressure lost resistance, the RR values for cymethrin and methomyl decreased from 83.5, 22.9 fold to 9.8, 8.4 fold, respectively after these generations.

Ninsin and Toshiharu 2005 reported that the acetamiprid resistant of *P. xylostella* (RR= 110 fold) when was reared without exposure to acetamiprid for

seven generations dropped rapidly to 2.42 fold. Pasteur and Raymond 1996 suggested that all resistance genes whether conferring a low or high level of resistance are selected as long as the selection pressure is maintained because they confer a high fitness to their carriers. It seems that in the absence of insecticide, the resistance genes have lower fitness than their susceptible counterpart. However, the intensity of selection against resistance genes varies widely with the nature of the genes. Evaluation of this fitness in natural conditions in the absence and presence of insecticides should allow us to devise control programs where both selection forces negate each other.

In contrast, the present results were not compatible to the results of Wyss, 2003 who published the resistant strain of tobacco budworm, *H. virescens*; the RR= 669-fold resistance to spinosad. When this strain was not exposed to spinosad for five generations, the RR decreased to 476-fold. He suggested that the resistant strain is highly homogenous with the predominant genotype, rr. As would be expected, in the absence of any immigration of susceptible genes into the population, resistance is stable.

In our investigation, the slope value is relative low in SDRS; it appears that the strain is not homogenous. The strain has susceptible genes. However in SFRS, these values are relatively high, and this means the strain is relatively more homogenous than SDRS. This can explain why resistance stability is higher in SFRS than SDRS. Our studying suggested that selection methods play a vital role in the ability of strain to back to susceptibility. In SDRS, the most path route of spinosad to reach insects is by dermal contact but in SFRS by ingestion. In the field application, the insect may be exposed to two ways together, so we expected that the resistance is different.

Abamectin was used as acaricide, although it has insecticidal activity (Jansson and Dybas1998; Corbitt et al 1985). Cotton is usually infested by mites with cotton leafworm. In this investigation, we compared the resistance of this bacterium product to spinosad. The cotton leafworm became resistant to this product in 25 selection generations. The reversion ratio is relatively slower than spinosad resistant strains, although the ARS selected by larval dip method like SDRS. These results are very important when using abamectin for controlling mites in cotton; we should consider the rate and the effects of this product to cotton leafworm. It may be the low concentrations that make selection to resistance and this resistance quite stable.

The reversion of spinosad resistance in spite of the high RR at the parental generation could be attributed to a heterogeneous resistant strain and the inability of the resistant individuals to compete effectively with the susceptible ones in terms of reproductive potential and other biotic factors. Unstable spinosad resistance implies that the rotational use of spinosad with insecticides such as pyrethroids and abamectin (Wang et al 2005; Liang et al 2003; Temerak 2003) that do not show cross-resistance would be an effective approach in maintaining susceptible individuals in field populations of *S. littoralis* so as to further retard the development of spinosad resistance. Although resistance development in cotton leafworm to conventional insecticides is well documented, the resistance is unstable and there is no cross-resistance between the insecticides. Thus, incorporating both of these insecticides in a rotational scheme with spinosad would be a reliable resistance management strategy.

In conclusion, our study recommended that spinosad and abamectin can be used for controlling cotton leafworm with IPM programs, especially with the tactics split spray or spray the product then stop at least 1-2 generations for spinosad and 2-3 generations to abamectin. Further studies need to be investigate especially the competition of resistant, reversion and susceptible individuals in the field. Also the selection and reversion of the two products by several methods should be investigated.

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Bioassay of Trichoderma fungal culture filtrate chitinase against cotton bollworms

INTRODUCTION

Recently the demand for alternatives to chemical methods of pest and diseases management has become stronger owing to concerns about the safety and environmental impacts of chemicals. Alternate strategies viz., pheromone traps, botanicals, cultural methods etc. tried with partial success. In coming days biological control (Biocontrol agents and their products; proteins/enzymes) may become an important component of pest management practices due to their specificity, safety to natural enemies, environment and humans.

Trichoderma species produce and secrete a number of hydrolytic enzymes, including several chitinases, which are thought to enable Trichoderma to degrade the chitin-containing cell walls of other insects. T. harzianum, is a soil borne, filamentous fungus known as an effective biocontrol agent of several plant-pathogenic fungi.

Exoskeleton and gut linings of insects (where chitin is found) are the target sites for many pest control agents. Insect cuticle is primarily made of chitin, an insoluble polysaccharide which acts as physic-chemical barrier to environmental hazards and natural enemies. It's degraded to soluble, low molecular weight oligosaccharides by the action of insect molting enzyme; chitinase.

These enzymes degrade chitin, it might be speculated that if applied on to the insect, or if it enters the gut of insect larvae, it can cause significant damage to the peritrophic membrane structure which will result in the larvae being not able to feed and consequently leads to death. Also if applied topically results in the disruption of cuticle which subsequently causes abnormal molting. Hence in the present study we made an effort to bioassay the culture filtrate chitinases against major cotton bollworms.

MATERIALS AND METHODS

Micro-organism and maintenance

Trichoderma harzianum, obtained from soil sample collected from Western Ghats of Karnataka was used for the study. The fungus was maintained on potato dextrose agar (PDA) slants, sub-cultured every 2 weeks and stored at 4^{o}C.

Submerged fermentation

Spore inoculums were prepared by dispersing spores from fully sporulated culture on PDA slants in 0.1% Tween 80 under aseptic conditions. One ml of inoculums was transferred to 30 ml of sterilized production medium of pH 5.5 having the composition (gl^{-1}): $NaNO_3$ 1.0;KNO_3 5.0;$(NH_4)_2HPO_4$ 5.0; $MgSO_4.7H_2O$ 1.0, NaCl 1.0;colloidal chitin 1.0. Further process of incubation, collection of supernatant, analysis of protein in culture filtrate, determination of chitinase activity were done as described in Binod et al (2007).

Bioassay studies

The larvae of all the bollworms were maintained on respective artificial diets under laboratory conditions and third instar larvae was used for bioassaying the culture filtrate in all the cases.

The topical bioassay experiment for each kind of insect worm (Helicoverpa, Earias and Pectinophora) was carried out with chitin activities of 2000, 1000, 500, 250 and 100 U ml^{-1} and five microlitre of each preparation was applied topically on the thorax of worm using a micropipette. 50 larvae were used for each concentration and insect in 3 replications. Control was maintained with culture filtrate without chitinase induction with colloidal chitin. The topical application was continued for 3 days and then onwards insects left undisturbed. Observation on general health, mortality and adult emergence were performed visually.

RESULTS AND DISCUSSION

Mortality of larvae/pupae because of the treatment was measured. The larval development was affected which was indicated by lesser number of pupae which was proportionate to the concentration of chitinase used. Also at the end of the test period, the percentage of larval/pupal mortality was measured. The percentage mortality in chitinase-treated groups showed clear dosage dependence as presented in Table 1. The highest mortalities (65-80%) were recorded for groups treated with 2000 U ml^{-1} for all the bollworm species put together, whereas the control and the lowest chitinase concentration (100 U ml^{-1}) showed nearly 0-10% mortality. The groups treated with 1000 U ml^{-1} chitinase showed mortality rate of 40-60% (Table 1). Chitinases are enzymes with a specific hydrolytic

activity directed towards chitin. Slow action is the major concern in exploiting the current commercial bioagents like Bt and NPV and hence need for new pest management tools, a possible alternative is fungal cultures that can secrete high levels of chitin-metabolizing enzymes that might enable the use of chitinase sprays combined with other pesticide formulations to facilitate faster kill.

Table 1: Effect of topical application of chitinase on larval mortality

Chitinase concentration ($U\ ml^{-1}$)	% Mortality		
	Helicoverpa	Earias	Pectinophora
2000	75	65	80
1000	40	50	60
500	32	30	55
250	25	25	40
100	10	10	25
control	5	0	10

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GENERATING BASELINE DATA FOR PHOSPHINE RESISTANCE MONITORING IN RHYZOPERTHA DOMINICA (FABRICIUS) COLEOPTERA: BOSTRICHIDAE

INTRODUCTION

India is one of the world's largest consumers of grains and strategic grain reserves are maintained by the Indian government in very large long-term storages. India produces about 96.43 million tones (MT) of rice, 78.40 MT of wheat, 19.31 MT of maize and 40.73 MT of millets and other coarse grains (DES, 2008). The grains are handled, transported and stored in gunny bags for sales. About 70 per cent of the total production is reported to be retained by the farmers for consumption, seed purpose and for sale later on. The remaining 30 per cent goes to central pools which are maintained for public distribution and export. The Food Corporation of India, Central Warehouse Corporation and State Warehouse Corporation are involved in storage of food grains in India.

A considerable loss occurs in stored food grains due to insects, rodents and fungi. Insect pests cause heavy loss of stored grain quantitatively and qualitatively throughout the world (Madrid *et al.*, 1990). Post harvest losses of food grains in India are estimated to be around 10 per cent of the total production, of which losses during storage alone are estimated to be 6.58 per cent (Gahukar, 1994). In India, the Pansay Committee estimated that the average storage loss is 2.5 per cent in central storage has been reported due to storage insect infestation (Sawhney, 1988).

At the 1997 Montreal Protocol Meeting, industrialized nations of the world agreed to phase out the fumigant, methyl bromide by 2005 due to the problem of ozone depletion, whereas, developing nations have committed to reduce the use by 20 per cent in 2005 and phase out in 2015 (WMO, 1995). At present, the choice of fumigants for controlling insect pests in stored products is limited to phosphine and it is the only registered fumigant for application directly to stored grains. Further the aluminum phosphide is cheaper and easy to apply than other available fumigants and hence the government agencies and private pest control firms prefer to use phosphine for the fumigation.

Phosphine resistance appears to be the most prominent in many parts of the world (Champ and Dyte, 1976) and Indian subcontinent (Mills, 1983; Rajendran, 1992), where the fumigation of whole stores without proper sealing is commonly practiced. Such practices result in repeated under dosing, inadequate exposure periods and use less air tight conditions which cause a gradual increase in the proportion of the total population showing resistance (Friendship, 1989; Collins and Dyte 1976).

There has been yet no systematic study on the incidence of phosphine resistance in different parts of the country to indicate the extent of the problem and need to tackle it. The problem of phosphine resistance in insects has led to search for suitable alternatives to this fumigant. There is an urgent need to assess the real situation with regard to the occurrence of phosphine resistance in our country. Therefore, the present study is undertaken to investigate the current situation with

regard to phosphine resistance among the insect pests of stored grain.

MATERIALS AND METHODS

The research work as per the objectives was carried out at Agricultural College and Research Institute, Coimbatore during 2008-2009. The materials used and the methods employed in the study are presented hereunder.

Mass culturing of test insects

Rice weevil and lesser grain borer

Maize and sorghum grains were infested with *R. dominica* collected from Millet Breeding Station, TNAU, Coimbatore, Tamil Nadu, India, and were reared in the laboratory at 28° C and 70 per cent relative humidity on whole wheat kernels outlined by Mohan *et al*. (2007).

Preparation of phosphine gas sources - FAO Method No.16

Phosphine gas of 86 per cent purity was generated in a gas generating apparatus by reacting 100 mg of aluminium phosphide formulation with 5 per cent sulphuric acid to provide phosphine for dosing purpose.

Fumigation bioassay

The fumigation procedure as given by FAO method was followed (Anonymous, 1974). Five different concentrations were tested against the test insects *R. dominica.* Thirty adults were taken in a vial (5 cm × 1.2 cm) and the mouth of the vial was covered with net (25 mesh) to prevent the insects from escaping. Three such vials containing thirty insects each were placed in the fumigation chamber (*i.e.*, 5.3 lit desiccators) and considered as three replications.

The required amount of phosphine gas was withdrawn from the source using the gastight syringe and applied to insects in fumigation chamber. The required dose volume of 86 per cent gas source at room temperature 30°C was determined by using the formula developed by FAO Method No. 16

$$d_1(\mu l) = \frac{298X\ x_i(mg/l)X\ V_i(l)X\ 22.414X1000X1000X100}{273X1000X\ 33.9977(GMWphosphine)X\ 86}$$

The five different concentrations from 0.02 to 0.06 mg l^{-1} were obtained from the preliminary range finding test to construct log dose probit mortality line for susceptible population. Required dose volume was taken and injected into the appropriate desiccators and the time when each dose applied was recorded. Following dosing, the desiccator was held at a temperature of 30°C throughout the exposure period of 20 hrs.

Mortality assessment

Following fumigation, the insects were transferred to 15 ml plastic container with small quantity of suitable medium and held at 25°C and 70 per cent relative humidity. Adult mortality was determined after 14 days from the end of the exposure period as fixed by FAO Method No.16 (Anonymous, 1974). The observation on numbers responding *i.e.* dead insects was taken and insects showing any movement were considered to be alive.

RESULTS

Acute toxicity of phosphine to *R. dominica*

LC_{50} of phosphine assessed for F_1 population was 0.033 mg l^{-1} and LC_{99} value was 0.088 mg l^{-1} (Table 1 and Fig. 13-17).

