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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Abstracts in Resistance Management

Letter from the Editors

Dear Subscribers,

In the April 2010 edition of the American/Western Fruit Grower online magazine, a video entitled, "Insecticide Rotation and Resistance Management in Tree Fruit" by Rick Weinzierl was published, examining different methods that fruit growers can utilize for insect control on tree fruit pests. The concept of his study focuses on two different methods, mixtures of insecticides and rotating pesticides used in the field. This video is available on www.growingproduce.com on Growing Produce TV, channel 2.

Also available in the April edition, the 2010 Organic Trade Association Organic Industry Survey stated that, "…despite a down economy, sales of organic goods rose during 2009." In 2009, organic produce sales reached almost $9.5 billion. For more on the results of this survey please visit www.growingproduce.com and search for the article title, "Survey: Organic Produce Sales Grow".
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

Susceptibility of *Anopheles species* populations collected from four localities in Juba County to DDT and deltamethrin, Central Equatoria State, Southern Sudan

Abstract: The study aimed to evaluate the susceptibility of *Anopheles species* to DDT 4% and deltamethrin 0.05%. Mosquitoes were tested for their susceptibility to both insecticides following WHO standards and using test kit provided by WHO. The study indicated that *Anopheles* mosquito was found tolerant to DDT 4% and deltamethrin 0.05% in Juba County in all mosquitoes collected from the sentinel sites. However, the mosquito species was found tolerant according to WHO criteria where the mortality rate during 24 hrs is less than 98%. The results of the present study showed that the DDT 4% KDT_{50} to *Anopheles species* exposure for 1 hr was found 6.7, 1.6, 1.4 and 0.9 minute in Northern Bari payam, Katour payam, Juba payam and Munuki payam respectively. The results of the study showed that the mortality rate after 24 hrs in the sentinel sites selected in Northern Bari payam, Munuki payam, Katour payam and Juba payam was found 98%, 96%, 96% and 97.6% for DDT 4% and 74.7%, 90.4%, 88% and 88.8 respectively, therefore according to

the WHO criteria all the Anopheline mosquito species found in Juba County seemingly tolerant to DDT 4% and deltamethrin 0.05%.
Keywords: *Anopheles species*, DDT, deltamethrin, knockdown time, Resistance, Juba County, Southern Sudan

INTRODUCTION

Malaria is a major health problem in the tropical countries, especially sub Saharan Africa, where about 90% of the clinical cases occur. There are nearly 500 million clinical cases of malaria worldwide each year and 1.1 to 2.7 million people die annually (WHO, 2000). The *Anopheles gambiae* complex is the main malaria vector (Collins and Besansky, 1994). N, malaria constitutes around 40% of all infectious diseases and *Plasmodium falciparum* is the predominant species, which is responsible for over 90% of the infections (El Gaddal, 1991). The emergence of chloroquine resistance and the deterioration of national control programs that applied DDT (and other insecticides) have been coincident with the upsurge of malaria. Currently, the Global Strategy for Malaria Control, which was designed as a lifeline for Africa, emphasizes early diagnosis and prompt treatment as the major line of attack (WHO, 1997). *An. arabiensis* is the major malaria vector reported from all parts of the country, coexisting with *An. gambiae* sensus stricto (s.s.) and *An. funestus* in Southern Sudan (Petrarca, *et. al.,* 2000). These three African mosquitoes are as efficient as malaria vectors because of their marked preference for human environments and humans as hosts and because they adapt so rapidly to changes in the environment. The intensity of malaria transmission by these mosquitoes is determined largely by environmental conditions. *An. gambiae* is usually predominant in humid environments while *An. arabiensis* is found in drier areas, but they coexist widely over much of their range of distribution (Coetzee, *et al.,* 2000).

An. Arabiensies is adapted to change in the breeding sites according to the change in the environment (WHO, 1975). In Sudan *An. arabiensis* found to utilize different type of breeding sites, such as pools made by the flood (receding floodwater) along the bank of the Nile River, rain pools, and artificial water storage containers. The *Anopheline* tend to breed in sluggishly moving streams or in stagnant pools, especially where there is a luxurious growth of weed's or grass and not apt to be found in rapidly flowing streams. Hence there is necessity for constant care of ditches and necessity to prevent them becoming obstructed by vegetation (WHO, 2001).

In the absence of methods to control adult mosquitoes, the strategy was to reduce breeding sites. Accordingly, a considerable effort should be made to drain swamps and marshes and to somehow limit the populations of mosquitoes, whether vectors or not (Clive, 2002**).** Desowitz **(**1999) stated that the Pontine Marshes near Rome and the Hula swamps in Israel are often used as examples of success in eliminating vector populations.

What has been learned over the past several decades is that specific control strategies should be developed for specific country conditions (Gillies, 2001). The most important tools to control malaria consist of properly trained personnel with authority to coordinate and carry out their scientific work (Roberts, *et. al.,* 2000).

Resistance is a potentially powerful, pervasive natural phenomenon. The development and severity of resistance to pesticides is controlled primarily by human action. Ignorance or a lack of concern in dealing with resistance can set the stage for explosions in pest populations leading to reversals in the effectiveness of public health protection programs. By 1966 the problem of *Anopheline* resistance to DDT had become clear. There were 15 species resistant to DDT and 36 resistant to dieldrin (WHO, 1970)**.** It has been argued that DDT can still be effective against resistant mosquitoes **(**Grieco, *et. al.,* 2007), and that the avoidance of DDT-sprayed walls by mosquitoes is an additional benefit of the chemical (Sharma, 2005). For example, a 2007 study reported that DDT-resistant mosquitoes still avoided DDT-treated huts. The researchers argued that DDT was the best pesticide for use in IRS (even though it did not afford the most protection from mosquitoes out of the three test chemicals) because the others pesticides worked primarily by killing or irritating mosquitoes modes of action the authors presume mosquitoes will develop resistance to **(**Grieco, *et. al.,* 2007). Contrary to fears based on experience with other insects such as House Flies, genes for DDT resistance in *Anopheles* do not generally give cross resistance to pyrethroids. Resistance has been selected to pyrethroids in a few *Anopheles* populations but so far has not been a major problem in the field. Like DDT, pyrethroids tend to irritate mosquitoes so that they do not rest on deposits for long. However, the pyrethroids paralyze the nervous system so fast that contact for a few minutes is enough to kill, whereas much longer contact is required with DDT and mosquitoes may escape it without picking up a lethal dose. For these reasons, when comparisons have been Control of Malaria Vectors in Africa and Asia made, better malaria control has generally been achieved with pyrethroids than with DDT (Curtis, 1996). Pyrethroid resistance is becoming a serious problem in several agricultural pests, such as the horn fly (Sparks *et al.,* 1985). DDT becomes ineffective so quickly now because DDT-resistant mosquitoes exist at low frequency in the global mosquito population and, when a local population is sprayed, a strong force of selection in favor of the resistant mosquitoes is immediately created. It is only a

matter of time before the resistant mosquitoes take over (Mark, 2004). Insecticide resistance has long been recorded in almost all West-African countries. Pyrethroid resistance due to *Kdr* mutation has recently been observed, other resistance mechanisms (resistant AChE, esterases, oxydases, GST) have also been recorded in West- and Central-African populations of *An. gambiae* (Weill *et. al.*, 2003). Although pyrethroid insecticides are a promising means of controlling *Anopheles* malaria vectors compare to DDT, there is a need to monitor for resistance, in order to provide the baseline information for development of an effective control measures for *Anopheles species* mosquitoes in Juba County, Central Equatoria State, the study aims at determining the DDT 4% and deltamethrin 0.05% knockdown (KDT50 and KDT95) to *Anopheles species* and evaluate the level of resistance to these insecticides.

MATERIALS AND METHODS

Study site: The study was conducted in Juba County, Central Equatoria one of the 26 states of Sudan, it is in the South of the Sudan. Juba is the capital of the State; it was formerly named Baher el Jebel (river of Mountain). The state comprise of eight county, Juba county, Tali county, Rokon county, Mangella county, Liria county, Terekaka county, Koje–Koji county, Morobo county and Yei county, and the study conducted in Juba county (Plate 1) which comprise fourteen payam (localities), four payam were selected for the study, these were Juba payam, Kator payam, Munuki payam, and Northern Bari payam.

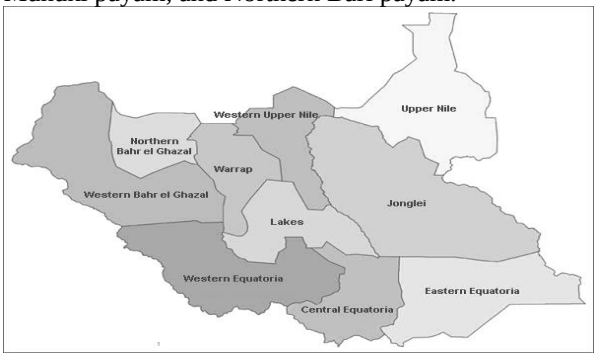


Plate 1: Study area in Central Equatoria State Southern Sudan

Sample size and sampling techniques: Samples of *Anopheles* larvae were collected from the four localities of the Juba county (plate: 1, Juba payam, Katour payam, Munuki payam, and Northern Bari payam) which have a high population density of mosquitoes. The field collection of *Anopheles species* and susceptibility tests were conducted between early March and April 2009, when the population of the mosquitoes reached high levels. The larvae collected were transferred to the laboratory for rearing. Emerging females were collected for testing.

Insecticides resistance test: Tests were conducted on female Anopheles of 1-3 days unfed emerged. WHO test kit positions (vertical and horizontal), one expose time is 1 hr. WHO test kit for adult mosquitoes were used, 10 impregnated papers were prepare using silicone oil with technical DDT 4%, 5% and deltamethrin 0.05 % (WHO diagnostic concentration). Insecticides impregnated papers prepared in accordance with WHO specifications were purchased from the WHO Collaborating Centre in Malaysia (Vector Control Research Unit, School of biological Sciences Universiti Sains Malaysia, Penang). Anopheles Adult females were used for susceptibility testing, for each insecticides, 5 replicates of 25 mosquitoes each were tested using WHO impregnated paper, each test including a control from the same mosquitoes tested. The exposure time was 60 minutes in exposure tube in the normal vertical position. The number of mosquitoes knockdown was recorded 10, 15, 20, 30, 45, 50 and 60 minutes after the start of the exposure, in addition to the mortality after 24 hrs. Mortality was noted immediately after exposure as percentage knockdown and after 24 hrs as percentage mortality. During the 24 hrs holding period the test kits were kept in a cardboard box covered with wet towel (WHO, 2001). The KDT_{50} and KDT_{95} were calculated.

Data analysis: The resistance/susceptibility status of the tested population was determined for each insecticide according to WHO criteria. This said that a resistant population is defined by mortality rates less than 80% after 24 hr observation period while mortality rates greater than 98% are indicative of susceptible population. Mortality rates between 80-98 % suggest a possibility of resistance (suspected resistance) that require confirmation (WHO, 1998).
The data was analysis using SPSS version 16 and computer software Program.

RESULTS

Assessment of resistance to DDT and deltamethrin insecticides and detection of knockdown resistance (*kdr*), were conducted in four payam (Juba payam, Kator payam, Munuki payam, and Northern Bari payam). The results obtained from this study suggest overall that *Anopheles species* is tolerant to DDT 4% (97.0±0.6) and resistant to deltamethrin 0.05% (84.9±4.3**),** Overall and by localities percent mortality of *Anopheles species* after 24 hrs exposed to the two insecticides are presented in Table 1.

Table 1: Percent mortality (Mean ± SE) after 24 hrs of tested insecticides at Juba County monitored localities

LOCALITY	INSECTICIDES	%MORTALITY (Mean ± SE)
Northern Bari payam	DDT 4%	98.0±0.9
	deltamethrin	74.7±14.9
	control	0.0
Munuki payam	DDT 4%	96.0±1.6
	deltamethrin	90.4±0.9
	control	0.0
Katour payam	DDT 4%	96.0±1.3
	deltamethrin	88.0±1.8
	control	0.0
Juba payam	DDT 4%	97.6±0.9
	deltamethrin	88.8±2.3
	control	0.0
Total	DDT 4%	97.0±0.6
	deltamethrin	84.9±4.3
	control	0.0

The results presented in table 1 show that in Northern Bari payam locality *Anopheles species* was found to be susceptible to DDT 4% (98.0±0.6), while to deltamethrin was found resistant (74.7±14.9), in case of Munuki payam *Anopheles* population was shown slightly tolerant to DDT 4% (96.0±1.6), and resistant to deltamethrin 0.05% (90.4±0.9). Similar results were obtained in Katour payam, and Juba payam, the *Anopheles* population showed tolerance to DDT 4%, and resistant to deltamethrin 0.05% (Table 1). During bioassay, control showed no mortality.