Table 1: Acute toxicity of phosphine gas to *R. dominica* by fumigant bioassay over generations

Generation	Regression Equation	Chi square χ^2	LC_{50} mg l^{-1}	Fiducial limits		LC_{99} mg l^{-1}	Fiducial limits	
				LL	UL		LL	UL
1	Y = 5.5918x + 13.245	3.279	0.033	0.032	0.036	0.088	0.076	0.103
2	Y = 5.2507x + 13.07	4.899	0.029	0.027	0.031	0.082	0.069	0.096
3	Y = 6.2571x + 14.656	4.150	0.028	0.027	0.031	0.074	0.064	0.087
4	Y = 5.5491x + 13.574	0.433	0.027	0.026	0.030	0.073	0.064	0.085

The susceptibility index (SI) of F_4 generation over F_1 was 1.2222 and 1.2054 based on LC_{50} and LC_{99}respectively. The rate of resistance decline (R) **was** negative indicating that susceptibility increased with the succeeding generations. The R value was - 0.0218. Thus, the number of generations required for a 10-fold decrease in LC_{50} was calculated as 45.90 (Table 2).

Table 2: Susceptibility index and rate of resistance decline of phosphine for *R. dominica*

Sl. No	Insect	Susceptibility index		Rate of resistance decline	
		LC_{50}	LC_{99}	R	G
1.	*R. dominica*	1.2222	1.2054	- 0.0218	45.90

DISCUSSION

LC_{50} of phosphine assessed for F_1 population was 0.033 mg l^{-1} and LC_{99} value was 0.088 mg l^{-1} (Table 1). White and Lambkin (1990) observed that the LC_{50} value of phosphine to different strains of *R. dominica* as 0.0057, 0.0061, 0.0064, 0.0073 and 0.0076 mg l^{-1} - where as the present study reveals that the LC_{50} value of *R. dominica* for phosphine as 0.033 to 0.027 mg l^{-1} which is higher than the earlier findings and similar to *T. castaneum*. This shows that there is an increase in LC_{50} value over a period of time which may be due to the geographical variations or development of resistance to phosphine.

The susceptibility of *R. dominica* population was tested with relaxation of selection pressure to phosphine by continuously rearing the population in laboratory for five generations. The results indicated that susceptibility slightly increases with succeeding generation as evident from decline in LC_{50} from 0.033 (F_1) to 0.027 (F_4) mg l^{-1} and the LC_{99} from 0.088 (F_1) to 0.073 (F_4) and the susceptibility index at LC_{50} and LC_{99} were 1.2222 and 1.2054 respectively and the number of generations required for 10 fold decrease in LC_{50} was 45.90. The rate of resistance decline is negative indicating that the susceptibility increased with succeeding generation.

The discriminating dose of phosphine against *R. dominica* based on LC_{99} value was fixed as 0.03 mg l^{-1} by FAO Method (Anonymous, 1975). But in the present study it was 0.07 mg l^{-1} for the laboratory cultured F_4 generation of *R. dominica*. Higher LC_{99} value to the extent of 2.33 times could be due to resistance buildup over the past three decades or due to the geographical variation.

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Variability in the response of *Helicoverpa zea* and *Heliothis virescens* (Lepidoptera: Noctuidae) to Cry1Ac and Cry2Ab2 in diet incorporation assays

ABSTRACT

Our dataset on susceptibility of *Helicoverpa zea* and *Heliothis virescens* to insecticidal proteins expressed in Bt cotton is large, spans nearly two decades and may be suitable for exploration of a number of insect resistance management questions. Both species exhibit wide variability among field populations in response to toxins incorporated into meridic diet, and both species can be selected for elevated responses to toxin challenge in diet assays. Shifts in measured susceptibility over time and geographic location may be important indicators of changing levels of field resistance, but more work is needed to associate variability observed in diet assays with variability in survival of insects on toxin expressing plants in the field.

INTRODUCTION

When Bt cotton was commercially deployed in the United States, the EPA required annual monitoring of pest susceptibility to the insecticidal proteins of *Bacillus thuringiensis* expressed in the transgenic plants. Potential resistance to Bt proteins was especially a concern for field populations of bollworm, *Helicoverpa zea* (Boddie), and tobacco budworm, *Heliothis virescens* (F.), targeted by the transgenic traits. Prior to commercial deployment of Bt cotton, field populations of both species were surveyed for susceptibility to Cry1Ac and Cry1Ab proteins by Monsanto Company scientists (Stone and Sims 1993) and our research group at Mississippi State University (Luttrell et al. 1999). Both research groups utilized diet incorporation assay procedures similar to that used by Dulmage et al. (1976) to compare potency of different Bt strains, and both research groups reported successful laboratory selection for resistance in at least one of the species. Stone et al. (1989) reported selection of a strain of *H. virescens* with 20-fold resistance levels, and Luttrell et al. (1999) reported selection of a *H. zea* strain with 100-fold resistance.

Responsibility for monitoring Bt susceptibility in *H. zea* and *H. virescens* was assigned to a public research group, the Southern Insect Management Research Unit of the USDA-ARS in Stoneville, Mississippi. Hardee et al. (2001) reported initial results of these efforts that involved spray table assays with a commercial Bt formulation (MVPII) and diet overlay assays with a freeze-dried formulation of the MVPII formulation. Blanco et al. (2008) and Blanco et al. (2009) report more recent efforts of this group to track Cry1Ac resistance in *H. virescens*.

In 2002, about a decade after our initial examination of Bt susceptibility in *H. zea* and *H. virescens* at Mississippi State University, we again examined variability in response of both species to Cry1Ac. We later developed baseline information for susceptibility of both species to Cry2Ab2, Vip3A and Cry1F. This work was done at the University of Arkansas in a different laboratory with different protein sources and different personnel, but procedures were generally the same as those used in the initial studies at Mississippi State University. Our initial interest was explaining control problems reported by Arkansas and Mississippi extension entomologists (Luttrell et al (2004)). Components of this research have been published in the entomological literature (Ali et al. 2006, Ali and Luttrell 2007, Ali and Luttrell 2009). In this report, we present a general summary of the response of both species to Cry1Ac and Cry2Ab2, the insecticidal proteins expressed in Bollgard® and Bollgard II® cottons (Monsanto Company, St. Louis, MO).

MATERIALS AND METHODS

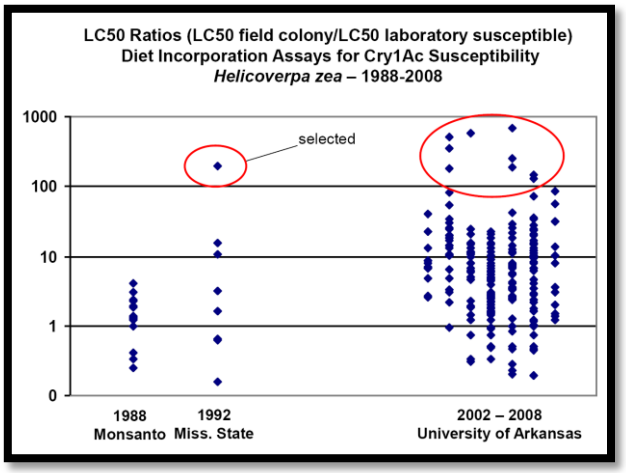
Detailed descriptions of research methods are contained in Luttrell et al. (1999), Ali et al. (2006) and Ali and Luttrell (2007). In general, insects were collected from the field as eggs, larva or adults and taken to the Margaret McClendon Insect Rearing Facility at the University of Arkansas. Colonies of these field collected insects were reared and progeny from the first to third generation were exposed to Cry1Ac or Cry2Ab2 in diet incorporation assays. Toxin sources were lyophilized MVPII powder and a Cry2Ab2 corn leaf powder obtained from Monsanto Company (Ali et al. 2006, Ali and Luttrell 2007). Each assay included five to seven concentrations of the toxin and an untreated control. All assays were replicated two to eight times with 16 larvae per replicate. Mortality at seven days was the standard response variable in all studies including early studies at Mississippi State University. Later studies included stunting as a response measured at seven days. Mortality was used to calculate LC (lethal concentration) regressions. Failure of larvae to molt to the second instar by seven days plus mortality was used to calculate MIC (molt inhibition concentration) regressions. Probit regression models were calculated for all response variables using SAS PROBIT. All studies included comparative assays with susceptible laboratory strains of both species. Resulting data were reported as susceptibility ratios with response of field strain divided by response of laboratory strain. This was done to eliminate the experimental influence of variable toxin potency.

RESULTS AND DISCUSSION

Stacked scatter graphs illustrate annual variability in the LC50 response of *H. zea* (Figure 1) and *H. virescens* (Figure 2) to Cry1Ac at the University of Arkansas relative to the previous benchmark

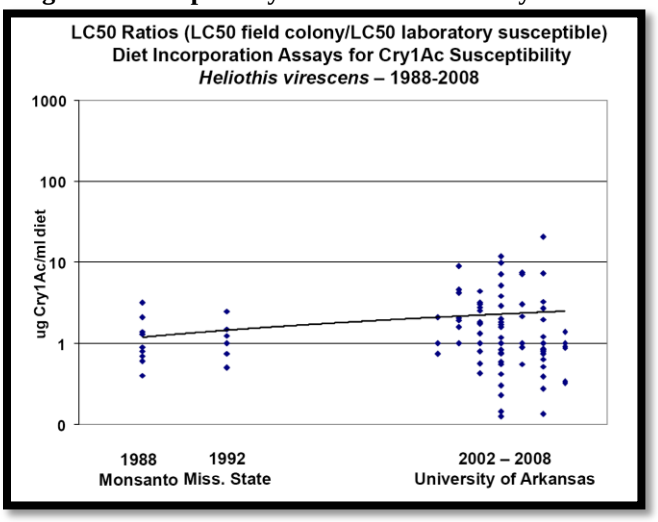
measurements. Figure 1 represents more than 300 individual regression estimates of colony susceptibility. The variability measured at Mississippi State University in 1992 was similar to that measured by Monsanto scientists in 1988. Included in the 1992 data is the regression estimate for a selected strain that had a LC50 greater than 100-fold that of the susceptible laboratory strain. This strain was selected from ~200 field collected insects and was lost due to insufficient reproduction after nine generations of selection. A large number (more than 300) of regressions representing different field strains were obtained in studies at the University of Arkansas from 2002-2008. Of these, 15 (~5%) had LC50s 100-fold or higher than those of a susceptible laboratory strain. The higher responses in 2002-2008 data as compared to 1992 data have been a concern for potential resistance evolution.

Figure 1. Susceptiblity of *H. zea* to Cry1Ac.



Bt cotton has been highly effective against field populations of *H. virescens*. Although no field population exhibited LC50s 100-fold those of the susceptible laboratory strains (Figure 2), there was variability in response of *H. virescens* to Cry1Ac. As with *H. zea*, the larger number of samples at the University of Arkansas was more variable than in benchmark studies. The resistant strain selected by Monsanto scientists in 1988 (Stone et al. 1989) had an LC50 about 20-fold that of the non-selected strain. Our assay data at the University of Arkansas included that much variability in samples of field strains of *H. virescens*. As reported for *H. zea*, there was a trend for higher LC50s in Arkansas data as compared to previous Mississippi data.

Figure 2. Susceptibility of *H. virescens* to Cry1Ac.



Assays conducted at the University of Arkansas included measurements of stunting as well as mortality after seven days of exposure to treated diet. Figures 3, 4, 5 and 6 provide comparative summaries of LC and MIC ratios for *H. zea* and *H. virescens* exposed to Cry1Ac and Cry2Ab2. The effective dose required for mortality at seven days was generally higher than that required for stunting and LC50 ratios are generally higher than MIC50 ratios. Variability in response tended to be greater with mortality data. With *H. zea* exposed to Cry1Ac there was no indication of increased survival with LC50 and MIC50 estimates over the seven years of study at the University of Arkansas (Figures 3). LC50 data for *H. zea* exposed to Cry2Ab2 suggested a trend for increased survival over the test period (Figure 4). Some LC50 ratios approached the 100-fold level in 2005 and 2006, but average values were similar across the years. MIC50 responses showed no trend for increased survival of *H. zea* exposed to Cry2Ab2. Collectively, the 2002-2006 studies at the University of Arkansas corroborate earlier benchmarks studies that documented wide variability in response among populations of both species to Cry1Ac and Cry2Ab2. *H. zea* exhibited more variability and was the less susceptible species.

Figure 3. LC50 and MIC50 ratios for *Helicoverpa zea* exposed to Cry1Ac.

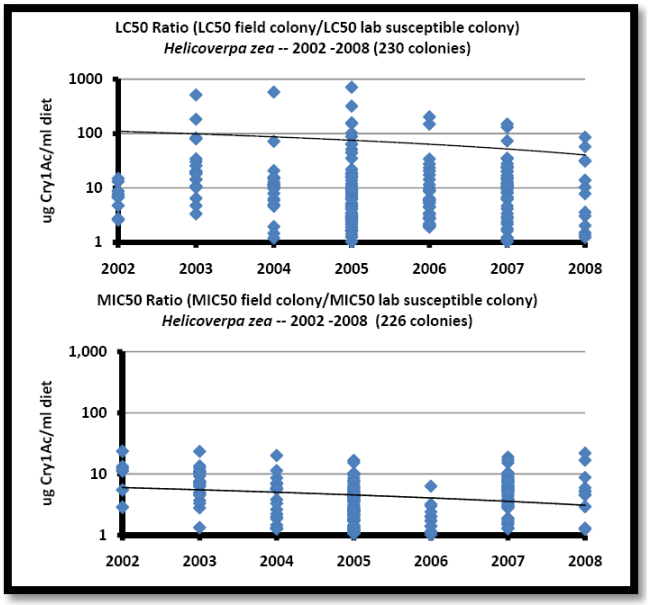


Figure 4. LC50 and MIC50 ratios for *Helicoverpa zea* exposed to Cry2Ab2.

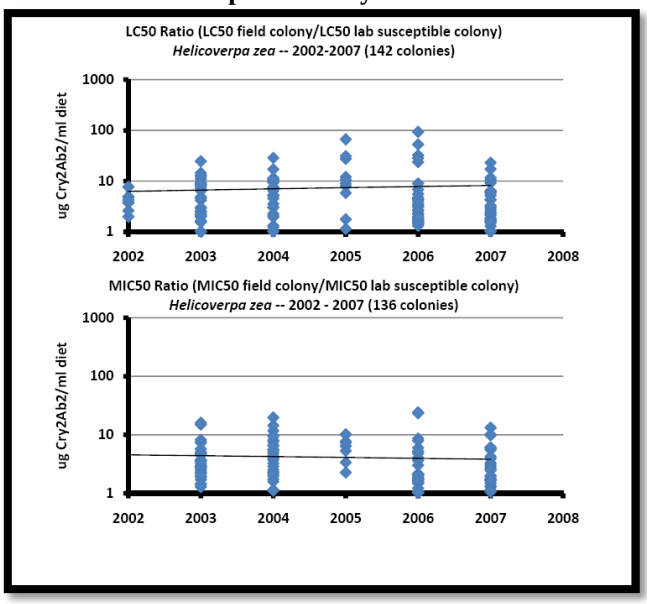


Figure 5. LC50 and MIC50 ratios for *Heliothis virescens* exposed to Cry1Ac.

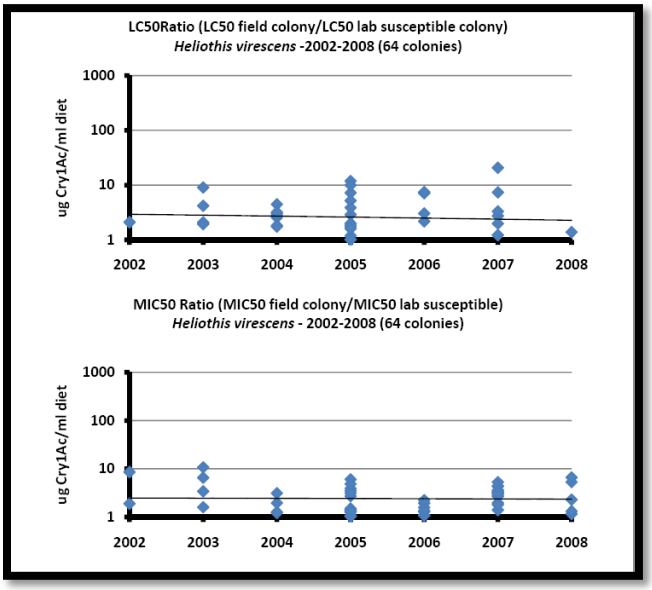
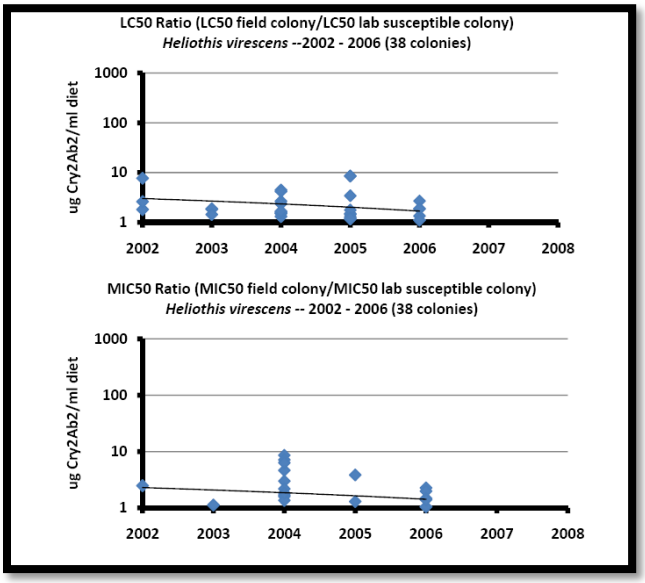


Figure 6. LC50 and MIC50 ratios for *Heliothis virescens* exposed to Cry2Ab2.



In summary, this large dataset describes wide variability in response of *H. zea* and *H. virescens* to Cry1Ac and Cry2Ab2 insecticidal proteins expressed in Bt cotton. This variability and the ability to select for elevated responses in the laboratory suggest that both species have genetic potential to evolve resistance to the toxins. The significant difference in responses for *H. zea* exposed to Cry1Ac in 1992 at Mississippi State University and those measured at the University of Arkansas a decade later suggest that resistance levels increased (Luttrell et al. 2003. However, these are not strong empirical comparisons given differences in research laboratories, toxin sources and sample

efficiency. Baseline studies at Mississippi State University included only a few colonies. Those at the University of Arkansas included 100s of colonies with a greater capacity to detect rare genotypes. Interestingly, a smaller but similar trend for higher LC50s was observed in *H. virescens* data comparisons between the two study sites.

While our data do not confirm the evolution of field resistance to Cry1Ac in *H. zea*, they also do not eliminate the possibility. If differences between 1992 Mississippi State University studies and 2002 University of Arkansas studies are due to increased frequencies of Bt resistance genes, the change must have occurred early in the deployment of Bt cotton. The lack of evidence for further changes in the University of Arkansas data from 2002-2008 suggest that environmental and/or genetic factors, perhaps strong fitness costs combined with effective use of mandated refugia, may be at work to limit resistance evolution. The continued measurement of variable responses is a reminder of the genetic plasticity of these pests and a caution for continued vigilance in managing the threat of resistance evolution. Additional research should be conducted to link diet assay results to actual field survival of insects on toxin expressing plants.

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Fitness traits of insecticide resistant and susceptible strains of diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae)

ABSTRACT
We compared the biological traits of insecticide resistant field populations and a laboratory reared susceptible strain of diamondback moth, *Plutella xylostella* L. The insecticide resistant field populations (Bangalore and Shimoga) differed from the susceptible strain in respect of larval and total developmental periods. The susceptible strain completed its development in a shorter period compared to the insecticide resistant field populations. No significant differences were observed between the populations in respect of fecundity, hatchability of eggs and sex ratios. The above results clearly demonstrated that certain fitness components in the resistant strains appear to be reduced, but not all.
Key words: Fitness traits, insecticide resistance, Diamondback moth (DBM), *Plutella xylostella*

INTRODUCTION

The diamondback moth (DBM), *Plutella xylostella*, is a major pest of cruciferous vegetables throughout the world causing huge crops losses, particularly to cabbage and cauliflower (Talekar and Shelton, 1993). The crop losses due to DBM may often go up to 100 per cent (Calden and Hare, 1986). The pest is considered to be difficult to manage in most parts of the world owing to its ability to develop resistance quickly to insecticides deployed in its management. It has developed resistance to almost all insecticides used for its control including *Bacillus thuringiensis* toxins (Sannaveerappanavar and Viraktamath, 2006). Insecticide resistance and control failure are now common in areas wherever crucifers are grown. Insecticide resistance in insects often involves deficiencies in fitness, vigor, behaviour or reproductive potential. Reduced biotic fitness of resistant phenotypes has been reported for several species of insects. Resistant strains of arthropods often have lower fecundity and longer developmental time than their susceptible counterparts (Geoghiou, 1977). The temephos (OP compound) resistant strain of southern house mosquito showed lower fecundity, took more time to complete development and had a poor viability than the susceptible strain (Farrari *et al*., 1981). Egg production, hatchability, mating success and adult viability were lower in resistant Hawaiian strains of DBM (Groeters *et al.*, 1994). However, a *B. t.* resistant population of DBM in Florida did not show any fitness cost (Tang *et al.* (1997). The information on the fitness cost of resistance in Indian populations of DBM is not available. Therefore, in the present study, an attempt has been made to compare the biological traits of insecticide resistant field populations with that of the laboratory reared susceptible strain of DBM.

MATERIAL AND METHODS

The susceptible strain of DBM which was maintained in the laboratory without exposing to any of the insecticides for more than 278 generations and field populations of DBM collected from major cabbage growing areas of Karnataka (south India) *viz.*, Bangalore, Hassan, Shimoga and Belgaum, and also field populations from north India *viz.*, Punjab and Delhi were used in the study to compare the life time fecundity. The field populations of Bangalore, Shimoga and the susceptible strain were compared for other fitness traits such as larval duration, pupal weight, sex ratio and developmental time.

Ten pairs of moths of each DBM population were used in the study. The sexes were separated in the larval stage itself by observing light yellowish spot on dorsal aspect of posterior part of the abdomen in male larvae. The larvae were transferred on to fresh mustard leaves in petri dishes and allowed for pupation. The resulting pupae were kept in specimen tubes at room temperature (28 ± 1^0C) for moth emergence. A few hours after emergence (<6 hours old), the moths were paired and allowed for oviposition in plastic boxes. Food for adult moths was provided in the form of 10 per cent honey solution in cotton. A mustard leaf disc was provided to each pair for oviposition. Each leaf disc was placed in a glass vial containing water to maintain freshness. Each day, fresh leaf discs were given to the moths and the eggs were counted. Leaf discs with eggs were placed in glass vials containing water to record the hatchability. For observations on larval duration and pupal weight, thirty newly hatched larvae were transferred on mustard leaves in petri dishes and maintained in a BOD incubator at 25 ± 1^0 C. Old leaves were changed with fresh leaves as and when required. The pupae were collected each day and weighed. Sex ratio was recorded when the larvae were in fourth instar stage.

RESULTS AND DISCUSSION

Resistant populations divert more body resources towards various resistance mechanisms to overcome the toxic effects of insecticides. This may often result in some biological deficiencies in the resistant populations when compared with the susceptible populations. Hence, in the present study, biological traits of insecticide resistant field populations were compared with that of the susceptible population of DBM. The results of this study are presented in Table 1. The insecticide resistant field populations of Bangalore and Shimoga differed from the susceptible

population in respect of certain fitness traits and were found to have lower fitness than the susceptible population. The susceptible and insecticide resistant populations differed only in respect of larval and the total developmental periods, and the pupal weight. The total larval period was significantly longer for the resistant field populations of Bangalore and Shimoga (13.03 and 12.63 days, respectively) compared to susceptible strain (12.16 days). Also, the mean larval periods of Bangalore and Shimoga populations were also significantly different from each other. This variation could be attributed to the insecticide usage (spray schedule, frequency and dose) or differential selection pressure on the DBM in these locations. The results are in agreement with Trisyono and Whalon (1997) who also reported larval development of resistant strain of Colorado potato beetle was significantly longer than that of susceptible strain.

Table 1: Life history traits of susceptible and insecticide resistant field populations of *Plutella xylostella* L.

Fitness traits / Populations	Fecundity (No. of eggs/female)	Egg hatchability (%)	Larval period (days)	Pupal weight (mg)	Developmental time (Days)	Sex ratio (M:F)
Susceptible	198.00^{a}	90.92^{a}	12.16^{c}	6.43^{b}	21.37^{b}	1: 1.02^{a}
Bangalore	174.67^{a}	84.10^{a}	13.03^{a}	7.10^{a}	23.10^{a}	1:1.12^{a}
Shimoga	185.33^{a}	85.12^{a}	12.63^{b}	6.93^{a}	22.73^{a}	1:1.14^{a}
Hassan	181.11^{a}	ND	ND	ND	ND	ND
Belgaum	176.89^{a}	ND	ND	ND	ND	ND
Delhi	190.12^{a}	ND	ND	ND	ND	ND
Punjab	176.17^{a}	ND	ND	ND	ND	ND

* ND: Not determined
* Means in each column followed by same letter are not significantly different at 5% level by DMRT

The mean pupal weight of susceptible strain (6.43 mg) was significantly lower compared to the mean pupal weight of field populations of Bangalore and Shimoga (7.10 and 6.93 mg, respectively). There was no difference in pupal weight between Shimoga and Bangalore field populations. Similarly, Cheng *et al.* (1999) reported resistance to dimehypo and cartap in DBM did not affect mean pupal weight of the resistant strains, thus indicating that no fitness cost was involved in resistance to those compounds. Similar results were also reported by Sayyed and Wright (2001) who also found that the mean pupal weight of Cry I Ac selected strain was not significant from that of unselected ROTH starin of DBM. However, in the present study the mean pupal weight of the susceptible strain was less than that of the resistant field populations. This could be due to the continuous rearing of the insect under laboratory conditions and inbreeding.

Developmental time of insecticide resistant field populations of Bangalore and Shimoga was significantly longer (23.10 and 22.73 days, respectively) compared to that of susceptible strain (21.37 days). However, there was no significant difference in the developmental period of Bangalore and Shimoga populations. The extended developmental period of more than one day compared to the susceptible population may increase the risk of being attacked by natural enemies. Similarly, Sayyed and Wight (2001) found variation in developmental time between the selected and unselected Malaysian strains of DBM. Similar results were also reported by Geoghiou (1977) who reported resistant strains of arthropods often have longer developmental time than their susceptible counter parts.