Knockdown bioassay: Knockdown effects of two insecticides DDT 4% and deltamethrin 0.05% to the *Anopheles* population collected from different localities of Juba County presented in table 2. The results indicated that the *An. species* was found to be resistant to deltamethrin in Munuki payam, Katour payam, Northern Bari payam and Juba payam. Deltamethrin KDT_{50} values ranged between 2.3 and 3.9 min and KDT_{95} values ranged between 127.8 and 293.2 min. The percent mortality of the tested *Anopheles* population collected from Munuki payam, Katour payam, Northern Bari payam and Juba payam after 1 hr were 87.2, 89.6, 90.4 and 91.2 respectively. The knockdown data indicate further precipitation of pyrethroid resistance in these species, particularly in Munuki payam and Katour payam to some extend.

Table 2: Knockdown time (KDT50 and KDT95) of *Anopheles species* exposed to deltamethrin 0.05% and DDT 4% for monitored Juba County.

Insecticide tested	Localities	KD %Mortality (1 hr)	KDT_{50} minute	KDT_{95} minute	Regression line equation
DDT	Northern Bari payam	90.4	6.7	151.9	Y=4.0+1.21X
	Munuki payam	94.4	0.9	59.9	Y=5.04+0.90X
	Katour payam	89.6	1.6	146.7	Y=4.82+0.84X
	Juba payam	96.8	1.4	43.3	Y=4.84+1.10X
Deltamethrin	Northern Bari payam	90.4	3.6	293.2	Y=4.4+1.07X
	Munuki payam	87.2	2.4	255.5	Y=4.69+0.81X
	Katour payam	89.6	3.9	138.5	Y=4.37+1.06X
	Juba payam	91.2	2.3	127.8	Y=4.66+0.94X

The susceptibility of *Anopheles species* to knockdown effects of DDT 4% in four locations of Juba County (Munuki payam, Katour payam, Northern Bari payam and Juba payam), reveal that the *Anopheles species* are fully tolerant to the DDT 4% (Table 2). DDT 4% KDT_{50} values and KDT_{95} values in different localities in Juba County ranged between 0.9 and 6.7 min. and 43.3 and 151.9 min. respectively. The percent mortality of the tested *Anopheles* population collected from four localities *i.e.* Katour payam, Northern Bari payam, Munuki payam, and Juba payam after 1 hr was 89.6, 90.4, 94.4 and 96.8 respectively.

The results in Table 2 shows that although the *Anopheles species* population suspected tolerance to DDT 4%, the KDT for *Anopheles species* population seemingly low than that of deltamethrin 0.05% particularly in Katour payam, Munuki payam, and Juba payam (KDT_{50} for DDT for three localities were 1.6, 0.9 and 1.4 min respectively, while for deltamethrin were 3.9, 2.4 and 2.3 min respectively).

The quick knockdown time minutes were obtained by DDT 4% to *Anopheles species* (KDT_{50}= 0.9) in Munuki payam locality, while the latest knockdown obtained by DDT 4% to *Anopheles species* (KDT_{50} = 6.7) in Northern Bari payam (Table 2 and Fig. 1, 2, 3 and 4). In comparison, the DDT 4% KDT_{50} to *Anopheles species* population in Northern Bari payam was found higher 7.4 fold than that of Munuki payam,

4.7 fold than that of Juba payam and 4.2 fold than that of Katour payam.

Fig.1. Regression line of log time knockdown of DDT to *Anopheles species* collected from Munuki payam after 60 minute of exposure.

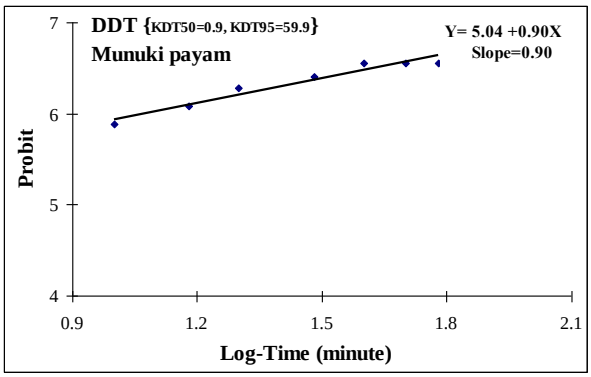


Fig.2. Regression line of log time knockdown of DDT to *Anopheles species* collected from Northern Bari payam after 60 minute of exposure.

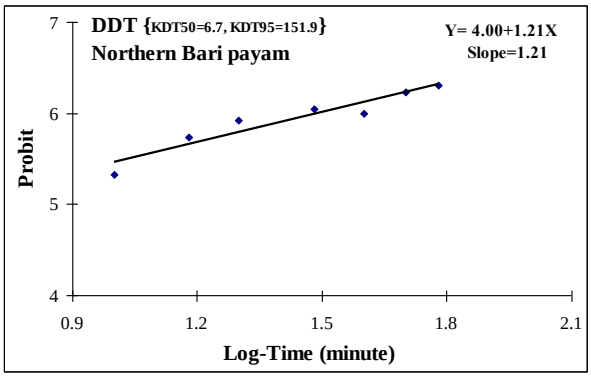


Fig.3. Regression line of log time knockdown of DDT to *Anopheles species* collected from Katour payam after 60 minute of exposure.

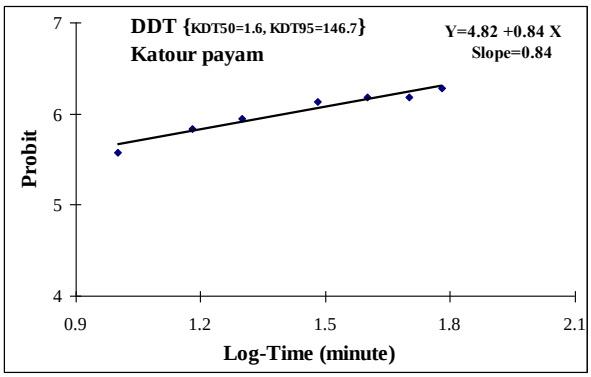
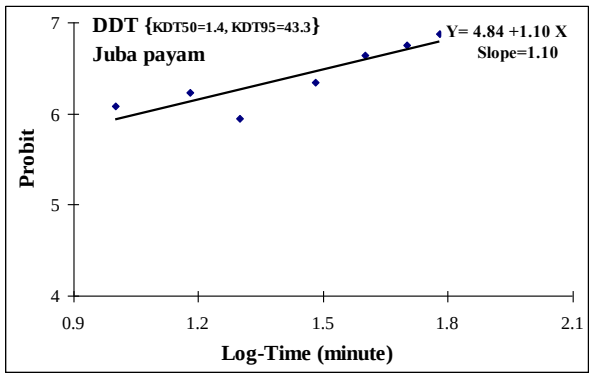


Fig.4. Regression line of log time knockdown of DDT to *Anopheles species* collected from Juba payam after 60 minute of exposure.



The fastest knockdown time minutes were obtained by deltamethrin against *Anopheles species* (KDT_{50}=2.3 and 2.4 min.) in Juba payam and Munuki payam respectively, while the latest knockdown time minutes were obtained by deltamethrin against *Anopheles species* ((KDT_{50}=3.9 and 3.6 min.) in Katour payam and Northern Bari payam respectively (Table 2 and Fig. 5, 6, 7, 8). In comparison, the deltamethrin 0.05% KDT_{50} to *Anopheles species* population in Katour payam was found higher 1.7 fold than that of Juba payam, 1.6 fold than that of Munuki payam and 1.1 fold than that of Northern Bari payam.

Fig.5. Regression line of log time knockdown of deltamethrin to *Anopheles species* collected from Juba payam after 60 minute of exposure.

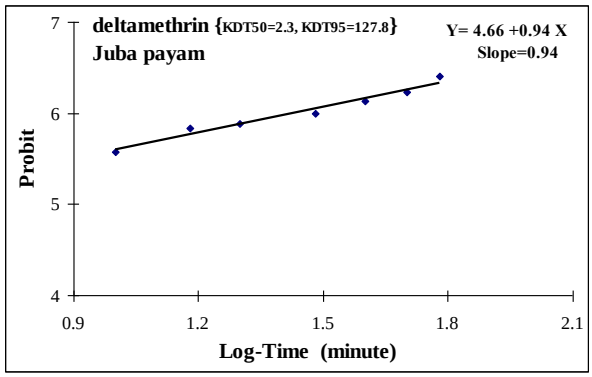


Fig.6. Regression line of log time knockdown of deltamethrin to *Anopheles species* collected from Munuki payam after 60 minute of exposure.

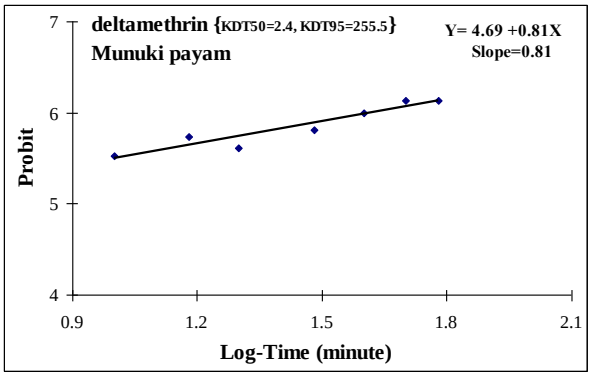


Fig.7. Regression line of log time knockdown of deltamethrin to *Anopheles species* collected from Katour payam after 60 minute of exposure.

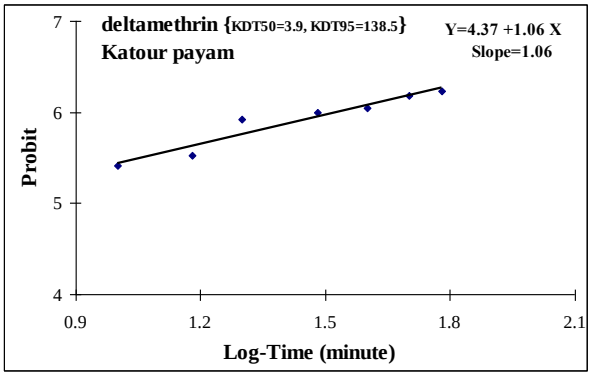
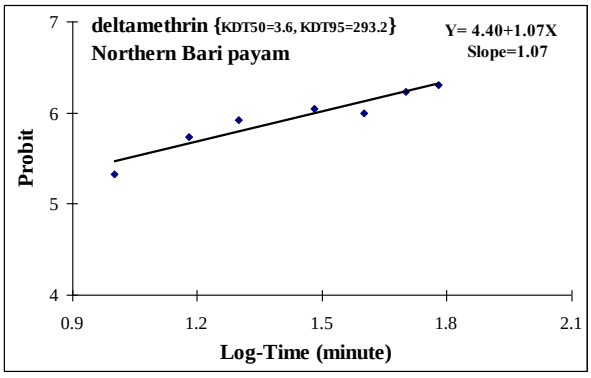


Fig.8. Regression line of log time knockdown of deltamethrin to *Anopheles species* collected from Northern Bari payam after 60 minute of exposure.



DISCUSSION

According to Petrarea *et al.,* (2000) *An. Gambiae sensus stricto (s.s)* and *An. funustus* in Southern Sudan consider the principal vector of the malaria; According to the WHO criteria for characterizing insecticides susceptibility, *Anopheles species* in Juba County was found to be tolerance to deltamethrin 0.05% and DDT 4%. The resistance occurred may be attributed to extensive use of insecticides donated by NGOs to Juba County after each peace agreement, improper application of indoor residual spraying by untrained volunteer and the lack of official labors. Additionally a massive free treated bet nets, which distributed to Juba County, need re-impregnation because the volunteers did not know about the techniques of using the insecticides dosages. This agrees with the findings of Hemingway and Bates (2003), they mentioned that because the prevalence and severity of resistance to DDT in several *Anopheles species*, pyrethroid remains the only insecticides authorized by the WHO for extensive use on nets in Africa's mosquito control campaigns. However, the current large-scale presence of pyrethroid resistance in mosquitoes, in West Africa, will affect control efforts and selection of multi-resistance mechanisms in both East and West Africa through exposure to DDT and pyrethroids may result in widespread failure of this strategy (Hemingway, *et al.,* 2002).

On the other hand, the results obtained by the present study was inconsistent with the same findings that obtained by Himeidan *et al.,* (2007). They found that DDT 4% was effective and ranged from 96.9%- 99.6% to *Anopheles arabiensis* in New Halfa, Eastern Sudan. The result obtained from the present study showed that at present DDT 4% is superior to deltamethrin 0.05% particularly to *Anopheles* population of three localities (*i.e*. Katour payam, Munuki payam, and Juba payam) in Juba County study areas, although *Anopheles species* population were tolerant to the both compounds.

In addition, tolerance for DDT and Deltamethrin in Juba County could be due to migration of cattle raider from neighboring countries such as Kenya and Uganda, where the passive dispersion may have taken place, so this may contribute in the importing of new resistance or tolerant generation to Equatoria State in general.