No significant differences were observed between the populations in respect of fecundity, hatchability of eggs and sex ratio. The mean fecundity of susceptible and six resistant field populations ranged from 174.67 to 198.00 per female. Though the females of susceptible population appeared to lay more number of eggs (198.00/female), the fecundity was not significantly different from that of six field populations. Similar results were also reported by Motoyama *et al.* (1991) who observed no differences in number of eggs laid by the selected and unselected strain of DBM. However, in another study, Groeters *et al.* (1994) reported that resistant DBM females produced fewer eggs than the susceptible strain. Likewise, Sayyed and Wright (2001) reported that significantly less number of eggs laid by Cry I Ac selected strain compared with the unselected ROTH starin of DBM.

The per cent egg hatching was compared between the susceptible and two insecticide resistant field populations (Bangalore and Shimoga). The egg hatching percentage ranged from 84.10 to 90.92, but the differences between the populations were not statistically significant. Similarly, a marginally significant difference in egg hatching was reported in DBM by Groeters *et al.* (1994). The sex ratio of susceptible population did not differ significantly from the sex ratios of resistant field strains. Similarly, Trisyono and Whalon (1997) also did not observe significant differences in the sex ratio of resistant and susceptible strains of Colardo potato beetle.

It is evident from the above results that the fitness cost of resistance in Indian populations of DBM was very limited with resistant populations taking longer time to complete their development. However, the resistant populations were as good as the susceptible strain in respect of fecundity, hatchability of eggs and sex ratios.

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Need for Generating Baseline Data for Monitoring Insecticide Resistance in new invasive mealy bug *Paracoccus marginatus* Williams and Granara de Willink (Insecta:Hemiptera: Pseudococcidae) , the Key Pest of Papaya and Biofuel Crop, *Jatropha curcas.*

Mealy bugs, cause serious damage considering their reproductive potential, invasive power and facilitation by the phoretic ants. About 5000 species of mealy bugs have been recorded from 246 families of plants throughout the world. Among these, 56 species have been reported from 15 genera of family Malvaceae including cotton and many other plants of economic importance (Ben-Dov, 1994). *Brevennia (Heterococcus) rehi* Lindinger on rice, *Kiritshenkella (Ripersia)sacchari* (Green), *Phenococcus (Dactylopius)saccharifolii* (Green), *Pseudococchus saccharicola* Takahashi, *Antonina (A.indica)graminis* (Maskell) and *Dysmicoccus carens* Williams, on sugarcane, *Coccidohystrix (Centrococcus) insolita* (Green) on brinjal and mango giant mealy bug, *Pseudococcus lilacinus* Green on pomegranate, mango,sapota and tamarind, *Pseudococcus brevipes* on papaya, *Rastrococcus* (*Phenacoccus*)*iceryoides* (Green), on sapota, , citrus, and curry leaf, *Cataenococcus theaecola* (Green) on tea, *Cataenococcus* sp. on coffee, *Pseudococcus brevipes* Ckll. on pine apple, *Nipaecoccus vastator* Maskell on wood apple, tamarind, oleander and potato tuber, *Phenococcus ballardi* Newst. on mango, Pseudococcus sp. on lily occur as regular or occasional pests. Root mealy bugs, *Dysmicoccus* sp. on tea, *Geococcus coffeae* on sweet potato and coffee, *Saccharicoccus sacchari* (Cockrell) and *Tetraneura javensis* on sugarcane and *Geococcus citrinus* on betelvine *Tetraneura nigriabdominalis* on finger millet occur on roots and tubers subterranially (Regupathy *et al*. 2003 , Suresh and Kavitha 2007 ,Nair ,1975). Few of them *viz.*, tailed/striped mealy bugs *Ferrisaina (Ferrisia) virgata*, (Cockerell), the pink hibiscus mealy bug or grapevine or mulberry mealybug, *Maconellicoccus hirsutus* (Green), the citrus mealy bug, *Planococcus citri* (Risso) and the coconut mealybug

Pseudococcus longispinus Targioni Tozzetti, and *Rastrococcus iceryoides* (Green), are the most serious and cause heavy damage to many of the horticultural crops in India.

Occurrence of a new mealy bug (believed to be *Phenacoccus solenopsis* Tinsley) was reported on *Parthenium hysterophorus* during April 2006 for the first time. This was followed by the reports of this pest occurring on many crops, including cotton. Simultaneously, reports were received on the occurrence of mealy bugs on cotton from almost every state in India, with *P. solenopsis*, *P. solani* Ferris and *F. virgata* (known as *Ferrisia malvastra* McDaniel in India) noted as a species complex in various States (Suresh and Kavitha,2007.). *P.solenopsis* emerged as serious pest of cotton in Gujarat (Jhala et.al. 2008) and Punjab (Dhawan, 2007). It has attained the status of a serious pest on a wide range of host plants in Pakistan as well (Abbas et.al 2005,2006; Arif et.al., 2009).

But recently during the routine field visits and rowing survey by the authors for the occurrence of pests on biofuel, *Jatropha curca* (Regupathy and Ayyasamy,2006,2007a,b,2008) , infestation of papaya mealy bug , *Paracoccus marginatus* Williams and Granara de Willink. in and around Coimbatore, Tamil Nadu, on papaya and Jatropha and various associated plants in the neighbourhood was observed (Regupathy and Ayyasamy 2009). *P. marginatus* incidence was observed on commercial papaya plantations in Udumalpet, Karur and Coimbatore and mulberry fields and on Jatropha in 11 locations in and around Coimbatore only. *F.virgata* only was observed in 16 locations spread over in Coimbatore, Theni, Dindigal, Thirunelveli and Virudhunagar Districts of Tamil Nadu. The occurrence of *P. marginatus* on papaya in Coimbatore had been reported by Muniappan (2009). Severe infestation was found on more than fifty hosts including horticultural crops like papaya bhendi, brinjal, curryleaf, guava, mango, pomegranate, silk, cotton, star gooseberry, and west indian cherry, ornamentals, flowering shrubs, foliaged shrubs, flowering trees,select ornamental foliage trees, shade trees ,hedge plants, voluntary plants and weeds. Heavy infestation could cause death to most of the host plants, except the deciduous Plumeria, Jatropha and teak and *Prosopis*. Apart from infesting papaya , attack of several genera of host plants (> 50), including economically important tropical fruits and ornamentals in Mexica and USA had been reported(Pest alert,2003 ; Alison Walker et.al,2003 and Miller et al.1999). A complete description of all instars of both sexes of the mealy bug, and the distinguishing characters from other closely related species had been provided by Miller and Miller (2000, 2002) .The biology of *P.marginatus* had not been studied in detail. However the biology may not be drastically different from the general pattern of other mealy bugs (Pest Alert, 2003).

In India Papaya is cultivated extensively on commercial scale and mulberry for silkworm rearing. Government of India is taking various measures to promote bio-fuel production. Planning commission has identified 200 districts spread over in 18 states *viz*., Andhra Pradesh, Bihar, Goa, Gujarat, Haryana, Himachal Pradesh, Jharkand, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Orissa,Punjab, rajasthan, Tamil nadu, Uttaranchal and Uttar Pradesh (Neelu singh, 2008). Cultivars are planted in extensive scale with high inputs. Persistent increase in the population of this mealy bug and its invasive nature on large number of weed and wild hosts serving as statutory inoculum may likely to be major threat to the economical production of papaya, mulberry and Jatropha unless effective precautionary measures are taken.

Insecticides effectively check the pest in the event of outbreak, but could be recommended only as a last resort in the integrated package of control measures. *P.marginatus* has never gained status as a serious pest in its native Mexico and/or Central America, due to the presence of an endemic natural enemy complex. Commercially available generalist predator, *Cryptolaemus montrouzieri* Mulsant, and naturally occurring other lady beetles, lacewings, and hover flies, have a potential impact on mealybug populations. Biological control through release of lab cultured *C. montrouzieri* had been successfully followed for the management of mealy bug on grapes, citrus, mango, guava, coffee, rubber, cocoa and mulberry (Regupathy et.al., 2003; CPG 2005;CPTHC 2003). However the coccinellid predators generally found on other species of mealy bug were seldom found feeding on *P.marginatus*. Muniappan (Personal communication) is of the opinion that *Cryptolaemus* is known to feed on *P.marginatus* but none of the coccinellid predators would be able to provide satisfactory control. Notable numbers of Lycenid larvae, *Spalgius epius* (Westwood) were found feeding on *P.marginatus* on teak, pomegranate, *Tecoma, Thespesia*, hibiscus and nerium plants during cooler months (Regupathy and Ayyasamy, 2009). With approach of summer, the population of *S. epius* also declined. Due to problem in ovipositon of *S. epius* under captive condition, there is a remote chance of mass

multiplication in lab and field release. In the absence of effective natural enemies, the other option at present containing this pest is use of insecticides. A number of insecticides *viz.* dichlorvos, monocrotophos,methyl demeton , quinalphos, fenitrothion, fenthion , dimethoate (Regupathy et.al., 2003) acephate, carbaryl, chlorpyrifos, diazinon, dimethoate, malathion, and white mineral oils (Alison Walker, 2003). are available to control mealy bugs. Generally higher doses of insecticides than that used to other pests may be required when treating for mealy bugs because mealy bugs are protected by thick waxy, cottony sacs, and often are concealed inside damaged leaves, flower racemes and fruit bunches. Chemical controls are only partially effective and require multiple applications. Moreover reinfestation from the infested hedge, ornamental and weed (Parhtenium) hosts around the fields in small scale farming system prevailing in India necessitates repeated application of insecticides. This might hasten the development of resistance to all or some of these chemistries and there is a need to monitor *P.marginatus populations* for the development of resistance.

In most practical situations the best monitoring method is the use of discriminating/ diagnostic dose (DD) i.e. the dose that kills 99 percent of susceptible individuals (Roush and Miller, 1986.). The population which has not undergone any exposure to insecticides provides the advantages of totally susceptible bench mark for calculating resistance ratios. At present the pesticide application is nil or at least, minimal and the exposure of *P.marginatus* to pesticides might be nil or negligible. This is the ideal time to get the susceptible population for generating base line susceptibility.

Out of number of bioassay techniques *viz*., topical assay, leaf residue/ leaf disc, foliar application bioassay, thin layer exposure bioassay/ surface residue vial bioassay, sticky card technique, slide dip bioassay and glass vial technique available, the leaf disc method (Anonymous, 1990) will be more suitable for *P. marginatus* considering contact as well as systemic poisons are used against this pest. This method had been followed for *P.solenopsis* by Dhawan et.al., (2008). Field collected *P.marginatus* reared on papaya /Jatropha flower could be used for bioassay. The control is treated with acetone alone. Not less than 50 newly emerged one day old nymphs / crawlers could be used per dose need to be used per dose/treatment. Mortality counts are taken 24 h after treatment. To assess the relative toxicity of insecticides median lethal dose (LD_{50}) to *P. marginatus* is determined. Preliminary range finding tests are made to fix the appropriate dosage range. The log -dose / concentration - response curves are fitted (Regupathya and Dhamu, 2001). The DD can be arrived at as detailed by Roush and Muller (1986).

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Susceptibility of larval leafminer *Liriomyza sativae* Blanchard (Dip: Agromyzidae) to Azadirachtin, Matrine and Spinosad under laboratory condition

ABSTRACT
The leafminer, *Liriomyza sativae* Blanchard**,** is one of the most serious pests in greenhouses of vegetable crops throughout the world because of its short generation time, high reproductive rate, conspicuous damage, and the ability to develop resistance to currently registered insecticides. Azadirachtin (Neem Azal 15%), Matrin (Kingbo 0.4%) and Spinosad (Spinosin 24% SC), has been tested on *L. sativae.* These treatments were conducted on cucumber plants under laboratory conditions (25°c, 65 RH, L: D 16:8). The leaf-dip bioassay method was adopted for assaying Azadirachtin, Matrin and Spinossad. The LC_{50} was estimated by POLO-PC, SAS softwars. The results indicated that the Spinosad had a lower LC_{50} compared with Azadirachtin and Spinosad insecticide (LC_{50} of Azadirachtin = 9.5 ppm, LC_{50} of Matrin = 8.86 ppm and LC_{50} of Spinosad= 4.4 ppm).
KEY WORDS: Azadirachtin, Matrine, Spinosad, LC_{50}, *Liriomyza sativae*

INTRODUCTION

The Leafmining flies (LMF), *Liriomyza trifolii* (Burgess), and *L. sativae* Blanchard are important 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, 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 control of leaf miner is the application of insecticides (Cox *et al.*, 1995). The effectiveness of some insecticides has retained for a few years after introduction (Leibee, 1981). In some countries such as Indonesia, many insecticides are routinely applied to control leaf miners (Rauf *et al.*, 2000). Insecticide applications can reduce the percentage of parasitism and *predation*, and these effects reduce to control leafminers. Botanical insecticides derived from the seed of the neem tree, *Azadirachta indica* (Meliaceae), have shown promise due to their physiological (insect growth regulating) and antifeedant effects on a diversity of phytophagous insects (Ascher, 1993). Neem insecticides are attractive for use in integrated pest management programs because of their low contact toxicity and need to be directly ingested by insects to be effective (Schmutterer, 1988). Neem formulations have been studied for their effects on feeding and oviposition in *L. trifolii* and *L. sativae*, but not *L. huidobrensis*, and results are dubtful, in deterrent effects and reduction in oviposition. It was found that *L. trifolii* larvae are adversely affected (reduction in pupation) by neem formulations when applied as a soil drench (Weeb *et al.*, 1983 and Meisner *et al.*, 1985).

Spinosad belongs to a new group of insecticides and it is originated from the fermentation process of soil bacteria *Saccharopolyspora spinosa*. This compound has activity on several groups of insects such as lepidopterous, dipterous, coleopterans and thysanopterous (Williams *et al*. 2003). This compound also proved to be safe to human beings receiving a LC_{50} above 5,000 mg and therefore it was classified as Class I (Florim and Nakano 1997). For example, in countries like the United States of America, this compound has also been registered for organic crops. In Brazil, spinosad is already registered to be used in potato crops to control *Liriomyza huidobrensis* (Branchard) (Diptera: Agromyzidae) at a rate range from 163.2 to 201.6 g ai/ha (Agrofit 2007). However, the use of adjuvant, as the surfactant polyether-polymethylsiloxanecopolymer (Break Thru®) helps to reduce the required rate to achieve acceptable control. The usage of lower rates helps to reduce technology costs and brings fewer side effects to the environment (Bueno *et al.*, 2007). Results indicated that Success applied without an adjuvant appeared to provide the most consistent adult mortality of Liriomyza leafminers. In contrast, the addition of a penetrating surfactant (crop oil concentrate) resulted in significantly greater larval mortality consistent with the leafminer feeding behavior (Palumbo 2002).

In this study the effects azadirachtin, matrin and spinosad were evaluated on larval leafminer *Liriomyza sativae* Blanchard (Dip: Agromyzidae) under laboratory condition in Plant Protection Research Institute of Tehran, Iran.

MATERIAL AND METHODS

Host plant culturing

Cowpea was seeded in 10-cm-diam. pots (4–6seeds 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 16:8 h (L: D), until two true leaves were fully expanded.
The cucumbers (var: negin that were grown in 10-cm-diam. pots (2 seeds per pot), were used for bioassay tests.

Leaf miner rearing

The infested cucumber leaves were collected from the greenhouse cucumbers 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 the cages (0.5*0.5*0.5 meter) were covered by white cloth screen that included the pots grown cowpea.

Insecticide

The insecticides that were used in our experiments were Azadirachtin (Neem Azal %15 EC, Trifolio-M GmbH, Botanical insecticides derived from the seed of the neem tree, *Azadirachta indica* (Meliaceae),) insecticide to control leafminers, and Kingbo (oxymatrine>0.2% & psoralen (natural regulater)>0.4%, Beijing Kingbo Biotech Co., Ltd) insecticides derived from that several wild plants, *Acarmicha sp. , Vertrum nigrum, Sophora flavescent.* Spinosad (Spinosin %24 SC) is a fermentation metabolite of the actinomycete *Saccharopolyspora spinosa*, a soilinhabiting microorganism.

Bioassays

The bioassay experiments were carried at 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 3-4-d-old flies for an oviposition access period (OAP) of 4-6 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 (<5 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 a part of the petiole were treated by submersion for 5 s into the appropriate serial dilutions of insecticides in distilled water. Five to seven doses were used in each bioassay. In the bioassays the leaf dipping technique was employed against first (1days old) and last (4days old) larval instars. Mortality of larva observed continuously and recorded at 24h intervals for two insecticides. Based on prior experience, bioassays of the were conducted with Azadirachtin at 15 ppm, which was reported to result in ~95% larva- mortality and Matrine at 12 ppm.

Data was analyzed using probit analysis procedure by POLO PC software.

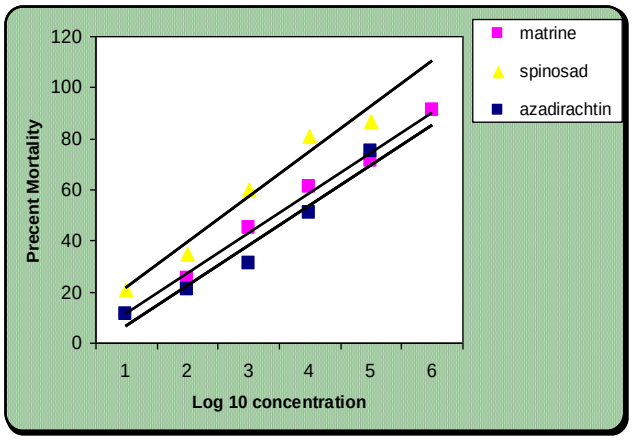
RESULTS

The results revealed that the LC_{50} for spinosad and azadirachtin that the first instars larvae were 4.4 and 9.5 ppm respectively. Additionally the results indicated that the LC_{50} value for matrine for first instars larvae were 8.8 ppm. Therefore the efficacy of spinosad was increased the mortality rate in first instars larvae in competition with Azadirachtin and matrine (Table- 1 and Figure- 1).

Table 1- Compression the LC_{50} value of azadirachtin, Matrine and spinosad on the first larval instars of *L. sativae*

Insecticide	Slope (±SE)	LC_{10} ppm	LC_{50} ppm	LC_{90} ppm	Chi-square	Df	heterogenicity
Spivosad	0.23 (±1.7)	0.8	4.4	23.8	0.11	3	0.09
Matrine	2.35 (±0.35)	2.5	8.86	30.98	0.55	3	0.18
Azadirachtin	1.13 (±0.29)	1.5	9.5	59	0.10	3	0.03

Figure 1: Effect of azadirachtin, matrine and spinosad on *Liriomyza sativae* larvae under constant laboratory condition



DISCUSSION

Laboratory tests resulted in higher mortality than tests at the same concentrations of insecticide. The effect shown by of the spinosad, azadirachtin and matrine allows a reduction in the commercially recommended concentration of insecticides without any loss in effectiveness.

Due to lower hazard of botanical pesticides for human and environment in comparison to conventional synthetic pesticides, it seems that these plant extracts 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.

This is supported by the findings of Weintraub and Horowitz (1997) who reported that similar results were obtained when drenching plants infested with 1st-instar larvae with 1-15 ppm azadirachtin (11.7% eclosion) *vs* dipping leaves at the same time period and

concentration (44.7% eclosion). In the current study, LC_{50} values for matrine of a collected strain *L. sativae* were 8.8 ppm. This is supported by the findings of Asghari (2006) who reported that matrine 9 ppm be able to control leaf miner fly.

Additionally the results indicated that the LC_{50} value for pure spinosad for first instars larvae were 4.4 ppm. This is supported by the findings Ferguson *et al.*, (2004) the defined value LC_{50} of spinosad were 2.53 and 1.5 for the first instar of leafminer *L. trifolii*. As result shows that the toxicity of three insecticides in larvae stages is more than adult insects. This is supported by the findings Bueno *et al.*, (2007) the use of adjuvant, as the surfactant polyether-polymethylsiloxanecopolymer (Break Thru®) helps to reduce the required rate to achieve acceptable control. The usage of lower rates helps to reduce technology costs and brings fewer side effects to the environment. Results indicated that Success applied without an adjuvant appeared to provide the most consistent adult mortality of Liriomyza leafminers.

CONCLUSIONS

This research confirmed that when used on cucumber plants, spinosad, azadirachtin and matrine are effective against the larvae of the leafminer fly (*L. sativae*) at the commercially recommended dosages. 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.

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Influence of phenological and physical characters of tomato on fruit borer, *Helicoverpa armigera (*Hubner) infestation.

Abstract
Among different phenological and physical factors of tomato varieties studied on the infestation of tomato fruit borer, the per cent fruit damage and weight loss (r = 0.9853), per cent fruit damage and pericarp thickness (r = 0.7677), per cent fruit damage and fruit hardness (r = 0.7363) showed positive correlation while the numbers of flowers per inflorescence (r = - 0.7525) were negatively correlated with the fruit damage.

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is a profitable vegetable crop, cultivated widely in the semi arid regions of Rajasthan. Among the various insect-pests responsible for lowering the yield of tomato crop, the fruit borer, *Helicoverpa armigera* (Hubner), is a highly destructive pest causing serious damage (Srinivasan, 1959, Krishnamoorthy and Mani, 1996). The monetary loss due to this pest in the country has been estimated over rupees one thousand crores per year (Jayraj *et al*., 1994). Use of resistant varieties is recognized as a promising tool in the bio-intensive pest management system. Plant resistance can provide an effective measure to combat the pest. The morphological and physical characteristics of plants and fruits are associated with attraction, feeding and oviposition of the insect pests. Udaipur district is no exception facing lower tomato yields due to pest attack. None of the studies have ever been conducted to evaluate relationship between plant resistance factors and fruit infestation. Therefore, present study was undertaken with an objective to see the effect of phenological and physical factors of tomato varieties on fruit borer infestation.

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Materials and Methods

With a view to work out a relationship the plant characters *viz*., flowers per inflorescence, trichomes per sepal, sepal size, fruits per truss, fruits per plant, fruit length and breadth, pericarp thickness, fruit hardness, juice content (%) juice/rag ratio and fruit yield were correlated with fruit damage. The phenological and physical factors were recorded from each of 10 tomato varieties as follows.

(a) Flower initiation and fruit formation period: Date of flower initiation and first fruit formation was recorded by daily visual observations of plants in each variety and days to flower initiation and fruit formation were counted.

(b) Number of flowers per inflorescence: Number of flowers in 45 inflorescence (15 inflorescence in each of the 3 repeats) was counted and average number of flowers per inflorescence was calculated.

(c) Number of trichomes per sepal: Number of trichomes in 45 calyces (15 calyces in each replication) was recorded with the help of magnifying hand lens and average number of trichomes per sepal was calculated.

(d) Size of sepal: The size of sepal was measured with the help of scale. In all 45 calyces (15 each in 3 repeats) from each variety were taken and average size of the sepal was recorded.

(e) Shape of fruit: Fifteen mature and uniform fruits from each variety were observed for this purpose. The fruits on the basis of their shape were grouped in to there categories *i.e.* round, pear and plum.

(f) Number of fruits per truss: Number of fruits in 45 trusses (15 trusses in each of the 3 repeats) was counted and average number of fruits per truss was calculated.

(g) Number of fruits per plant: For each variety total number of fruits per 15 plants (5 plants in each of the 3 repeats) was recorded at each picking. Thus total number of fruits per plant was calculated.

(h) Size of fruit: Ten randomly selected fruits from each replication of each variety were taken and the length and breadth (cm) were measured with the help of Varnier's caliper. Thus the average size of the fruits was calculated

(i) Pericarp thickness: For determining the pericarp thickness, the fruit was cut horizontally and thickness (cm) was thus recorded with the help of Varnier's caliper. Fifteen fruits (5 fruits from each

of the 3 repeats) were used for recording the data in each variety.

(j) Hardness of fruit: Fifteen uniform sized fruits from each repeat of every variety were observed for this character. The hardness of the fruit (kg/cm^2) was measured with the help of "Pressure Tester".

(k) Juice /rags ratio: Ten uniformly matured fruits from each repeat of every variety were crushed in a mixture and sieved. The quantity of juice and rag was measured separately. The per cent juice content and juice/ rag ratio was worked out.

(l) Yield /plant: Fruit yield (healthy and damaged) of 10 randomly selected plants from each replication at each picking was recorded and cumulated after each picking. The average yield per plant was worked out.

Results and Discussion

In the present study, plant type and fruit shape showed poor correlation with fruit damage. Hence it was concluded that these factors have no effect on the fruit damage by *H. armigera*. Kashyap and Verma (1987) also reported that shape of the fruit in normal genotypes had no relationship with fruit damage.

The days to flower initiation (r = - 0.4097) and fruit formation (r = - 0.3383) showed negative correlation with fruit damage in both the years. Fery and Cuthbert (1973) and Mishra *et al.* (1996) observed a negative correlation with the earliness attribute in tomato and fruit damage, support the present finding.

The plant characters, fruits per truss and fruit yield per plant had no correlation with fruit damage. Kashyap and Verma (1987) reported similar results regarding number of fruits per truss and fruit size. Fery and Cuthbert (1973) also reported no correlation between shape and size of fruit with fruit damage. However Canerday *et al*. (1969) observed that cultivars having small fruits were less susceptible. Cosenza and Green (1979) also stated that large fruits were more damaged as compared to small fruits. In the present investigation trichomes per sepal and sepal size were found to have no correlation with fruit damage while studies conducted at AVRDC (1977) revealed that lack of trichomes lead to more susceptibility. Rath and Nath (1995) found trichome density and sepal length, to have a significant and positive impact on infestation level. Sivaparakasam (1996) observed that more number of eggs were laid on hairy than glabrous cultivars. According to Chandrakar *et al.* (1998) cultivars having highly hairy peduncles were less susceptible to the pest than those with less hair on the peduncles.

Figure 1.

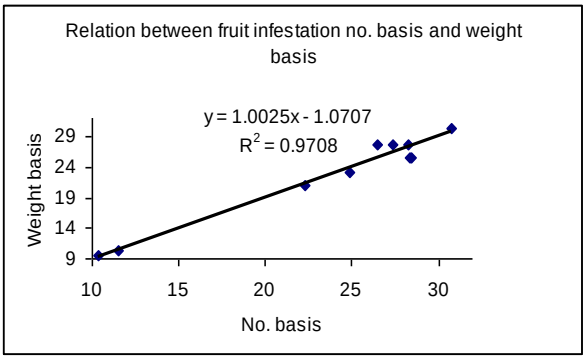
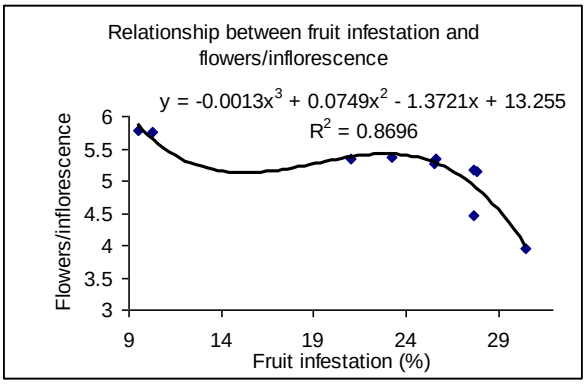


Figure 2.