A judicious use of insecticides followed by regular monitoring of insecticide resistance is required to avoid the rapid emergence of insecticides resistance in *Anopheles species* in Juba County.

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SUSCEPTIBILITY OF *PERIPLANETA AMERICANA* L. (ORTHOPTERA: BLATTIDAE) POPULATION FROM WAD MEDANI (SUDAN GEZIRA) TO THREE PUBLIC HEALTH INSECTICIDES

ABSTRACT

This American cockroach, *Periplaneta Americana* (L.) (Orthoptera: Blattidae), as a public health pest, did not receive the attention it deserves by the Sudanese researchers. The study was initiated to investigate the susceptibility of this pest to three recommended public health insecticides in Wad Medani town as a representative of the Gezira State cockroach population.

Adults were collected from the sewage at night, topically treated with different concentrations of each insecticide, *viz*. lamdacyhalothrin, deltamethrin, and pirimiphos-methyl. Results were taken after 24 hr in terms of LC_{50}, LC_{90}, LD_{50}, LD_{90}, the slope of the log -dose probability line (Ldp-line), and the prime resistance ($RR'=LC_{90}/LC_{50}$).

The results revealed that the adult American cockroach exhibited acceptable levels of susceptibility to all the tested insecticides (LC_{50}

ranged between 0.02 and 11 µg/roach). The prime resistance ratio, *i.e.* RR' that shows the performance of the LC_{90} or LD_{90} compared to that of the LC_{50} or LD_{50} revealed that this ratio was as follows: Pirimiphos methyl 1.4x, deltamethrin 1.4x, lambda cyhalothrin 3.2x.

All populations proved to be homogeneous to pirimiphos-methyl (slope=7.93) and deltamethrin (10.62), and heterogeneous to lamda-cyhalothrin (2.45).

Generally, the American cockroach was more sensitive to lamda-cyhalothrin (LC_{50} = 0.022 µg/roach), and less sensitive to pirimiphos-methyl (LC_{50} =11 µg/roach); the cockroach tolerated double the dose required from lambda-cyhalothrin when treated with deltamethrin (LC_{50} =0.04 µg/roach).
Keywords: *Periplaneta americana, Adults, lamdacyhalothrin, deltamethrin, pirimiphos-methyl.*

INTRODUCTION

The American cockroach belongs taxonomically to class Insecta, order Orthoptera, sub order Blattaria, family Blattidae, genus *Periplaneta* and species *americana* L. (Imms, 1957; Stetsen, 2001).

According to Hagenbuch *et al*., (1988) cockroaches dwell outside, but will wander indoors for food and water or during extremes in weather conditions. During the day, the American cockroach, which responds negatively to light (nocturnal), rests in harborages close to water pipes, sinks, baths, and toilets, for example, where the microclimate is suitable for survival (Bell and Adiyodi, 1981).

Cockroaches (Orthoptera: Blattidae) are among the most common of insects. Fossil evidence indicates that cockroaches have been on earth for over 300 million years. Roaches are considered one of the most successful groups of animals. Because cockroaches are so adaptable, they have successfully adjusted to living with humans. About 3,500 species of cockroaches exist worldwide. Only four species are common pests. These are the German cockroach, *Blattella germanica* (Linnaeus), the brown-banded cockroach, *Supella supellectium* (Serville), the Oriental cockroach, *Blatta orientalis* (Linnaeus), and the American cockroach, *Periplaneta americana* (Linnaeus).

The American cockroach, *P. americana* can become a public health problem, due to their association with human waste and disease, and their ability to move from sewers into homes and commercial establishments, can transmit several human diseases, such as typhoid fever, diarrhea, dysentery and leprosy. In addition, cockroaches carry the eggs of parasitic worms, and may cause allergic reactions, including dermatitis, itching, swelling of the eyelids, and more serious respiratory conditions (Stankus, *et. al.*, 1990, and Jacobs, 2002). Roaches can cause allergic reactions in some people. The response is caused by roach "allergen" that is ingested with contaminated food or inhaled when dried fecal particles and fragments of ground-up bodies of dead roaches are mixed with house dust (Shripat and David, 1993 and Anon. 1997).

Cockroaches (especially the American cockroach, which comes into contact with human excrement in sewers or with pet droppings) may transmit bacteria that cause food poisoning *(Salmonella* spp. and *Shigella* spp.). Cockroaches cause serious economic losses by eating, staining or tainting our possessions. They can even wreck computers and other electronic equipment by causing short circuits. Cockroaches move freely from building to building or from drains, gardens, sewers and latrines to human habitations. Because they feed on human feces, as well as human food, they can spread germs that cause disease (Cornwell, 1968, and Roth and Willis, 1957). Cockroaches like house flies, can spread disease by contaminating human food with germs they pick up in latrines, garbage dumps (Stankus, *et. al.*, 1990).

Cockroaches are controlled by applied insecticides to the resting and hiding places as residual sprays and insecticidal dusts. Such applications are effective for periods ranging from several days to months, depending on the insecticide and the substrate on which it is deposited. Insecticides can also be combined with attractants as toxic baits. Wooster and Ross (1989) stated that cockroaches are difficult to control with insecticides for several reasons, one of which is that they may become resistant to commonly used compounds. Moreover, many insecticides are repellent to them and are, therefore, avoided. Chemical control gives only temporary relief and, wherever possible, it should be accompanied by environmental sanitation and house improvement (Schal, 1988).

Many studies have addressed insecticides resistance in German cockroach and most investigators have mainly examined the genetics and mechanisms of resistance (Siegfried and Scott, 1992). Field resistance to most current insecticides is found only in German cockroaches. American and smoky brown cockroach populations were found to be resistant to chlorinated hydrocarbon insecticides during the period of time when these insecticides were used extensively for cockroach control. Insecticide resistance in the German cockroach was first identified in 1952 in populations exposed to chlordane, in 1964 to organophosphates, in 1968 to carbamates, in mid-1980s for pyrethroids, in 1992 to sulfluramid, and in 1994 to abamectin. Little resistance has been developed by the American and the large brown cockroaches *P. Americana and P. brunnae* respectively, mainly to DDT and chlordane. The American cockroach has been found to be resistant to trichlorfon in China, and the large brown cockroach to diazinon in the USA (Cochran, 1989).

Resistance to insecticides has been a problem in all insect groups that serve as vectors of diseases. There are several key mechanisms responsible for cockroach resistance to insecticides, such as detoxification (metabolic) mechanisms, mutations or modifications in sites of action (*e.g*. nervous system) mechanisms or structural (anatomical or morphological) modifications.

Because of the development of resistance, and for environmental reasons, the chlorinated hydrocarbons (CHCs) have been replaced by the biodegradable organophosphorus (Ops), carbamates insecticides and synthetic pyrethroids are compounds that are highly toxic to insect. The German cockroach is resistant to several organochlorine, OP, carbamate and pyrethroid insecticides (Cochran, 1989).

Pest resistance to pesticides has now been wide spread, particularly in the developed countries and in some of the developing countries. Extensive usage and heavy reliance on insecticides have led to the development of insecticide resistance in cockroach. Insecticide resistance is a major threat to chemical and pest control industries worldwide. The development of resistance to modern organic insecticide has attracted so much attention (Oppenoorth and Welling, 1979).

Cockroaches resistance to insecticides was first detected in the German cockroach to chlordane in Corpus Christi, Texas, USA in 1952 (Heal *et al.*, 1953). An increasing number of cases have been documented in the USA (Bennett and Spink, 1968; Cochran, 1989; Rust and Reierson, 1991, Zhai and Robinson, 1991), Canada (Batth, 1977), Europe (Chapman *et al*., 1993., Jensen, 1993), and Japan (Umeda *et al.*, 1988). Currently, resistance to all the major groups of insecticides (organochlorines, OPs, carbamates and pyrethroids) in *B. germanica* has been reported (Cochran, 1995). Increased tolerance, and potential resistance to other novel insecticides, such as sulfluramid (Schal, 1992) and abamectin (Cochran, 1994), along with behavioral changes in responding to glucose attractant (glucose- aversion) in cockroach bait (Silverman & Ross, 1994) have been reported recently.

Repeated and frequent applications of an insecticide usually reduce the effectiveness and, inevitably produce resistance of the target pest to the insecticide. Resistant strains are derived from the initial population, due to the selection pressure on the pest population (Bettini *et al.*, 1970; Green *et al*., 1977, and Sawicki, 1979). Generally, the resistant strains arise when the whole population is selected by killing off the susceptible individuals (Green *et al*., 1977).

High levels of resistance ordinarily occur only where there is a history of exposure to the poison. The sign of resistance in cockroach has been indicated by control failure or usually persistent infestation, despite the apparently proper usage of the pesticide. Usually, the first response by insecticide user when a pesticide is loosing effectiveness is to increase the dosage applied and the frequency of application, which is probably a major cause of resistance (Bettini *et al.*, 1970; Brown and Pal, 1971; Busvine, 1980, and Georghiou, 1986).

According to Brown and Pal (1971) control failure should be confirmed by the standard test of the insect. These tests compare the insect population suspected of resistance with a normal or baseline population of the strain, *i.e.* with untreated population elsewhere, when the development of resistance has dictated a change from one insecticide to another, the original resistance may be progressively lost.

Brent (1986) stated the aims of detection and monitoring are: (a) to check for the presence and frequency of occurrence of the basic genetic potential for resistance in target organism population (b) to gain early warning that the frequency of resistance is rising and for that practical resistance problems are starting to develop (c) to determine the effectiveness of management strategies introduced to avoid or delay resistance problems.

Monitoring is an integral part of pesticide resistance management. Monitoring denotes different operations ranging from global surveillance program to a much smaller investigation of cases of suspected resistance. The effective management of resistance to a pesticide depends on the continued development of a new compound, as well as the judicious use of existing materials (Hommock and Soderlund, 1986).
Green *et al*. (1977) concluded that efforts should be expanded to develop IPM system, which is the most permanent solution to delay or to avoid pest resistance to pesticide.

For this reason, the present work investigates the effect of the public health insecticides Actellic® 50% EC (pirimiphos-methyl), Icon® 10%WP (lamda-cyhalothrin) and K-othrine® 250% WG (deltamethrin) on the control of the American cockroach and the susceptibility of the adults to these insecticides.

The specific objectives are:
a) Determination of the LC_{50} and $LC_{90,}$ their respective LDs, and the slopes of the log-dose probability lines (Ld-P lines),

b) Answering the question about which chemical might cause the development of resistance faster than the others in (terms of prim resistance ratio, RR'),

c) Offering options to the users when the three insecticides are available (in terms of susceptibility and RR').

MATERIALS AND METHODS

Site: This study was conducted in the Biology Laboratory of the Faculty of Agricultural Sciences (FAS) , University of Gezira (U of G), Wad Medani, Sudan (14 24 N 33 29 E, 408 m above sea level).

Equipment: The equipment used were graduated cylinders 100 ml (B.S. 604), flasks 100 ml (glass 3.3), syringes 10µl (HP 9301- 0246), Mettler balances (BDF-460), pipettes (pmk-380), plastic containers with glass covers (can see through), and several ventilation pores. The base diameter for the plastic container is 12 cm, the peak diameter is 8 cm, and the height is 8 cm. Fig. (1).

Figure (1): plastic container

Collection of cockroaches: The collection of the adults occurred at the periods from October 2003 to May 2004. Adults were collected at night from sewers and manholes using flash light. The insects were kept in plastic containers. Wet bread was used in adult nutrition until use for the bioassay.

Insecticides used: The insecticides used for this study are Actellic® 50% EC (Pirimiphos-methyl *O-(2-diethyl amino-6-methyl pyrimidin-4-yl) O,O dimethyl phosphorothioate*). Icon® 10%WP (lamda-cyhalothrin *α-cyano-3-phenoxy benzyl 3-(2-chloro-3, 3, 3-trifluro-prop-1-enyl)-2,2-dimethyl cyclopropane carboxylate*). K-othrine® 250% WG (Deltamethrin *(s)–α–cyano–3-phenoxybenzyl (IR, 3R)–3-(2,2-dibromoviny)-2,2 dimethyl cyclo propane carboxylate*).

Dilutions

Deltamethrin Dilution: Deltamethrin 250WG was brought from The Malaria Administration, Ministry of Health (MOH) and Social Welfare, Gezira State, Wad Medani, the Sudan. One g of K-othrine was placed into a 100 ml flask, and 100 ml of distilled water was gradually added followed by shaking to dissolve the WG. The flask was shaken until the WG of Deltamethrin was completely dissolved in the flask. One ml of the previously prepared solution was taken by pipette (1ml) and placed into another 100 ml flask and completed by adding 99 ml of water. Fifty ml from this solution was taken and placed into 100 ml flask and to it another 50 ml of water was added. The concentration of this solution is then 1250µg/100ml (12.5µg/ml).

L**amda-cyhalothrin dilution:** Lamda-cyhalothrin 10 WP was brought from Agways Company Wad Medani, Sudan. One g of the formulation was placed into a 100 ml flask, and to it 100 ml of water gradually added with shaking. The flask was shaken until the WP was completely dissolved in the flask. One ml of the previous solution was taken by pipette (1ml) and placed into another 100 ml flask and 99 ml of water added. The concentration of the solution then was 1000µg/100ml (10µg/ml).

Pirimiphos-methyl Dilution: Pirimiphos-methyl 50% EC was also obtained from Agways Company, Wad Medani. Sudan. One ml of the formulation was placed into a 100 ml flask and to it 99 ml of water was gradually added with shaking. The flask was shaken until the milky appearance was obtained. The concentration of the solution then was 500,000µg/100ml (5000 µg/ml).

Treatments: Topical application method using micro-syringes was adopted in treating cockroaches. The mean weight of the American cockroaches were measured before the treatments, it was 0 .75g.

Volumes of 3.0, 3.2, 3.4, 3.6, 3.8, and 4 µl from the diluted Deltamethrin (equivalent to 0.0375, 0.04, 0.0425, 0.045, 0.0475, 0.05 ppm or µg/g), 1.8, 2.0, 2.2, 2.4, 2.6, and 2.8µl from the diluted Pirimiphos-methyl (equivalent to 9, 10, 11, 12, 13, 14 ppm or µg/g), and 1, 2, 3, 4, and 5µl from the diluted lamda-cyhalothrin (equivalent to 0.01, 0.02, 0.03, 0.04, 0.05 ppm or µg/g) were dispensed under the pronotum of the adult cockroaches by a microsyrnge. The treatments start from low to high concentrations. The treated adults were placed in plastic containers for 24 hr. The control was always included. For each test freshly prepared solutions were used. Each concentration was replicated 3 times, and each test was repeated at three different days. Mortality was determined after 24hrs. Abbott's formula was used to correct for the natural mortalities.

Data Analysis: The percentage mortality for each concentration was calculated on the basis of the mean %mortality. The percentage mortality was corrected

using Abbott's formula (Abbott, 1925, and Busvine, 1980) as follows:

$$\text{Correct \% mortality} = \frac{\text{Test \% mortality} - \text{Control \% mortality}}{100 - \text{Control \% mortality}} \times 100$$

When the control mortality exceeded 20%, the test was repeated (Busvine, 1980).

The corrected percentage mortality data was plotted against the corresponding concentration in a probit paper to obtain a log-Dose line (Ld-P), i.e. regression lines, which were fitted by eye. LC_{50} and LC_{90} were graphically estimated. The standard error (S.E.) and fiducial limits (F.L.) were also calculated for the LC_{50} and LC_{90} (Finney, 1980 and Busvine, 1980).

RESULTS AND DISCUSSION

When treating the American cockroach adults with deltamethrin using the concentrations specified (0.0375, 0.04, 0.0425, 0.045, 0.0475, 0.05 ppm or µg/g) for 24 hr, the results showed that the LC_{50} value found to be 0.04 ppm, whereas the LC_{90} value was 0.054 ppm (the difference was as little as 0.014 ppm). The results indicated that the population prove to be more homogeneous to this insecticide (slope =10.62). The difference between the LC_{50} and LC_{90} values was small and this was reflected on the RR' (1.4) As the R R' is generated by dividing the LC_{90} of the strain with the LC_{50} strain (Table 1).

Table 1: The Susceptibility of the American cockroach *Periplaneta americana* (L.) to three public health insecticides in terms of acute toxicity

Insecticides	No. of insects Tested	LC_{50} (µg/insect*)	LC_{90} (µg/insect)	LD50 (µg/g)	LD90 (µg/g)	RR' Ratio (LC_{90}/LC_{50})	Calculated Slope	Regression line equations
lambda-cyhalothrin	324	0.02 ± 0.001	0.07 ± 0.002	0. 029	0. 093	3.2	2.45	Y= 1.7 + 2.45x
Deltamethrin	270	0.04 ± 0.01	0.054 ± 0.02	0. 053	0. 072	1.4	10.62	Y = -1.43 + 10.62x
Pirimiphos-methyl	378	11 ± 1.00	15.9 ± 1.20	14.67	21.2	1.4	7.93	Y = -3.25 + 7.93x

* Average weight of adult = 750 mg/insect

Lambda-cyhalothrin was also tested on the American cockroach adults at the concentrations specified (0.01, 0.02, 0.03, 0.04, 0.05 ppm or µg/g) for 24 hr. The results showed that the LC_{50} value was 0.022 ppm, whereas the LC_{90} value was 0.07 ppm (the difference = 0.048 ppm). The findings prove that the population heterogeneous to this insecticide (slope =2.45). The difference between The LC_{50} and LC_{90} values were big (RR' = 3.2) (Table 1).

Regarding Pirimiphos methyl, which was tested on the American cockroach adults at the concentrations specified (9, 10, 11, 12, 13, 14 ppm or µg/g) for 24 hr, reflected an LC_{50} value of 11 ppm and LC_{90} value of 15.9 ppm. The results indicated that the population might prove to be homogeneous to this insecticide (slope =7.93) (Table 1). The range between the LC_{50} value and LC_{90} value, as expected, was small (RR' = 1.4).

When comparing the LD_{50s} values of the two pyrethroids, that of Lambda- cyhalothrin (0. 029 ppm) was smaller than that of deltamethrin (0. 053 ppm), which in other words, indicates that the former was more potent than the latter. Thus, the American cockroach was more susceptible to the former than the latter. In the contrast, the LD_{90} value of deltamethrin (0.072 ppm) was smaller than that of lambda-cyhalothrin (0.093 ppm). This indicates that the American cockroach was more susceptible to deltamethrin than lambda-cyhalothrin. That is why the term RR' was introduced since 1984 by Bashir (personal communication) (R R' is generated by

dividing the LC_{90} value of the strain with the LC_{50} value strain). Therefore, it is not advisable in presenting the bioassay results in terms of LD_{50} value alone. These results would mislead. To be on the safe side, the LD_{50} value, in addition to both values of LD_{10} and LD_{90} should be presented in the tables. The larger the range, the more heterogeneous the species is, the smaller the slope of the Ld-P line.

The American cockroach, as expected, tolerates higher doses from the OP pirimiphos methyl. The results showed that the values of LD_{50} and LD_{90} were proved to be much higher than those of deltamethrin and lambda-cyhalothrin (Table 1). For lambda cyhalothrin, the cockroach required 500 to 505x the concentration to achieve the same mortality.

However, when compared to deltamethrin, doses required were ranging between 273 – 276x. The slope of the Ld-p line of lambda-cyhalothrin (2.45; Table 1) proved to be the smallest one, the population is more heterogeneous towards this compound. The slope of the Ld-p line of deltamethrin (10.62; Table 1) proved to be the biggest one. Therefore, it can be said that the population is more homogeneous towards this compound, followed by pirimiphos methyl (7.36; Table 1), which showed relative homogeneity of the population, according to their slopes.

In conclusion, the insecticidal properties of deltamethrin make it well-suited for the control of cockroaches in an urban setting. Based on our characterization of the activity of deltamethrin, this compound can be effective as a toxic contact insecticide against *P. Americana*.

Further research will be needed to develop insecticides treatment strategies for cockroach management programs.

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

Abstract: Two field population of *Bactericera cockerelli* (Sulc) from Raices and San Rafael with a susceptible line to determine the resistant levels. Results show that Raices population was 4.2, 2.6, 6.5, 29.3 and 2.1 times more resistant to abamectin, cypermethrin, endosulfan, imidacloprid and profenophos respectively than the susceptible line. San Rafael population values were 4.2, 1.4, 11.7, 9.2 and 1.3 times more resistant to the same products.

Key words: Potato psyllid, Resistance, LC_{50}

Introduction

Potato crop represents a very important agricultural commodity in Mexico. The potato region of Coahuila and Nuevo Leon represents 15% of the national production (SAGARPA, 2008), which is affected by potato psyllid *Bactericera* (*Paratrioza*) *cockerelli* Sulc. Munyaneza et al. (2007) mention that *B. cockerelli*

causes direct damage by sucking plant fluids and injecting toxins. The indirect damage by transmitting a phytoplasm that causes purple top disease (Garzón *et al*., 2004). Flores *et al*. (2004) mention that this disease is the most important factor limiting potato production that caused in years 2003 and 2004 yield reductions up to 90%. Many chemical products are used to control the pest; Almeyda *et al.* (2008) state that 5 to 30 insecticide applications were required to control it. Due to the above reason research was carried out to determine the level of insecticide resistance of two populations of *B. cockerelli* from the Nuevo Leon potato growing area.

Materials and Methods

This work was carried out at Universidad Autonoma Agraria Antonio Narro (UAAAN). Insects were sampled on potato fields from Raices (Ra) and San Rafael (SR) and a susceptible line (SL) and were later reared in the toxicology laboratory of UAAAN. In each sampling site, 200 leaves infested with nymphs were collected. As a susceptible line a greenhouse population without selection pressure reared since 2004 was used, and all populations then were reared in a greenhouse to carry out the experiments.

Bioassays were done using the leaf immersion technique used for the pear psyllid (*Psylla pyricola* Foerster), with slight modifications (IRAC, 2005). Insecticides used were Abamectin®, Cypermethrin ®, Imidacloprid®, Endosulfan®, and Profenophos®. Purified water and Bionex as dispersant were used to prepare the different concentrations from 0.01 to 2000 ppm. Mortality readings were done 24 and 48 h after treatment, considering dead nymph the ones that were dehydrated or did not respond to stimulus. Once determined the LC_{50}, the resistant proportion was determined by dividing the LC_{50} values of the field population against the susceptible (Georghiou, 1962), Data was analyzed by a probit analysis with the maximum verisimilitude method and using a SAS system Windows 9.0 (2002).

Results and Discussion

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

Raices population LC_{50} for abamectin, cypermethrin, endosulfan, imidacloprid and profenophos had values of 0.29, 214.83, 410.27, 107.37 and 3.63 ppm respectively (Table 2) and SR population LC_{50} (Table 3) had values of 0.29, 120.74, 732.63, 33.80 and 2.29 ppm to the same products. Raices population had the LC_{50} higher values to cypermethrin and imidacloprid, whereas SR population to endosulfan. This variation is probably due to the wide range of products and application methods used by growers; Vega *et al* (2008), estate that lack of control is due to deficient equipment calibration, management diversity and irrational pesticide use.

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

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

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

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

Raices Line (Ra)						
			Ppm			
Insecticide	n	df	LC_{50}	Fiducial limits 95 %	LC_{95}	Ra_{vs} SL
Abamectin	480	5	0.29	(0.2241-0.3720)	4.2277	4.2 X
Cypermethrin	480	5	214.83	(69.803-717.656)	4914.3	2.6 X
Endosulfan	480	5	410.27	(363.481-460.037)	1877.6	6.5 X
Imidacloprid	480	5	107.37	(33.445-336.764)	1037.8	29.3 X
Profenophos	480	5	3.63	(0.8589-9.1608)	60.295	2.1 X

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

Table 3: Lethal concentration and fiducial limits resistant proportion against of the San Rafael (SR) population and it´s the susceptible line

San Rafael Line(SR)						
			Ppm			
Insecticide	n	df	LC_{50}	Fiducial Limits 95 %	LC_{95}	SR_{vs} SL
Abamectin	480	5	0.29	(0.1800-0.4219)	3.1669	4.2 X
Cypermethrin	480	5	120.74	(17.746-734.100)	1978.2	1.4 X
Endosulfan	480	5	732.63	(477.883-1189.05)	5331.3	11.7 X
Imidacloprid	480	5	33.80	(9.3117-95.946)	1055.1	9.2 X
Profenophos	480	5	2.29	(0.2765-7.3794)	44.961	1.3 X

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

Tables 2 and 3 also show resistance ratio of Ra and SR populations against the SL. Ra population was 4.2, 2.6, 6.5, 29.3 and 2.1 times more resistant to abamectin, cypermethrin, endosulfan, imidacloprid and profenophos respectively, and for SR population was 4.2, 1.4, 11.7, 9.2 and 1.3 times more resistant to the same products tan the SL. Resistance threshold for imidacloprid was 29.3 times higher on RA population and endosulfan on the SR population, the resistance threshold was 11.7 times higher than the SL. The reason to find imidacloprid with resistance problem is probably due that it is being used in the area since 1993, with 2 or 3 sprays pear season (Anónimo, 2003). However, on the other hand, Gutiérrez *et al.* (2007), reported a reduction on the resistant proportion on *Bemisia tabaci* (Gennadius) from 29.8 ppm in the F3 to 6.3 ppm in the F6. Resistance in endosulfan is probably due to the fact that the product is also used to control other insect pests in the same crop.

Conclusion

The susceptible line can be used as a reference line for other susceptibility studies on *Bactericera cockerelli.*Imidacloprid presented resistant levels on both populations; however, its use could be restricted to critical phases of the crop as a management alternative.