The per cent fruit damage showed positive correlation (r = 0.9853) with weight loss (Fig. 1). Similar results were obtained by Kashyap and Verma (1987). In the present findings the pericarp thickness (r = 0.7677) and fruit hardness (r = 0.7363) were found to have positively correlated (Fig. 3 and 4) with fruit damage but Cosenza and Green (1979), Juvik and Stevens (1982) and Rath and Nath (1995) found tough skinned fruits to be responsible for resistance to borer however, Kashyap and Verma (1987) observed no correlation between pericarp thickness and fruit damage. The green tomato fruits were preferred more as compared to ripe fruits because green fruits were harder than the ripe fruits and may have provided better grip to the mandibles of *H. armigera* larvae. The numbers of flowers per inflorescence were negatively correlated (r = - 0.7525) with the fruit damage (Fig. 2). These results are in conformity with that of Canerday *et al.* (1969) and Kashyap and Verma (1987) who reported negative correlation between number of flowers per inflorescence and fruit infestation.

Figure 3.

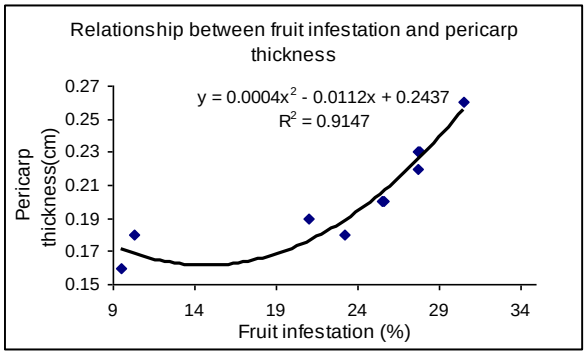
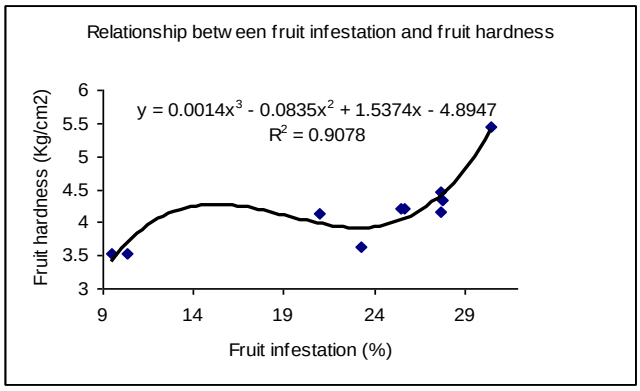


Figure 4.



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Host plant resistance in tomato (*Lycopersicon esculentum* Mill.) against fruit borer, *Helicoverpa armigera* (Hubner) - A review

Tomato (*Lycopersicon esculentum* Mill.) is a profitable vegetable, cultivated widely. Among the various insect-pests responsible for lowering the yield of tomato crop, the fruit borer, *Helicoverpa armigera* (Hubner), is a highly destructive pest causing serious damage (Srinivasan, 1959, Krishnamoorthy and Mani, 1996). The monetary loss due to this pest in the country has been estimated over rupees one thousand crores per

year (Jayraj *et al*., 1994). Use of resistant varieties is recognized as an important tool in the bio-intensive pest management system. Plant resistance can provide an effective measure to combat the pest. The morphological and physical characteristics of plants and fruits are associated with attraction, feeding and oviposition of the insect pests. Many types of insecticides have been used to control this pest. As a result this species is subjected to a considerable selection pressure and thus resistance to all the major classes has been observed (Wolfenbarger *et al*., 1981). The identification of chemicals in resistant varieties is of most practical significance. The analysis of plant leaves and fruits of tomato for these chemicals may be used as a selection tool. The presence of antibiotic factor might be a valuable source of resistance for commercial cultivars (Fery and Cuthbert, 1975). Thus evolving a pest resistant variety and use it in the integrated pest control programme will be very much desirable. A lot of information on the estimation of losses caused by *H. armigera* to the fruits of tomato has been generated throughout the country. A few notable studies have been discussed here. Studies conducted in Tamilnadu showed fruit damage by this insect-pest in tomato to be 40-50 per cent (Srinivasan, 1959). Damage by this pest in tomato fruit was recorded to be 18.2 to 55.8 per cent in Punjab (Singh and Singh, 1970 and 1975; Singh and Chahal, 1978). Tiwari and Krishnamoorthy (1984) observed 18.90,18.00 and 21.64 per fruit damage (no. basis) and 13.27,12.67 and 17.88 per cent (weight basis) in crops harvested during October – November, January – February and March – April, respectively. Tomato fruit infestation by *H. armigera* during 2nd week of April ranged between 33 to 55 per cent (Parihar and Singh, 1986). Kashyap *et al* (1988) recorded more than 30.60 per cent fruit infestation by *H. armigera* in the tomato variety HS – 101. Fruit damage ranging from 18.7 to 23.3 per cent (number basis) and 1.5 to 27.4 per cent (weight basis) were observed in the variety HS-101 at weekly interval (Kalra, 1992).

Physical/phenological factors:

Canerday *et al.* (1969) reported that tomato cultivars differed for the damage done by tomato fruit worm, *H. zea*. Fery and Cuthbert (1973) screened 22 genotypes of tomato revealed significant differences in fruit worm damage which varied from 31.4 to 75.3 per cent in genotypes TF-2 and Epoch, respectively. Later Fery (1974) also observed a high degree of variation in the borer incidence among 1030 tomato genotypes, though none were found free from damage by *H. armigera.* Fery and Cuthbert (1974) reported Tiny-Tim as a resistant cultivar having 83.1 percent less fruit damage than the susceptible genotype. Parker Barbosa (1974) observed entry 38 as resistant to fruit worm and pinworm (*Keiferia lycopersicella*) in a large number of processing tomatoes. The insect pest incidence in tomato influenced by earliness, fruit – size, vine- size and plant density reported that large and marketable fruits were damaged more and the fruit number and vine size were negatively correlated with the borer damage. They also observed 50.2 and 46.1 per cent fruit damage in untreated tomato varieties Campbell – III, repectively. Juvik and Stevens (1982) reported that the fruit skin, particularly the toughness of the pericarp, was principally responsible for resistance to borer. Lal (1985) found Parker, Bonus and VNF-8 cultivars to be highly resistant (1.2-5%) while Super Marmand, Bonset F_1 hybrid and No. 502 VNF F_1 hybrid highly susceptible (22.6- 44.7 % damage).

According to Kashyap and Verma (1986), cultivars HT 64 and HT 50 suffered the least fruit damage by *H. armigera* (1.7- 2.9 and 2.4- 23.7 %, respectively) while HS 172 and HS 173 were damaged the most (42.6-55.05 %). Kashyap and Verma (1987a) reported that the survival of *H. armigera* larvae on a wild food plant (*Lycopersicon hirsutum f. glabratum*) was less (16.6 %) as compared to 90 per cent in susceptible cultivated genotypes. Kashyap and verma (1987) worked out a positive correlation between fruit damage on number and weight basis while a negative correlation existed between flowers/inflorescence and fruit damage. It was also pointed out that the surface texture of the calyx also influenced the susceptibility of the genotype. Asian Vegetable Research and Development Center (1987) studied that comparison of a number of tomato accessions with very pubescent leaves and stems with susceptible TK70 and resistant 76W PI134417 for field damage by *H. armigera.* All the accessions were more susceptible than TK70 and the lack of resistance, despite the pubescence was attributed to lack of glandular trichomes. Sharma *et al*. (1990) observed least mean per cent reduction in the fruits and yield (16.31 and 10.45, respectively) in Kanchan-3 among 4 cultivars screened against *H. armigera.* Mishra and Mishra (1993) found BT1 least susceptible among 16 varieties tested against *H. armigera*. Singh *et. al.* (1994) categorized cultivars Punjab Chhuhara, US 2, J14-1-12, Pant Bahar, AzadT-2. Pusa selection1, Pusa selection 4, Hybrid KT4 and Pusa hybrid 4 as resistant and KS6 and selection 18 as susceptible to *H. armigera.* Brar *et.al* (1995) reported different tomato species like *L. hirsutum, L. pimpenellifolium, L.peruvianum* and *L.chimeliwshii* as highly resistant to fruit borer. Sivaprakasam (1996a) studied the ovipositional preference of *H. armigera* on tomato cultivars and observed that more number of eggs lay on hairy than glabrous cultivars and less on cultivar Paiyur-1, which has less trichome density and long calyces. Sivaprakasam (1996 b) found that the consumption and growth rate of *H. armigera* larvae were highest on cultivars Co3 followed by Madanpalli.

Gc *et al*. (1997) screened 4 commercial cultivars, 6 local cultivars and 12 hybrids found that Altair F_1 , Mercur F_1 , F_1 958930, Rupali hybrids and Roma , a commercial cultivar were resistant.

Bio-chemical factors:

Chandrakar *et al.* (1998) who observed a negative correlation between ascorbic acid content of the fruit and fruit damage. Salvanarayanan and Narayanasamy (2006) found that acidity of the fruits exerted a significant negative correlation on larval feeding. Similarly, in gram the foliage of desi type cultivars, which are resistant to *H. armigera*, exude very acidic (pH 1.3) droplets (Rembold and Winter, 1981). Salvanarayanan and Narayanasamy (2006) who found non-reducing sugars in the foliage had a significantly positive correlation with larval feeding. Isman and Duffey (1982) found inhibition in larval growth of *H. zea* when semi purified extracts of phenolics from tomato foliage were added in the artificial diet. Dose responses for extracts were equal to those obtained with pure chlorogenic acid and rutin-major phenolic constituents of tomato foliage. Similarly, Benerjee and Kalloo (1989) also found that high phenol content in tomato leaves imparted resistance against fruit borer *H. armigera.* Salvanarayanan and Narayanasamy (2006) found that ortho-dihydroxy phenols of the fruits exerted a significant negative correlation on larval feeding On the basis of high phenol content in plants, pest resistant lines could be identified and used for breeding resistant varieties. Sharma *et al* (2008) found that the reducing sugars were positively correlated while ascorbic acid, acidity, zinc, ferrous and total phenols were negatively correlated with fruit infestation.

Conclusion:

Use of insect-resistant crop varieties is economically, ecologically, and environmentally advantageous. Economic benefits occur because crop yields are saved from loss to insect pests and money is saved by not applying insecticides that would have been applied to susceptible varieties. In most cases, seed of insect-resistant cultivars costs no more, or a little more, than for susceptible cultivars. Ecological and environmental benefits arise from increases in species diversity in the agro-ecosystem, in part because of reduced use of insecticides. Increase in species diversity increase ecosystem stability, promoting a more sustainable system far less polluted and detrimental to natural resources.

The IPM concept stresses the need to use multiple tactics to maintain insect pest abundance and damage below levels of economic significance. Thus, a major advantage to the use of insect-resistant crop varieties as a component of IPM arises from the ecological compatibility and compatibility with other direct control tactics. Insect-resistant cultivars synergize the effects of natural, biological, and cultural insect pest-suppression tactics. The "built-in" protection of resistant plants from insect pests functions at a very basic level, disrupting the normal association of the insect pest with its host plant. The compatible, complementary role plant resistance to insect pests playing with other direct control tactics is, in theory and practice, in concert with the objectives of IPM. All crop cultivars should contain resistance to insect pests. Plant resistance to insect pests has advantages over other direct control tactics. For example, plant resistance to insects is compatible with insecticide use, while biological control is not. Plant resistance to insects is not density dependent, whereas biological control is. Plant resistance is specific, only affecting the target pest. Often effects of use of insect-resistant cultivars are cumulative over time. Usually the effectiveness of resistant cultivars is long-lasting. The role of plant resistance to insects in IPM has been well defined, at least in theory. However, the specific role a resistant cultivar in a particular IPM situation is crucial to successful deployment of the resistant cultivar. The impact of the resistant cultivar on standard cultural, biological, and insecticidal control methods should be well defined. Likewise, the impact of each of these control tactics on the resistant cultivar also must be defined.

Several definitions have been used to convey the relative level of resistance in a plant. However, the problem of quantifying resistance continues to be a problem influencing farmer acceptance of insect-resistant cultivars. A better way to define resistance levels in agronomically improved resistant cultivars is through quantified comparisons of insect pest damage or plant yield loss of susceptible cultivars. Once insect pest abundance or damage to yield-loss relationships have been determined, economic threshold levels can be determined and combined with factors such as crop value and insect pest control costs to develop dynamic thresholds for use by producers. Dynamic thresholds provide a description of resistance and can reduce crop loss risk because limitations are known and remedial action can be taken when necessary. By using this system to define relative differences in insect pest resistance between cultivars, it may be possible to simply indicate that a resistant cultivar has a higher economic threshold level than a traditional susceptible cultivar.

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Susceptibility of *Phthorimaea operculela* (Zeller) to old and new generation of spinosyn products on two potato varieties in Egypt.