Acknowledgment

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A survey of insecticide resistance in the *Colorado potato beetle* (*Leptinotarsa decemlineata*) among northern *Xinjiang* Uygur autonomous region

Abstract

A discriminating dose bioassay for monitoring insecticide resistance in 4th instar larvae and adults of the Colorado potato beetle (*Leptinotarsa decemlineata*) was developed and the susceptibilities of some field populations to several insecticides were tested. In the bioassay, we first used a topical bioassay to assess the susceptibilities of Tekes field population and found that this population was susceptible to all tested insecticides when compared to the baselines having been documented. Tekes field population, therefore, was selected as a reference susceptible strain. Based on discriminating doses (LD_{99} values) obtained from Tekes reference strain, the susceptibilities of 4th instar larvae originating from 5 counties (cities) to 16 synthetic insecticides and the susceptibilities of adults from 8 counties (cities) to 5 synthetic insecticides were tested. Several field populations have developed detectable levels of resistances to cyhalothrin, isocarbophos, malathion, carbofuran, carbosulfan, and imidacloprid. Some alternative control strategies have also been discussed.

Key words: Topical bioassay; Discriminating dose; LD-P line; Alternative control strategy

Introduction

Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say), a leaf beetle (Coleoptera: Chrysomelidae) native to the southwestern United States and Mexico, is the most important insect defoliator of potatoes in the world. The beetle invaded China in the 1990s from Kazakhstan, and finally established in Huocheng county, Qapqal county, Yining county, and Taicheng city in Xinjiang Uygur autonomous region in 1922. Since then, it has spread eastward. Currently its distribution covers almost all northern Xinjiang, including Urumqi city, Qitai county and Jimsar county.

Colorado potato beetle causes significant damage to potato and eggplant in Xinjiang. When on potato plant, the beetles consume leaves, causing a 30% to 50% yield reduction each year. One beetle consumes approximately 40 cm^2 of potato leaves during the larval stage (Ferro et al., 1985; Logan et al., 1985), and close to an additional 10 cm^2 of foliage per day as an adult (Ferro et al., 1985). Once the foliage is gone, the beetles can feed on stems and exposed tubers (Alyokhin, 2009). If left uncontrolled, the beetles can completely destroy potato crops. In addition to impressive feeding rates, the Colorado potato beetle is also characterized by high fecundity, with one female laying 300 to 800 eggs (Harcourt, 1971).

Chemical control is widely practiced. Since 1864, hundreds of compounds were tested against this pest. Currently, insecticides still remain a very important component among the strategies for effective control of Colorado potato beetle on commercial potato farms. More than 30 active ingredients are registered for use against this pest around the world (Alyokhin et al., 2008).

Not surprisingly, high selection insecticide pressure eventually resulted in the development of insecticide resistance. Therefore, a survey of insecticide resistance in Colorado potato beetle is quite necessary in Xinjiang Uygur autonomous region.

Materials and Methods

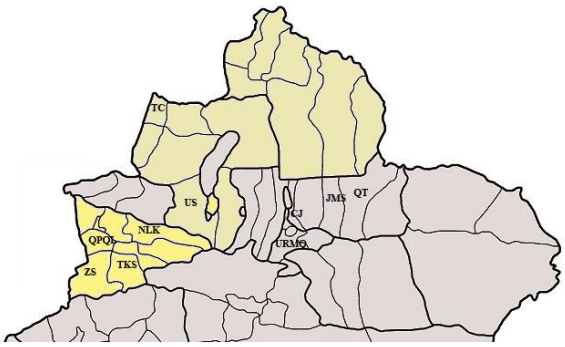
1.1 Test insects

Ten samples of Colorado potato beetle were collected from 2008 to 2009 in northern Xinjiang Uygur autonomous region (Table 1). Collection sites of these samples were showed in Figure 1. CPB adults and larvae were routinely reared in an insectary at 28±1℃ under a 14h:10h light-dark photoperiod and 50-60% relative humidity using fresh potato foliage as food.

Table 1: Ten samples of CPB collected in Xinjiang Uygur autonomous region

Sub-level division	Collection site	Population name
Changji prefecture	Qitai, Jamsar, Changji	QT, JMS, CJ
Urumqi city	Urumqi	URMQ
Tacheng prefecture	Tacheng, Usu	TC, US
Ili prefecture	Tekes, Nilka, Zhaosu, Qapqal	TKS, NLK, ZS, QPQL

Figure 1: The collection sites of 10 local populations of CPB in northern Xinjiang Uygur autonomous region



1.2 Insecticides

Seventeen technical grade insecticides were used in this study and their details were given in Table 2. These insecticides were kept in a refrigerator and temperature was maintained at -20 ℃ between the experimental sessions.

Table 2: Details of seventeen technical grade insecticides

Insecticide	Purity (%)	Manufacture
Cyhalothrin	95	Nanjing Red Sun Group Limited Company
α-Cypermethrin	95	Haili Chemical company limited by shares
Deltamethrin	98	Nanjing Red Sun Group Limited Company
Permethrin	93	Jiangsu Nanjing Aijin Chemical Group Limited Company
Azinphosmethyl	98.9	Sigma-Aldrich
Diazinon	95	Nanjing Hongliyuan Chemical Industry
Isocarbophos	98.4	Nanjing Red Sun Group Limited Company
Malathion	95	Nanjing Red Sun Group Limited Company
Phoxim	90	Jiangsu Tongzhou Zhengda Pesticide Factory
Carbayl	99	Haili Guixi Chemical Industry Pesticide Factory
Carbofuran	97	Nanjing Red Sun Group Limited Company
Carbosulfan	90	Nanjing Red Sun Group Limited Company
Acetamiprid	94.8	Wuxian Pesticide Factory
Imidacloprid	97	Nanjing Red Sun Group Limited Company
Thiamethoxam	95	Nanjing Red Sun Group Limited Company
Abamectin	95	Nanjing Baofeng Pesticide Factory
Fipronil	95	Bayer CropScience China Co., Ltd.

1.3 Bioassays

Topical bioassays were used to assess CPB susceptibilities to the insecticides. For Tekes local population, a reference susceptible strain in the present paper, insecticides were dissolved in acetone and, for each of the insecticides, at least five concentrations that resulted in more than 0% and less than 100% mortality based on preliminary assays were used. Ten 4th instar larvae were treated with 0.22 µl of insecticide solution on the fourth dorsal abdominal segment with a 10 µl microsyringe connected to a microapplicator (Hamilton Company, Reno, NV). For adults, the applied amount was 1.1 µl and the application spot was on the ventral area of the abdomen. The control larvae and adults were treated with 0.22 µl and 1.1 µl of acetone, respectively. Control mortality was typically less than 10%. Three to five replications per concentration were performed.

For other local populations, a discriminating dose assay was performed. The discriminating dose for each of the insecticides was the LD_{99} value of corresponding insecticide to Tekes reference strain. *Ten replicates* of 10 *larvae or 10 adults* per *sample were* used for each of the insecticides in the discriminating dose assay.

After treatment, larvae or adults were placed in Petri dishes (9 cm in diameter and 1.5 cm in height) containing fresh potato leaves and kept at 28 °C, 50%-60% relative humidity, and 14:10 h light:dark photoperiod. For pyrethroids, organophosphates, and carbamates, mortalities were assessed 24 hour later. For neonicotinoids, 16-membered macrocyclic lactones, and pyrazoles, mortalities were evaluated 72 hour later. Beetles incapable of becoming upright or who could not walk a distance equivalent to their own body length, when disturbed, were counted as dead.

1.4 Data analysis

Abbott's formula was used to correct the data for control mortality. Probit analysis was used to Tekes reference strain to estimate the doses needed to cause 50% mortality (LD_{50}) and 99% mortality (LD_{99}), their fiducial limits, and the slope of the line relating probit mortality to the log dose.

To quickly identify resistant beetles from susceptible ones, a discriminating dose bioassay was used in our laboratory bioassay. The mortality difference between Tekes reference strain and other local populations was compared by a chi-square test. Any local populations showing statistically significant mortality difference from Tekes reference strain were classified as resistant ones.

Results and discussion

2.1 Tekes local population as a susceptible strain

Topical bioassays were used to evaluate the susceptibilities of the 4th instar larvae of Tekes local population to several insecticides. The LD_{50} values of azinphosmethyl, carbofuran, imidacloprid and abamectin were 0.33, 0.023, 0.003 and 0.001 µg/larva, respectively. It has been reported that the LD_{50} value of azinphosmethyl to Raleigh susceptible strain was 0.20 µg/ larva (Argentine et al., 1989), of carbofuran was 0.58 µg/ larva (Rose & Brindley, 1985), of imidacloprid to Hughes Organic Farm susceptible strain was 0.0006 µg/ larva (Zhao et al., 2000), of abamectin to Raleigh susceptible strain was 0.001 µg/ larva (Gouamene-Lamine et al., 2003). The susceptibilities of adults of Tekes local population to some insecticides were also determined by topical bioassays. The LD_{50} values of azinphosmethyl, carbosulfan, imidacloprid and abamectin were 3.69, 0.94, 0.013 and 0.016 µg/adult, respectively, whereas the LD_{50} values of azinphosmethyl to Monroe-2 susceptible strain was 1.88 µg/adult (Bishop & Grafius, 1991), of carbosulfan to Majur susceptible strain was 1.76 µg/adult (Stankovi et al., 2004), of imidacloprid to Hughes Organic Farm susceptible strain was 0.021-0.026 µg/adult (Zhao et al., 2000), and of abamectin to Raleigh susceptible strain females and males were 0.006 and 0.005 µg/adult (Gouamene-Lamine et al., 2003).

Our results demonstrated that the susceptibilities of Tekes local population to tested insecticides were completely comparable to those to some susceptible strains. Consistent with this, Tekes County is located in the *Tekes* steppes in the northern side of Tian-shan Mountains at about 1500 m above sea level. The climate is cool and the frost-free growing season is

short. Farmers always use insecticide-free strategies to control agricultural pests. Therefore, we selected the Tekes local population as an insecticide susceptible reference strain.

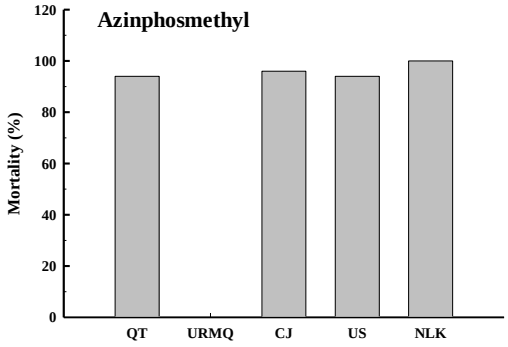
2.2 Determination of discriminating doses

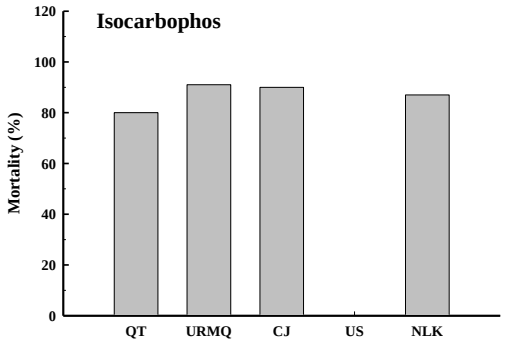
In order to obtain discriminating doses of CPB to conventionally used insecticides, log-dose probit lines of three pyrethroids cyhalothrin, deltamethrin, and α-cypermethrin, five organophosphates azinphosmethyl, isocarbophos, malathion, diazinon, and phoxim, three carbamates carbosulfan, carbofuran, and carbayl, three neonicotinoids acetamiprid, imidacloprid and thiamethoxam, a 16-membered macrocyclic lactone abamectin, and a pyrazole fipronil to 4th instar larvae and to adults of Tekes reference strain were obtained using topical bioassays. LD_{99} values of these insecticides were used as discriminating doses.

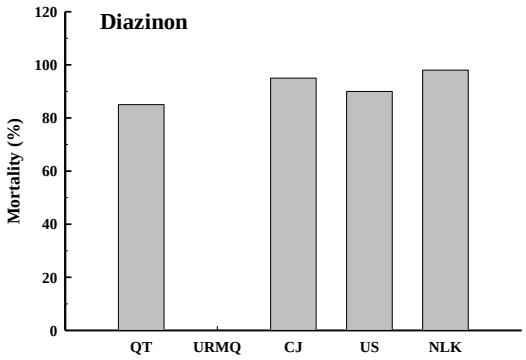
2.3 Susceptibility level of 4th instar larvae

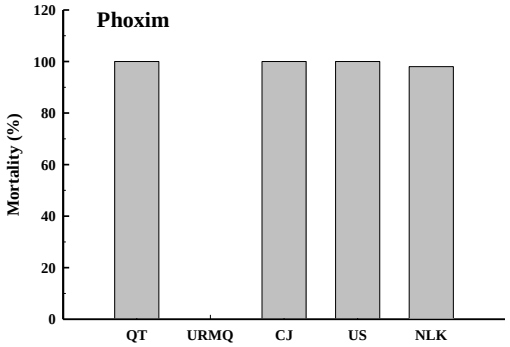
To test the susceptibility level of CPB in northern Xinjiang Uygur autonomous region, discriminating doses of 16 insecticides were applied to 4th instar larvae of 5 local populations including QT, URMQ, CJ, US and NLK. The mortalities were given in Figure 2.

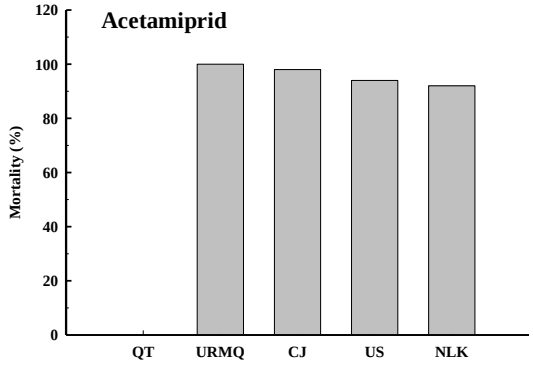
Figure 2: Mortalities of 5 local populations (QT, URMQ, CJ, US and NLK) of CPB 4th instar larvae at discriminating doses. * and ** represent statistically significant mortality difference at α=0.05 and α=0.01 level between local population and Tekes reference strain compared by a chi-square test

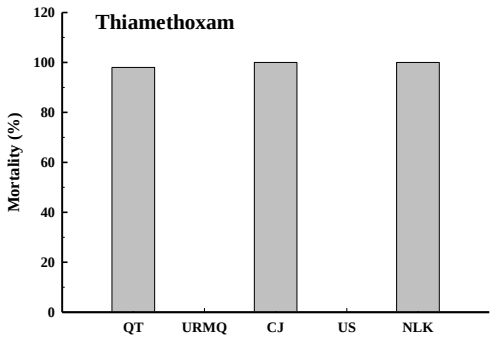


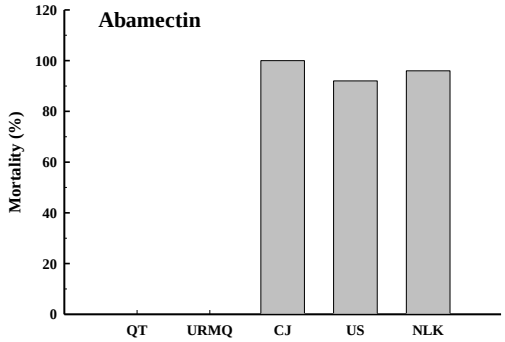


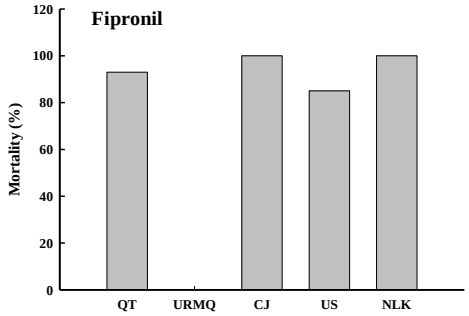






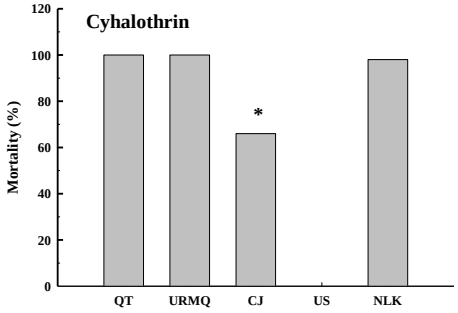


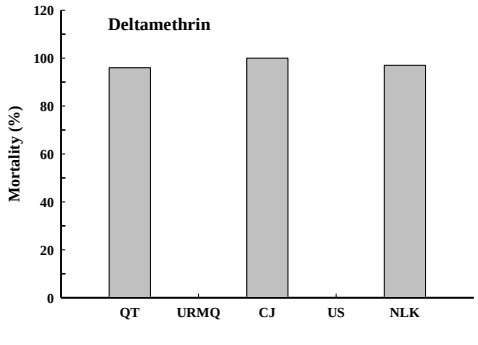


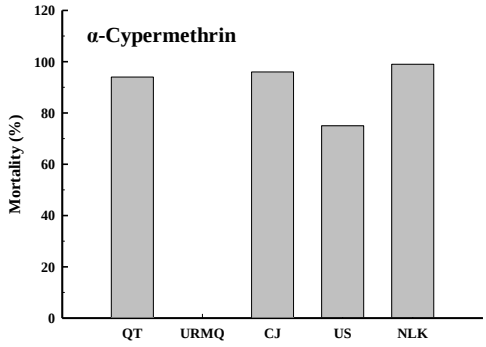


The discriminating dose bioassay was a conventional laboratory bioassay for quick identification of resistant beetles. The evaluation criterium used previously, e.g., the concentration that kills >90% of susceptible beetles kills only <5% of beetles from resistant populations (Bishop & Grafius, 1991; Heim et al., 1990) seemed too insensitive to determine resistant levels. Therefore, in the present paper, we compared mortality difference between Tekes reference strain and other local populations by a chi-square test, and classified those local populations that showed statistically significant mortality difference from Tekes reference strain as resistant ones.

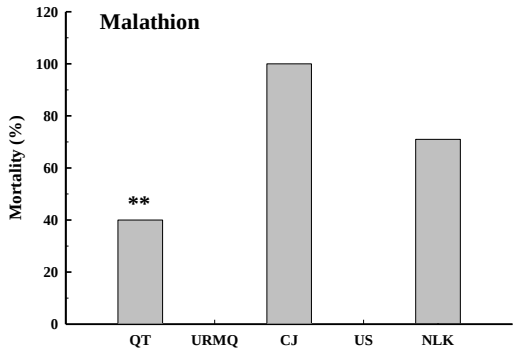
Among the five local populations, CJ populations (χ^2=3.86, P<0.05) caused obvious resistance to cyhalothrin, in contrast to deltamethrin and α-cypermethrin. Other four populations, however, were susceptible to these pyrethroids (Figure 2).



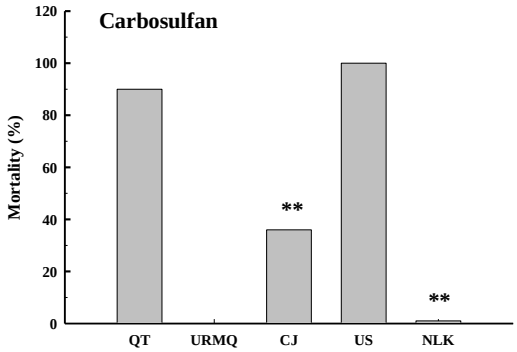


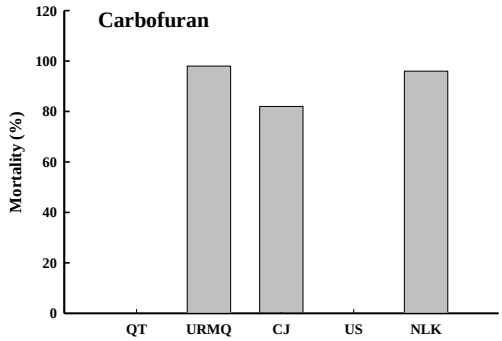


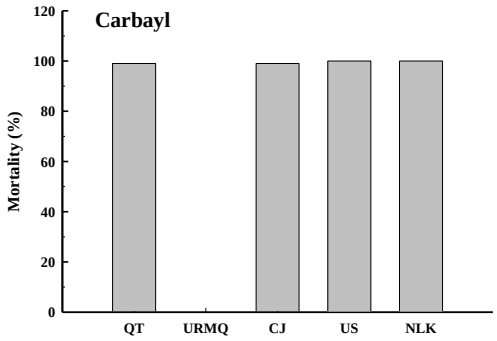
QT population (χ^2=16.51, P<0.0005) showed resistance to malathion among five organophosphates. Other four populations were susceptible to tested organophosphates (Figure 2).



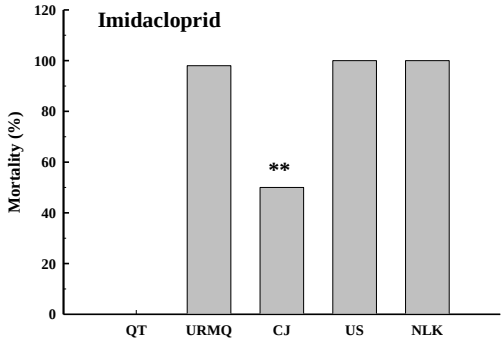
Of the five populations, CJ (χ^2=19.59, P<0.0005) and NLK (χ^2=73.88, P<0.0001) populations showed statistically significant resistance to carbosulfan. All the five strains were susceptible to carbofuran and carbayl (Figure 2).







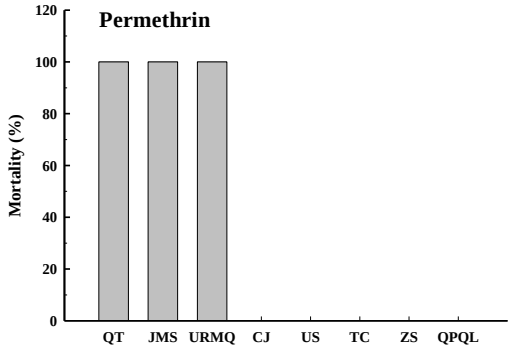
Moreover, CJ population had a resistance to imidacloprid (χ^2=10.10, P<0.001) among tested neonicotinoids, 16-membered macrocyclic lactone and pyrazole (Figure 2).

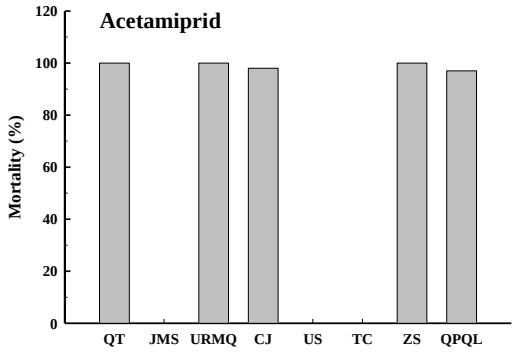


2.4 Susceptibility level of adults

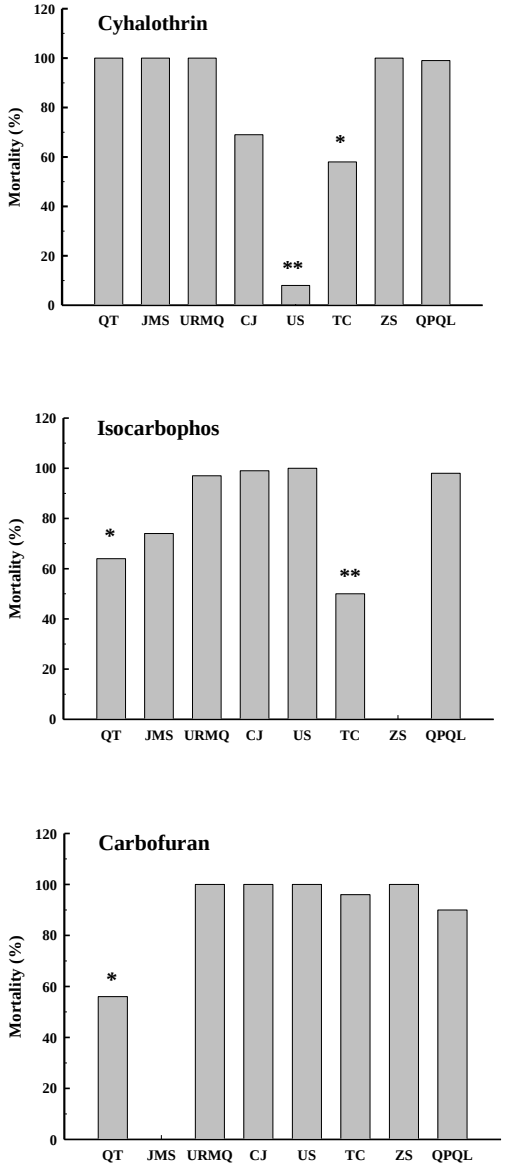
Since resistance levels vary greatly between beetle life stages (Pourmirza, 2005; Silcox et al., 1985; Zehnder, 1986; Zehnder & Gelernter, 1989), the susceptibility levels of adults originating from QT, JMS, URMQ, CJ, US, TC, ZS and QPQL to cyhalothrin, permethrin, isocarbophos, carbofuran and acetamiprid were also evaluated by topical bioassays (Figure 3).