Abstract
Two new potato varieties were planted in two plantation timing to investigate the performance of the new generation of spinosyn product namely spinetoram 12 SC (radiant) in comparison to the old recommended spinosyn product spinosad 24 SC (tracer).
Regardless of variety, earlier plantation showed significantly less infestation of the potato tuber worm *Phthorimaea operculella* (PTW) on foliage as well as tubers. Sackson variety was significantly more susceptible to the infestation of PTW on foliage as well as tubers than Bambino variety in both timing of plantation.

Radiant at 100 ml /fed was significantly the best treatment based on foliage or tuber infestation. Also, this product showed the lowest number of moths emerged from the harvested tubers. However, protecto was the weakest product for the same criteria.

Regardless of any variety or timing of plantation, 80 ml of radiant 12 % SC was significantly equal or better than 120 ml of tracer 24 SC. The last based on foliage or tuber infestation or emerged moths (from the harvested tubers). By taken gram active in consideration, radiant was 3 times stronger than tracer.

Radiant 12 SC will be an excellent addition to our IPM programs, (however, because it has the same mode-of-action as tracer, it will not provide an additional rotational partner for our resistance management programs).
Key words: Potato tuberworm, spinetoram , spinosad, two varieties

Introduction

In Egypt, potato is considered one of the most important exporting vegetable crops. The potato tuber worm (PTW), Phthorimaea operculella (Zeller) (Gelechiidae:Lepidoptera), is one of the most important pest which damage potato tubers in the field and during storage in many countries . Tunnels conducted in the tubers facilitate the growth of many bacterial and fungal diseases and reduce exportation value .This pest attacks solanaceous crops throughout the year including potato, tomato, pepper and egg-plant in Egypt.

In the field, different control measurements have been used to combat this pest; planting date, hilling and sowing depth of, distance of cultivation, intercropping systems, managing hosts / season, transgenic varieties, varietal resistance, sex pheromone, management and manipulation of levels of irrigation and chemical conventional insecticides (cited from Temerak, 2003), biological control agents as Bacillus thuringiensis or granulas virus or release of parasitoid were also used (Ali 1991 ; Kurjade and Pokharkar 1997, respectively).

Temerak 2003 indicated that Spinosad showed rapid and high level of efficacy and should replace the old conventional insecticides for the control of PTW. Temerak 2007 confirmed that spinetoram 12 SC was 5 times stronger than spinosad 24 SC in the field to combat egg masses of cotton leafworm based on gram active rate /fed.

Potatoes are exportation crop and Ministry of Agricultural (MOA) is looking for green products, safe, low dose in the environmental, fast-acting, fit in Integrated pest management (IPM) and low human toxicity. Spinosad and spinetoram are classified by EPA as reduced-risk product and awarded the green chemical from the white house in USA in 1999 and 2008, respectively. Current studies were undertaken to evaluate the susceptibility of PTW to the new product spinetoram (radiant 12 SC) in comparison to the old generation spinosad (tracer 24 SC), in the field.

Materials and Methods

Experiments were conducted at the farm of the Environmental Studies and Research Institute, El Sadat area at Minufiya University, at El Sadat City, in an area about one Faddan (4200m2) during season 2008 as summer plantation. Randomized complete block design with three replicates was followed. Two potato varieties such as Sakson and Banbino were planted.

Products used

Spinosad 24%SC (tracer): is a metabolite of the Actinomycete, Saccharopolyspora spinosa Martz & Yao. It is a naturally occurring mixture of two active spinosyns (spinosyn A & D). It is a trademark of Dow AgroSciences Co. It is considered the 1st generation from the spinosyn group.

Spinotoram 12% SC (radiant): is the 2nd new generation of the spinosyn group with the same mode of action as spinosad. It is a mixture of two active spinosyns (spinosyn J & L).
It is a trademark of Dow AgroSciences Co.

Spinotoram + 1 L mineral oil 96%

Protecto:is Protecto (WP) 10%, a commercial product formulation contains 32x106 IU/mg of Bacillus thuringiensis Subsp Kurstaki

Plantation dates were 6/2/2008 and 12/2/2008
Soil type is sandy soil. Number of spray was three with 10 days interval. Volume of spray was 200L /Fed. plot size was 525 sq. meter.

Assessment

Foliage infestation was determined by examining 100 compound leaves taken randomly prior to every spray. Three samples after 10 days from each spray were investigated to count number of living larvae /100 leaves. Henderson and Tilton were used to calculate % reduction.

The obtained results were subjected to the analysis of variance test (ANOVA) with mean separation at 5% level of significance LSD according to Senedcor and Cochran (1973). During harvest 200 tubers were inspected to count surface hole infestations as well as number of galleries. Tubers were placed on sand inside cages (50 x 50 x 50 cm) to count emerged moths.

Results and Discussion

1st Plantation of 6/2/2008 (Early plantation)

Table 1a and 2a, showed performance of radiant 12SC versus tracer 24SC to combat PTW on the foliage of Sakison and Bambino varieties planted in the field, respectively.

Table 1a: Performance of Radiant12SC versus tracer 24SC to combat potato tuber worm on foliage of Sakson variety, early plantation in the field, El Sadat area, Minufiya Governorate, 2008

Treatments	Rate/fed.	Mean. No. of alive larvae per 100 compound leaves at10days interval*								Groups
		Inspections				% Reduction			Aver. larvae	
		Pre spray	1st	2nd	3rd	1st	2nd	3rd		
Radiant 12 SC	100	51	11	5	0	85	94	100	5.3	a
	80	49	13	6	1	81	92	99	6.7	b
	40	53	16	12	6	78	86	94	11.3	d
	40+oil	50	14	9	3	80	86	97	8.7	c
Tracer 24 SC	120	48	13	8	3	80	90	97	8.0	c
Protecto	300	56	19	13	7	76	86	94	13.0	e
Untreated		49	69	83	99				83.7	f
LSD at 0.05		1.11								

Date of inspection, just before spraying, April 6, April 16, April 6, May 6

Table 2a. Performance of Radiant12SC versus tracer 24SC to combat potato tuber worm on foliage of Bambino variety, early plantation in the field, El Sadat area , Minufiya Governorate, 2008

Treatments	Rate/fed.	Mean. No. of alive larvae per 100 compound leaves at10days interval*								Groups
		Inspections				% Reduction			Aver. larvae	
		Pre spray	1st	2nd	3rd	1st	2nd	3rd		
Radiant 12 SC	100	42	8	3	0	87	96	100	3.66	a
	80	39	8	5	1	86	93	98	4.66	a
	40	38	14	11	5	74	84	94	10.0	c
	40+oil	41	12	7	2	80	90	97	7.0	b
Tracer 24 SC	120	51	13	5	3	82	93	97	7.0	b
Prqtecto	300 gm	48	16	12	8	77	86	92	12.0	d
Untreated		43	61	80	96				79.0	e
LSD at 0.05		1.11								

*Date of inspection , just before spraying ,April 6, April 16, April 26, May 6.

Table 1 b and 2 b indicated performance of radiant12SC versus tracer 24SC to combat PTW on Sakison and Bambino varieties planted in the field at harvest, respectively.

Table 1b: Performance of Radiant12SC versus tracer 24SC to combat potato tuber worm on Sakson variety, early plantation in the field at harvest, El Sadat area, Minufiya Governorate,2008

Treatment	Rate/fed	Mean. No. of infested tuber/ 200 tuber	% potato tuber infestation	Mean No. of galleries / 200 tubers	Mean. No. of emerged moths /200 tuber
Radiant12 SC	100	3^{a}	1.5%	1^{a}	4^{a}
	80	5^{b}	2.5%	2^{ab}	14^{b}
	40	19^{d}	9.5%	9^{d}	54^{d}
	40+oil	9^{c}	4.5%	5^{c}	18^{c}
Tracer 24SC	120	5^{b}	2.5%	3^{b}	19^{c}
Protecto	300	19^{d}	9.5%	13^{e}	60^{e}
Untreated		60^{e}	30%	93^{f}	509^{f}
LSD at 0.05		1.76		1.81	3.23

Table 2b. Performance of Radiant12SC versus tracer 24SC to combat potato tuber worm on Bambino variety, early plantation in the field at harvest, El Sadat area, Minufiya Governorate, 2008

Treatments	Rate/fed	Mean. No. of infested tuber/ 200 tuber	% potato tuber infestation	Mean No. of galleries / 200 tubers	Mean. No. of emerged moths /200 tuber
Radiant12SC	100	1^{a}	.0.5%	0^{a}	2^{a}
	80	2^{a}	1%	0^{a}	5^{a}
	40	5^{c}	2.5%	1^{a}	12^{b}
	40+oil	3^{ab}	1.5^%	0^{a}	8^{ab}
Tracer 24SC	120	2^{a}	1%	1^{a}	9^{b}
Protecto	300	12^{d}	6%	5^{b}	35^{c}
Untreated		29^{e}	14.5%	33^{c}	177^{d}
LSD at 0.05		1.76		1.81	3.23

2nd Plantation of 12 /2 2008 (late plantation)

Table 3a and 4a indicated performance of radiant 12SC versus tracer 24SC to combat PTW on the foliage of Sakison and Bambino varieties planted in the field, respectively.

Table 3a: Performance of Radiant12SC versus tracer 24SC to combat potato tuber worm on foliage of Sakson variety, late plantation in the field, El Sadat area , Minufiya Governorate, 2008

Treatments	Rate/fed.	Mean. No. of alive larvae per 100 compound leaves at 10days interval*								Groups
		Inspections				% Reduction				
		Pre spray	1st	2nd	3rd	1st	2nd	3rd	Aver. larvae	
Radiant 12 SC	100	66	20	7	0	77	93	100	9	a
	80	66	22	10	3	74	90	97	11.66	b
	40	68	27	17	8	69	84	94	17.33	d
	40+oil	65	23	11	4	73	89	97	12.66	b
Tracer 24 SC	120	63	20	11	5	75	89	95	12.0	b
Protecto	300	59	24	13	7	75	76	93	14.66	c
Untreated		59	77	93	115	77	93	100	95	e
LSD at 0.05		1.11								

* Date of inspection, just before spraying ,April 13, April 23, May 3 , May 13.

Table 4a.Performance of Radiant12SC versus tracer 24SC to combat potato tuber worm on foliage of Bambino variety, late plantation in the field, El Sadat area , Minufiya Governorate, 2008.

Treatments	Rate/fed.	Mean. No. of alive larvae per 100 compound leaves at10 days interval*								Groups
		Inspections				% Reduction				
		Pre spray	1st	2nd	3rd	1st	2nd	3rd	Aver. larvae	
Radiant 12 SC	100	58	17	5	0	78	94	100	7.30	a
	80	61	20	8	2	75	92	98	10.00	b
	40	63	24	15	6	71	85	95	15.00	d
	40+oil	56	18	10	2	75	88	96	10.00	b
Tracer 24 SC	120	59	18	7	3	77	92	97	12.66	c
Protecto	300	65	27	16	7	68	84	94	16.66	e
Untreated		155	73	89	105				89.00	f
LSD at 0.05		1.11								

* Date of inspection , just before spraying ,April 13, April 23, May 3 , May 13.

Table 3b and 4b indicated performance of radiant12SC versus tracer 24SC to combat PTW on Sakison and Bambino varieties planted in the field at harvest, respectively.

Table 3b: Performance of Radiant12SC versus tracer 24SC to combat potato tuber worm on Sakson variety, late plantation in the field at harvest ,El Sadat area ,Minufiya Governorate, 2008

Treatments	Rate/fed	Mean. No. of infested tuber/ 200 tuber	% potato tuber infestation	Mean No. of galleries / 200 tubers	Mean. No. of emerged moths /200 tuber
Radiant12SC	100	10^{a}	5%	6^{a}	19^{a}
	80	11^{a}	5.5%	6^{a}	24^{b}
	40	25^{d}	12.5%	13^{b}	68^{d}
	40+oil	14^{c}	7%	6^{a}	28^{bc}
Tracer 24SC	120	12^{ab}	6%	7^{a}	27^{b}
Protecto	300	26^{d}	13%	13^{b}	83^{e}
Untreated		91^{e}	45.5%	120^{c}	753^{f}
LSD at 0.05		1.76		1.81	3.23

Table 4b: Performance of radiant12SC versus tracer 24SC to combat potato tuber worm on Bambino variety, late plantation in the field, at harvest El Sadat area , Minufiya Governorate,2008

Treatments	Rate/fed	Mean. No. of infested tuber/ 200 tuber	% potato tuber infestation	Mean No. of galleries / 200 tubers	Mean. No. of emerged moths /200 tuber
Radiant12SC	100	5^{a}	2.5	3^{a}	10^{a}
	80	8^{b}	4	3^{a}	14^{b}
	40	11^{c}	5.5	7^{b}	31^{c}
	40+oil	9^{b}	4.5	4^{a}	15^{b}
Tracer 24SC	120	9^{b}	4.5	4^{a}	16^{b}
Protecto	300	19^{d}	9.5	9^{c}	59^{d}
Untreated		42^{e}	21	51^{d}	251^{e}
LSD at 0.05		1.76		1.81	3.23

Table 5, showed the statistical analysis for all interactions among treatments, tuber infestation, varieties and timing of plantation.