Figure 3: Mortalities of 8 local populations (QT, JMS, URMQ, CJ, US, TC, ZS and QPQL) of CPB adults at discriminating doses. * and ** represent statistically significant mortality difference at α=0.05 and α=0.01 level between local population and Tekes reference strain compared by a chi-square test





Among these populations, US (χ^2=57.76, P<0.0001) and TC (χ^2=6.87, P<0.0001) beetles showed obvious resistance to cyhalothrin, QT (χ^2=4.83, P<0.05) and TC (χ^2=10.41, P<0.005) beetles have developed resistance to isocarbophos, QT beetles were resistant to carbofuran (χ^2=7.66, P<0.01) (Figure 3). Our results demonstrate that insecticide resistance levels vary between larvae and adults.



In summary, we found both larvae and adults of Colorado potato beetle in some local populations in northern Xinjiang Uygur autonomous region have developed resistance to some pyrethroids, organophosphates, carbamates, and even to neonicotinoids and pyrazoles, and suggested that characteristic differences in resistance risk can exist among different insecticides.

Reducing insecticidal pressure on pest populations is a common strategy to delay evolution of resistance (Alyokhin, 2009; Alyokhin et al., 2008). Selection towards insecticide resistance can be alleviated with non-chemical control methods. Some alternative control strategies such as crop rotation, manipulation of planting time, use of trap crops, plastic-lined trenches along field borders, and destruction of overwintering habitats, as well as classical biological control have been proven to be effective (Alyokhin et al., 2008) and should be practiced in potato culture system in northern Xinjiang Uygur autonomous region.

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Need and scope for insecticide resistance management for the invasive papaya mealy bug *Paracoccus marginatus* Williams and Granara de Willink in small scale papaya farming system in Tamil Nadu, India

Introduction

India leads the world in papaya production with an annual output of about 3 million tones compared to estimated annual world production of 6 million tones of fruits. Other leading producers are Brazil, Mexico, Nigeria, Indonesia, China, Peru, Thailand and Philippines. Papaya is mostly cultivated in the states of Andhra Pradesh, Karnataka, Gujarat, Orissa, West Bengal, Assam, Kerala, Madhya Pradesh and Maharashtra (NHB). Out of 8000 hectares of area in India, papaya is cultivated in about 3000 hectares area Tamil Nadu alone. In Tamil Nadu papaya cultivation has been taken up by progressive small scale farmers with farm holdings of 1-5 acres in and around Salem, Namakkal, Krishnagiri ,Coimbatore, Thiruppur, Erode and Dindigul Districts for papain production facilitated by papain industry providing latex collection centres and cold storage facilities (Regupathy and Ayyasamy, 2010b). Before 2006, plant protection measures are seldom taken though occasional and sporadic incidence of insect pests like fruit borer, fruit flies (*Bactrocera cucurbitae Dov.*), ak grasshopper (*Poekilocerus pictus* F.), aphids (*Aphis gossypii* Glov.), red spider mite (*Tetranychus cinnabarinus* Boisd.), stem borer (*Dasyses rugosellus* Stainton), grey weevil (*Myllocerus viridanus* Fb.), cotton whitefly *Bemesia tabaci* Genn., spiraling whitefly *Aleurodicus dispersus* Russel, and scale *Aspidiotus destructor* Sign. (Regupathy et.al., 2003; NHB; CPTHC, 2004) was reported. Recently the papaya was affected seriously by a new mealy bug *Paracoccus marginatus* Williams and Granara de Willink (Muniappan, 2008, 2009; Anonymous, 2009; Regupathy and Ayyasamy, 2009, 2010a,b ; Suresh et.al., 2010).

Intensity of papaya mealybug incidence

Recent survey taken in 81 farmers fields indicated the seriousness and invasive nature of *P. marginatus*

(Regupathy and Ayyasamy, 2010b). *P. marginatus* incidence was observed in 76 fields. In fields where the infestation was so severe, the entire plant population was covered with bugs. Bugs after debilitating the papaya plants started moving to hedges and adjacent hosts including banana and neem. The fruit set was affected when male inflorescence was severely infested, reducing pollination. When female flowers were infested heavily, the flowers dried up and the fruit drop was observed. In five fields, where the intensity was high, almost all plant parts were covered with bugs, honey dew and sooty mould. The fields in clusters that are contiguous or in close proximity are affected more or less uniformly. The fields that are secluded or isolated have less chance of infestation. The invasive nature of the bug is mainly due to three factors:

1. **Absence of Natural Enemy Complex:** In its native Mexico and /or Central America, it has never gained status as a serious pest, due to the presence of an endemic natural enemy complex. But introduction of this mealy bug in the absence of acclimatized native natural enemy complex enhanced the invasiveness of this papaya mealy bug in the new home, Tamil Nadu.

2. **Green Bridge:** The bug has enormous feeding potential and has more than sixty hosts including crops like bhendi, brinjal, curryleaf, guava, mango, pomegranate, mulberry, silk cotton, star gooseberry, and west Indian cherry, ornamentals, flowering shrubs, foliaged shrubs, flowering trees, select ornamental foliage trees, shade trees, hedge plants, forest trees, voluntary plants and weeds (http://www.bioone.org/doi/pdf/10.1653/00154040(2006)89%5B212:CBCOTP%5D2.0.CO%3B2); Muniappan,2008; Vennila, 2009; Amutha et.al., 2009; IFGT 2009; Regupathy and Ayyasamy, 2009,2010 a,b,; Thangamalar et.al.,2010).The crop mosaics prevailing in Tamil Nadu aids quick spread of this bug.

3**. Favourable Weather Conditions:** Mealybug occurs throughout the year in tropics and active in warm dry weather. Prolonged drought with scanty rainfall and less number of rainy days prevailing in Tamil Nadu, favors faster multiplication.

Need for Developing Insecticide Resistance Management Strategy

It becomes imperative to contain the spread through vigilant quarantine of seeds/root stocks/vegetables/ fruits/ ornamentals/other food materials and its management at Coimbatore so as to prevent invasion and establishment of *P. marginatus* into other districts of Tamil Nadu and the neighboring states within the country. Insecticides effectively check the bugs in the event of outbreak. A number of insecticides having systemic (monocrotophos, methyl demeton, dimethoate, acephate,and methomyl), translaminar (fenthion, imidacloprid, thiamethoxam) and contact (dichlorvos, quinalphos, profenofos., fenitrothion, acephate, carbaryl, chlorpyriphos, diazinon, malathion, FORS(Fish Oil Rosin Soap) and white mineral oils) actions are used (Alison Walker, 2003; Regupathy et.al., 2003). Systemic poisons are very effective on foliage and flowers. Translocation of the poison to the pericarp of the developed fruits is very limited. Contact poisons are more suitable to check bug infestation on fruits. Moreover the bugs present on the hidden surface of the fruit bunches and on leaf axils poses the problem taking the contact poisons to these hidden targets. Hence 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. Reinfestation occurs from the infested hedge, ornamental and weed hosts around the fields and clustered / contiguous farm holdings in small scale farming system prevailing in Tamil Nadu in spite of repeated application of insecticides. Chemical controls are only partially effective and require multiple applications. Most of these organophosphorus and carbamate insecticides except mineral oil and neonicotinoids, imidacloprid and thaimethoxam are with similar mode of action of inhibiting cholinesterase. During the survey taken in farmers holdings, in their anxiety to save the crop the farmers are trying various insecticides like chlorpyriphos, quinalphos, imidacloprid, acepahate, dimethoate in bizarre combinations and at very high doses. The combo may likely to hasten the development for all the chemistries simultaneously. As the effective period of insecticides like profenophos, chlorpyriphos, acepahte and FORS was up to 7- 10 days (Suresh and Kavitha, 2007 ; Dhawan *et al*., 2008) repeated applications are needed to check the population build up. Higher doses and repeated application of mostly chemistries of similar action may accelerate selection pressure. The possibility of mealy bug developing resistance and need for monitoring development of resistance to insecticides had been indicated (Regupathy and Ayyasamy, 2009.).

Scope for Developing Insecticide Resistance Management Strategy

The factors that hasten development of resistance could be due to unchecked multiplication, easy spread, and hidden target necessasitates, high dose and repeated applications. Hence there is a need to develop pest management strategy through rotation of chemistries and use of other means of pest control like botanicals and bioagents in conjunction with phytosanitary measures and mechanical methods as discussed below.

Rotation of chemistries: To reduce the selection pressure and thereby to prevent the resistance development, rotation of chemistries with different modes of action and avoiding repeated application of same chemistries are to be practiced. FORS @ 25 g/ l alone or in combination with insecticides *viz.*, FORS + profenophos (25 g + 2 ml lit 1), acephate + FORS (2 g + 25 g lit 1) and dichlorvos + FORS (1ml + 25 g lit 1) were also found to be effective in reducing the infestation of *P. marginatus* (Suresh et.al. 2010). FORS act as contact and physical poison. The neonicotinoids, imidacloprid and thaimethoxam act on acetyl choline receptors. The novel compound rynaxypyr, the first insecticide from a new class of chemistry, the anthranilic diamides was found to be effective against *P.marginatus* (Unpublished). It activates insect ryanodine receptors (RyRs) which play a critical role in muscle function. Contraction of muscle cells requires a regulated release of calcium from internal stores into the cell cytoplasm (Du Pont). Ryanodine receptors act as selective ion channels, modulating the release of calcium and binds to the RyR, causing uncontrolled release and depletion of internal calcium, preventing further muscle contraction. Insects treated with rynaxypyr exhibit rapid cessation of feeding, lethargy, regurgitation and muscle paralysis ultimately leading to death. Growth regulator buprofezin and other new compounds chlorantraniliprole and spirotetramate with different modes of action were found to be effective against cotton mealybug *Phenococcus solenopsis* Tinsley *(Dhawan et.al, 2009)*. Such new chemistries may be evaluated against *P.marginatus* to give more choice in the rotation of chemistries.

Apart from insecticides, the option of using other methods of pest control wherever possible may be considered to reduce the number insecticide applications.

Nursery treatment: In new plantation the nursery stock should be free from pest infestation. Seedlings obtained from nurseries provided with insect proof netting is preferred. The seedlings are to be inspected for bug infestation and if necessary are to be treated with preferably systemic poisons one or two days prior to planting to prevent carryover of pests from nursery to main field.

Gamma-irradiation for phytosanitary treatment: Sethi et.al (2009) had attempted to use gamma-irradiation as quarantine treatments or systems to eliminate, sterilize, or kill cotton mealybug, *P. solenopsis* to prevent their introduction and establishment into new areas. Radiation susceptibility by exposing different life stages viz., ovisac, third nymphal instar and gravid female to a range of ionizing doses had been assessed. The study had revealed that 40Gy irradiation of 0-1 day ovisacs inhibited male adult formation, and 70Gy was enough to inhibit the transformation of crawlers (first instar nymph) up to third instar nymph in male; whereas it caused more than 90 per cent reduction in female adult formation. Irradiation of ovisacs at 100Gy inhibited female adult formation and transformation of crawlers to next instars was inhibited at 400Gy. The estimated radiation dose range needed to disinfest the agrocommodities from *P. solenopsis* was between 100 and 400Gy. The possibility of extending this study to *P. marginatus* to ensure complete quarantine security could be considered for phytosanitation.

Field sanitation: In high intensity of bug infestation, the fallen leaves and fruits (Fig 1) when not removed served as reservoir of bugs for further spread. The adults moved and stationed on the stem near the ground level. Mealy bug egg masses from under the bark or fallen infested leaf materials, fruits, soil clods hatch, crawlers migrate, settle on new flush and later migrate to top canopy of the plants preferring flowers/fruits. The laterals of drip irrigation served as better path than the clods or soil from one plant to other plant. Collection and safe disposal of fallen leaves and fruits will prevent spread to other plant/fields and reduce the buildup of population to some extent.

Figure 1: Swabbing with mineral oil

Swabbing of stem with mineral oil as followed by farmers (Fig.1) had some effect in preventing the migration of bugs from shed infested leaves to new growth in the upper canopy Addition of contact poisons like quinalphos, chlorpyriphos, profenofos etc. will give synergy. The trapped bugs will be exposed to these contact poisons. Swabbing of infected stem and branches with fish oil rosin soap (FORS) @ 25ml/l will eliminate egg masses and different stages of bugs (Regupathy and Ayyasamy, 2010a). Follow up spray of FORS or insecticides are needed when residual infestation is noticed on foliage in later stages. FORS is byproduct of the fish processing industry. Like plant

oils fish oils are are chemically classified as lipids containing long-chain hydrocarbons (Sams and Deyton 2002). FORS is the product marketed by Kerala government soap industry and many commercial formulations are now available. It is a physical poison effective against whitefly, *Bemesia tabaci* on cotton and mealy bug on grape vine (Regupathy et.al. 2003).