Table 5: Statistical analysis as LSD of interactions among planting date , varieties and treatment on PTW in harvested tubers

		M. No. of infested tuber / 200 tuber				M. No. of galleries/ 200 tubers				M. No. of emerged moths/200 tubers			
Planting Date		6 / 2/ 2008		12 / 2/ 2008		6 / 2/ 2008		12 / 2/ 2008		6 / 2/ 2008		12 / 2/ 2008	
	Rate / fed	Sakson	Bamb.	Sakson	Bamb.	Sakson	Bamb.	Sakson	Bamb.	Sakson	Bamb	Sakson	Bamb.
Radiant 12SC	100	3	1	10	5	1.00	0.00	6.00	3.00	4.00	2.00	19.00	10.00
	80	5	2	11	8	2.00	0.00	6.67	3.00	14.00	5.00	24.00	14.00
	40	19	5	25	11	9.00	1.00	13.00	7.00	54.00	12.00	68.00	31.00
	40 + oil	9	3	14	9	5.00	0.00	6.00	4.00	18.00	8.00	28.00	15.00
Tracer24 SC	120	5	2	12	8	3.00	1.00	7.00	4.00	19.00	9.00	27.00	16.00
Protecto	300	19	12	26	19	13.00	5.00	13.00	9.00	60.0	35.00	83.00	59.00
Untreated		60	29	91	51	93.00	33.00	120.00	51.00	509.00	177.00	753.00	251.00
LSD at 0.05		1.76				1.81				3.23			

Foliage infestation was expressed as number of living larvae inside leaves. Tuber infestation was expressed as % of tuber infestation (may it has one or more surface holes) also as number of galleries and furthermore as emerged moths from the harvested tubers)

Sackson variety was significantly more susceptible to the infestation (as foliage leaves or tubers) in both timing of plantation. This variety produced significantly more moths indicating that this variety was significantly more suitable to complete life cycle of potato tuber worm for next generation.

2nd timing showed high level of infestation of PTW (as foliage leaves or tubers) than earlier timing regardless of any variety. It may recommend going further earlier.

The number of living larvae at last inspection reached zero (100% reduction) and 7-8 ones /100 leaves for any variety and any timing of plantation for Radiant at 100 ml and protecto at 300g, respectively. However, it ranged from 96-115/ 100 leaves in the untreated.

Tuber infestation as surface infestation without real galleries or with real galleries indicated the accumulated effect of the whole season. Also, the emerged moths from that tuber in a specific variety may reflect that this variety was nutritionally suitable to produce next generation moths.

For that reason, it can be detected that Radiant at 100 ml /fed was significantly the best treatment based on foliage or tuber infestation. Also, this product showed the lowest number of moths emerged from the tubers. However, protecto was the weakest product for the same criteria. The last product may be its performance affected by sun photo period.

Regardless of any variety or timing, 80 ml of radiant 12 % SC was significantly equal or better than 120 ml of tracer 24 SC. The last based on foliage or tuber infestation or emerged moths from the tubers. By taken gram active in consideration, radiant was 3 times stronger than tracer.

Radiant as 40 ml mixed with oil was significantly better than 300g of protecto. The last is valid as foliage infestation or tuber infestation or emerged moths from tubers. Adding mineral oil to the 40 ml of radiant decrease significantly foliage infestation as well as tuber infestation in both timing and the two varieties. Moreover, radiant mixed with oil at 40 ml was equal with tracer at 120 ml in most of the cases as foliage or tuber infestation.

This result is in line with Temerak 2007. Temerak confirmed that spinotoram 12 SC was stronger as efficacy than spinosad 24 % SC. to combat eggmasses of cotton leafworm based on Gram active/fed in the field.

Temerak 2003 stated that Adding mineral oil at 250 ml to spinosad at 20 ml/100L showed equal performance with double rate (40ml) of spinosad without oil. The last half dose as 20ml mixed with oil was equal of profenfos, c.ethyl and significantly better than fenitrothion at their recommended rates. Temerak indicated that Spinosad showed rapid and high level of efficacy and should replace the old conventional insecticides for the control of PTW.

Adding oil may protect the spinosad formulation from the light and consequently elongate the residuality as well as performance. Mohamed, 2000 indicated that the virulence of B.t. to control cotton leaf worm was greatest under shady conditions than under sunny conditions. Omar et al, 1987 found out that adding mineral oil prolonged residual activity of some insecticides. El-Deeb, 1993 indicated that the enhancement to certain insecticides by adding mineral oil can reach 4.54 as synergism factor. Mourad et al, 1994 revealed that adding oil could reduce the recommended rate of certain insecticides to half dose.

The weakest product in these trials was Protecto. The last product may be its performance affected by sun photo period. however, Gomaa and Ibrahim 2003 indicated that the microbial PTM control methods was economically and ecologically efficient more than the chemical control method in both field and store.

Spinosad is a new class of polyketide-derived macrolide effective against a broad range of pests belonging to orders lepidoptera, diptera, and hymenoptera (Sparks et al., 2001). It contains a mixture of two very active principles: Spinosyn A and Spinosyn D, and is derived from a new species of soil bacterium, Saccharopolyspora spinosa which acts both as a contact and a stomach poison. Electrophysiological evidences have demonstrated that it alters nicotinic currents in neuronal cell bodies and also disrupts the functions of GABA receptors of small neurons in the central nervous system (Salgado et al., 1997).

The above data resulted in an addition resource to combat PTW in the field. However, using one of the spinosyn products is generally recommended to avoid the build up of resistance and to be alternated with another recommended class of chemicals .Radiant 12 SC will be an excellent addition to our IPM programs, however, because it has the same mode-of-action as tracer, it will not provide an additional rotational partner for our resistance management programs.

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Abstracts in Resistance Management

Performance of transgenic cotton in Tungabhadra project area of Northern Karnataka

The Tungabhadra project command area in Northern Karnataka has seen phenomenal changes in cotton cultivation. Indiscriminate use of inorganic fertilizers, particularly nitrogenous fertilizers, to obtain higher yields and synthetic pyrethroids to combat various boll worms though initially resulted in a substantial amount of pest control, simultaneously led to secondary pest outbreak of sucking pests, resurgence of boll worms and dwindled cotton yields. With the advent of genetic engineering, transgenic cottons have been developed for combating the boll worm menace effectively. In this backdrop, the KVK, Raichur had taken up a large scale demonstration of Bt cotton with IPM strategy to show farmers the advantages of advanced technology and ecofriendly pest management practices.

The demonstration was taken up during 2004-05 in an area of 20 ha and 20 farmers were selected for the purpose. Each demonstration consisted of one ha of Bt cotton (RCH 2 Bt) where IPM tools were implemented and it was compared with another 0.5 ha of conventional hybrid. The demonstration fields were visited once in every week to know the crop condition and also to monitor pest population. Accordingly the field visits were made during which farmers were taken to fields and made to identify pests, their stages, incidence levels, nature of damage and their natural enemies.

Table 1: Incidence of sucking pests, bollworms, yield parameters and yield in Bt. and conventional hybrid

Pest/item	RCH-2 Bt	NCS-145
Thrips/leaf	0.84	1.08
Leafhoppers/leaf	0.28	0.64
Whiteflies/leaf	0.12	0.20
Aphids/leaf	1.64	2.12
Helicoverpa egg/terminal shoot	0.44	0.56
Helicoverpa larva/plant	0.08	0.76
Rosette flowers/plant	1.68	5.36
% bollworm damage	3.20	18.72
GOB/plant	32.40	19.80
BOB/plant	4.60	8.20
Yield (q/ha)	18.10	14.18

* Pest observations are average of 25 plants

Table 2: Cost economics of Bt Cotton

Item	RCH-2 Bt	NCS-145
Yield (q/ha)	18.10	14.18
No. of sprays	4	11
Agronomic practices (Rs/ha)	12550	10800
Plant protection (Rs/ha)	1936	5158
Total cost (Rs/ha)	14486	15958
Gross income (Rs/ha)	30770	24106
Net income (Rs/ha)	16284	8148
Net Profit of Bt. with IPM (Rs/ha)	8136	-

* Price of cotton @ Rs. 1700/q

It is evident from the results that Bt cotton does not offer protection against sucking pests. However because of timely application of systemic insecticides the incidence of sucking pests was low in Bt cotton. Similarly Helicoverpa adults did not differentiate Bt. and conventional cotton hybrids as far as oviposition is concerned. The combined effect of Bt. Toxin along with IPM strategies resulted in recording higher yield parameters which ultimately culminated in higher yields in Bt cotton (18.10 q/ha) compared to conventional hybrid (14.18q/ha). Economic analysis of the demonstration has shown that Bt cotton grown by using IPM tools had incurred a mere Rs. 1936 per ha towards plant protection where as Rs. 5158 per ha was spent on plant protection measures in farmers practice.
It can be concluded that transgenic cotton grown by using IPM tools helps in combating the bollworm menace effectively and realizes more net profit compared to conventional hybrids and farmers practice of plant protection.

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Farmers Field School (FFS) - A novel approach for dissemination of IPM in Cotton

Increased complexity in the pest scenario has led to a parallel increase in the cost of plant protection along with increased environmental pollution problems. In this context, the farmers of TBP area have developed a tendency to spray the crop with as many as 25-30 rounds of pesticides with a practice of higher doses and mixing of two or more insecticides which also could not realize in desirable level of pest control resulting in low productivity of cotton with gradual dwindling of cotton cultivation. Krishi Vigyan Kendra (KVK) Raichur demonstrated a sound, viable and effective IPM package developed for irrigated cotton through an innovative approach called Farmers field School in Manjarla village whose farmers have also been cultivating cotton since many years. Main objectives of FFS: To develop farmers' skills and knowledgeable attitude in identifying problems and taking decisions to get a healthy crop. It is an informal discovery based education, which not only helps the farmers to solve the problems but also gives insight on future problems. It aims at the farmers to become experts in their own fields by understanding the role of different components of ecosystem and their inter relationship. The principles of FFS: To grow a healthy crop, to conserve the crop defenders, to make field observations weekly through AESA and to make farmers experts. Results of FFS were listed below.

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Evaluation of Bt cotton vis-à-vis conventional hybrids through on farm testing

The unscientific way of bollworm management through indiscriminate application of insecticides has resulted in the development of resistance to insecticides by bollworms. Similar situations have developed in northern Karnataka as well. Once, a very potential area for cotton production, The Tungabhadra Project area, because of the sole reliance on inorganic fertilizers and indiscriminate use of pesticides particularly synthetic pyrethroids led to many ill effects like pesticides losing their efficacy, secondary pest outbreak and dwindling yields which resulted in escalated cost of cultivation. The introduction of transgenic cotton is a mile stone which could effectively manage pests leading to increased yields. In this context, the KVK, Raichur made an attempt in farmers' fields to evaluate performance of transgenic cotton.

Farm testing was conducted during 2003-04 in farmers' fields by using Bt cotton in comparison with conventional hybrids. The trial consisted of three hybrids viz., MECH-162 Bt, MECH-162 non Bt and NCS-145, a popular hybrid in the locality, each in an area of half an acre. Observations on incidence of various pests were made on 25 plants in each plot. The insecticidal applications were made on the basis of incidence levels of both sucking pests and bollworms.

The results revealed that Bt cotton did not differ from conventional hybrids in recording sucking pest population thus warranting two rounds of insecticidal sprays. However, the efficacy of Bt toxin was much convincing in terms of damage where as non Bt and NCS-145 have recorded 14.24 and 17.82 per cent fruiting body damage respectively. Similar observations were recorded in per cent rosette flower incidence which was 1.26 per cent in MECH-162 Bt. While it was 5.64 and 7.63 respectively in MECH-162 non Bt and NCS-145. Good opened bolls per plant were 40.32 in MECH-162 while non Bt and NCS 145 have recorded 26.54 and 20.26 per plant respectively.

Incidence of sucking pests and bollworms and yield in Bt., non Bt. and conventional hybrid

Pest/Item	MECH-162 Bt	MECH-162 Non Bt	NCS-145
Leafhoppers/leaf	1.28	1.46	1.14
Aphids/leaf	4.16	3.62	4.34
Thrips/leaf	2.28	2.76	1.82
Whiteflies/leaf	1.12	1.54	2.42
Helicoverpa larva/plant	1.20	4.12	4.84
Earias larva/plant	0.20	0.62	0.90
% fruiting body damage	4.52	17.24	17.82
% Rosette flowers	1.26	5.64	7.36
GOB/plant	40.32	26.54	24.26
BOB/plant	5.66	11.12	9.44
Yield (q/ha)	16.42	11.26	10.84

It can be concluded from the demonstration that transgenic cotton (MECH-162) offer better inbuilt tolerance for combating American bollworm resulting less plant protection expenditure and higher yields compared to conventional hybrids.

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Announcements and Submission Deadlines

Thank you to those who contributed to this issue - you have really made the newsletter a worthwhile reading experience! Our contributors truely increase the newsletter's success at sharing resistance information worldwide.

We encourage all of our readers to submit articles, abstracts, opinions, etc (see the newsletter online at http://whalonlab.msu.edu/Newsletter/submission.html for submission information).

The Newsletter is a resource to many around the globe. It is also a wonderful and effective way to enhance the flow of ideas and stimulate communication among global colleagues.

We appreciate your efforts to support the newsletter and we look forward to your continued contributions.

The next two **submission deadlines** are:

Monday, March 15, 2010
Monday, September 13, 2010

We hope you continue to consider the newsletter as a forum for displaying your ideas and research

Libraries that wish to receive a printed version may send a request to:

rpmnews@msu.edu, or

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