Stem banding: Some of the farmers even attempted to stem banding by tying black polythene paper (Fig. 2) around stem above ground level to prevent the migration from infested material. But the efficacy was limited. The dripping of latex from the incised fruits for papain and gathering of dust reduces the slippery nature of black polythene used as band. Moreover the trapping of moisture caused oozing of fluid and growth of saprophytic and other fungi (Fig.3.). Improvisation of the stem banding with oil coated paper may check the infestation to some extent. Detailed study is needed to optimize the type of sticker material to be used.

Figure 2: Stem banding with black polythene sheet

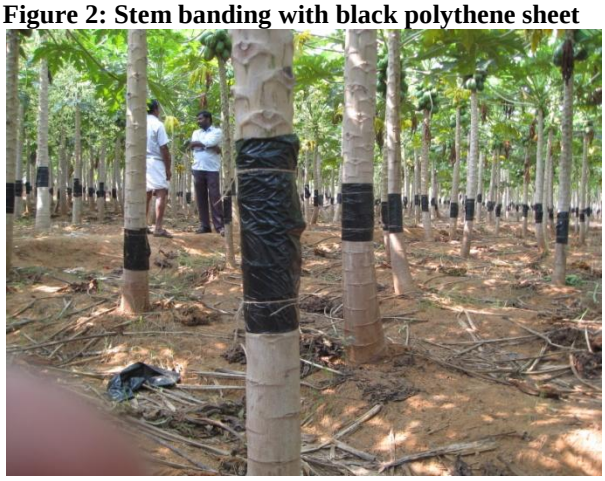

Figure 3: Stem bleeding and fungal growth

Neem: Eco-friendly nature of neem formulations offers greater scope by selectivity favouring honey bees and natural enemies. It has potential as repellent and physical poisons. When compared to insecticides neem oil was less effective against mealybug (Suresh et.al. 2010). However neem oil as spray supplement with insecticides is better option instead as sole method of control of *P. marginatus* as it may serve as sticker apart from repellent and cidal actions.

Biological control: Release of lab cultured commercially available generalist coccinellid predator, *Cryptolaemus montrouzieri Mulsant* had been successfully followed for the management of other species of mealy bugs feeding on grapes, citrus, mango, guava, coffee, rubber, cocoa and mulberry in Tamil Nadu. Naturally-occurring other lady beetles, lacewings, and hover flies, have a potential impact on existing species of mealybug populations. However these predators generally found on other species of mealy bug were seldom found feeding on this new invasive papaya mealy bug (Regupathy and Ayyasamy, 2010b). Notable numbers of lepidopteran (blue butterfly) predatory larvae, *Spalgis epius* (Westwood) were found feeding on *P. marginatus* on other hosts like bread fruit, teak, pomegranate, *Tecoma*, *Thespesia*, hibiscus and nerium, thevetia etc. during cooler months only but not on papaya. The predatory potential of *Spalgis epius* on a pseudococcid complex comprising *Planococcus citri* (Risso), *P. lilacinus* (Cockerell)and *Ferrisia virgata* (Cockerell)in coffee varied from 54.72 to 68.38 per cent (Rahiman, and Vijayalakshmi, 1998) and on *P. marginatus* on mulberry (Thangamalar, 2010) . The mass multiplication *S.epius* is not possible due to non-laying of eggs in captive conditions. The predatory potential of *S.epius* on *P. marginatus* on papaya have to be assessed by collection of pupae and fixing in infested papaya fields or on the preferable host plants on the hedges. 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 as the exotic parasitoids.

Recently parasitoids *Torymus* sp (Torymidae) and *Prochiloneurus aegyptiacus* (Mercet) (Chalcidoidea) had been recorded on *P. marginatus* on cotton with 21 and 7 per cent parasitisation respectively and other alternate hosts of *P. marginatus* like *Parthenium hysterophorus, Abutilon indicum, Phyllanthus niruri, Commelina bengalensis, Convolvulus arvensis* and *C.viscera* (Amutha et.al., 2009). However this needs verification as these are generally reported as hyperparasites on other mealybug parasitoids. Field survey for natural enemy complex of *P. marginatus* on existing flora is to be given top priority as had been done in the case cotton mealy bug *P. solenopsis* (Jhala et al., 2009 and Patel et al., 2009). Research need in

enhancing the impact potential of climatically adapted strains is emphasized (Sithanantham, 2008).

The entomopathogenic fungi *Metarrhizium anisopliae, Verticillium lecanii* and *Paecelomyes pictus* were not very effective against *P. marginatus* even during wet – humid season (Unpublished.). Suresh et.al. (2010) had also reported less effective nature of. *Beauveria bassiana* @ 5 g / l, Consortia @ 10 g/ l, Consortia + *B. bassiana* @ 5 g+5 g /l . Developing host adapted predators and parasitoids are needed for successful biocontrol of *P.marginatus* on papaya. In the absence of effective (host adapted) native natural enemies, the other option is to import natural enemies found in their native home. These local natural enemies (predators and parasitoids) might take some time to shift on to this new pest. The experiences obtained in the classical biological control program initiated as a joint effort between the US Department of Agriculture, Puerto Rico Department of Agriculture, and Ministry of Agriculture in the Dominican Republic in 1999 could be availed. Four genera of encyrtid endoparasitoid wasps specific to mealybugs were collected in Mexico by USDA and ARS researchers and Mexican cooperators as potential biological control agents: *Acerophagus papaya* (Noyes and Schauff), *Anagyrus loecki* (Noyes and Menezes), *Anagyrus californicus* Compere, and *Pseudaphycus* sp. (USDA 1999, 2000; 2002, Meyerdirk and Kauffman 2001). A fifth collected species was later reared and identified as *Pseudleptomastix mexicana* Noyes and Schauff *(*Noyes and Schauff 2003). The introduction of parasitoids *A. loecki*, *P. mexicana* and *Acerophagous papayae* Noyes and Schauff from Puerto Rico and field released in Guam from June to October, 2002 effected reduction of over 99 per cent *P.marginatus* was observed about a year after the introduction of these parasitoids. This has reduced risk of introduction of this mealybug to neighbouring islands in the Pacific Region (Meyerdirk et.al., 2004 and Muniappan et.al., 2006).

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Polymorphism *cox1* gene of Colorado Potato Beetle in South Ural populations

INTRODUCTION

It is known, that population is both unit of evolution, and unit of management of biologic species (Kaidanov, 1996, Yablokov, 1987). Analysis of polymorphism is presented to be basis for population research.

For the last time ground reason of polymorphism in animal populations in total and in insect's populations in particular was analysis performed by protein polymorphism (Levontin, 1991). In last decades due to progress of molecular biology methods possibility arrived to apply the molecular markers for different studies, from phylogeny and systematic to quantitative train loci analysis, intra- and inter-specific polymorphism etc. In this article presented results of our investigations of genetic variability in South Urals populations of Colorado potato beetles using cytochrome oxidase subunit I (*cox*I) mitochondrial DNA (mtDNA) sequences.

MATERIALS AND METHODS

Sample collection. Samples of adults of *Leptinotarsa decemlineata* Say were collected from 5 populations from Bashkortostan republic (South Urals, Russian Federation), 2 populations from Russia (Pskovskaya oblast and Leningradskaya) and 1 population from Ukraine (Kharkov) (Table 1, Fig. 1).

Table 1: Populations of Colorado Potato Beetles sampled including sample sizes and Ass. No in GenBank

Districts of Bashkortostan republic	Populations, No in map	Samples (n)		Ass. No in GenBank
		PCR-RFLP	Sequen-sing	
Tatishlinskiy	Shulganovo, 1	20	3	DQ649094 DQ649095 DQ127908
Ufimskiy	Dmitrievka, 2	20	3	DQ649098 DQ649099 DQ127906
Mijakinskiy	Kirgiz-Mijaki, 3	20	1	DQ011111
Beloreckiy	Mezhgorie, 4	20	3	DQ649100 DQ649101 DQ127907
Fedorovskiy	Dedovo, 5	20	3	DQ649096 DQ649097 DQ127909
Regions out of Bashkortostan				
Pskovskaya	Pskov, 6	10	–	–
Leningradskaya	Belgorod, 7	10	–	–
Ukraine	Kharkov, 8	10	–	–

Figure 1: Map showing the localities sampled in our study (for details about localities see Table 1).

DNA extraction and PCR reaction. Total genomic DNA was extracted from three legs of each beetle using carried out in 1.5 ml tubes by described by Chomczynski et al. (1987) with some modifications. DNA samples stored at -20°C until use.

For a 10 – 20 sample of beetles from each population (Table 1), a 750-bp fragment of mtDNA containing part of the *coxI* gene was amplified using the primers LCO1490 and HCO2198 (Folmer et al., 1994; Hebert et al., 2003).

Enzymatic digestion by restriction enzymes. Digestion of *cox*I gene was carried out restriction enzymes by manufacture protocols (SybEnzime, Russia). Two restriction enzymes were used in the present study: *Hinf*I and *Kzo*9I.

For a 1-3 sample from Bashkortostan populations was sequenced in one direction on an ABI PRISM™ 310 Genetic Analyser, Applied Biosystems using the Big Dye v. 3 sequencing kit. All nucleotide and amino aside sequences have been submitted to GenBank (Udalov et al., 2005, 2006), their accession numbers are provided in table 1. Multiple alignment and phylogenetics tree was carried out using the software package Lasergene (DNASTAR, USA).

RESULTS AND DISCUSSION

We could not find polymorphism neither by the length after PCR (fig. 2), and by PCR-RFLP after digestion two restriction endonukleases – *Hinf*I and *Kzo*9I (fig. 3).

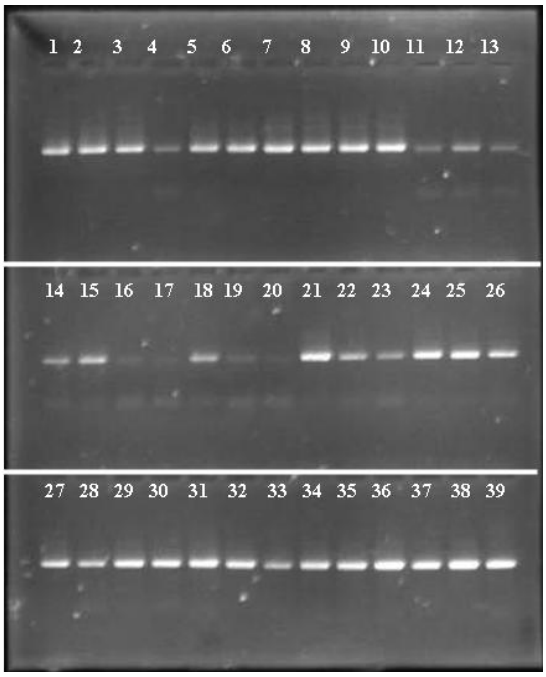

Figure 2: Agarose gel electrophoresis of PCR *cox*I Colorado potato beetles.
Lines 1-10 – sample from Dmitrievskaya local population, 11-20 – Pskovskaya, 21-30 – Kharkovskaya, 31-39 – Leningradskaya.

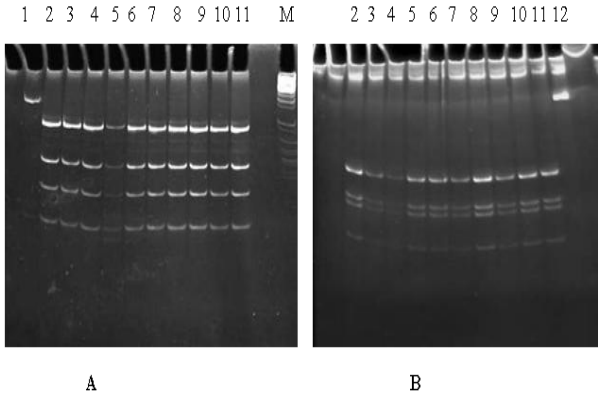

Figure 3: Agarose gel electrophoresis of a restriction enzyme analysis. А – *cox*I/*Hinf*I, Б – *cox*I/*Kzo9*I.
Lines 1, 12 – undigested PCR product, 2 - 3 – samples from Shulganivskaya population, 4- 5 – Dmitrievskaya, 6 - 7 – Mezhgorievskaya, 8 - 9 – Kirgiz-Mijakinskaya, 10 - 11 – Dedovskaya. Lane M – molecular size markers, DNA*ó* λ*Pst*I.

A sequencing of 750-bp fragment of mtDNA containing part of the *cox*I gene from 5 populations from South Urals showed us 11 nucleotide substitutions (Table 2) distinguishing from early published in Gene Bank (Ass. No AY165708; Hebert et al., 2003) which we considered as the "standard" mitotype.

Table 2: Sequence position parts of *coxI* gene from compare of samples from South Ural and sample from GenBank*

Position / Samples	3	25	31	46	91	115	268	295	385	442	463
AV165708	G	T	T	T	A	T	C	T	A	T	T
DQ011111	•	C	•	•	G	•	T	A	G	C	C
DQ127909**	C	C	•	•	G	•	T	A	G	C	C
DQ649096	•	C	•	•	G	•	T	A	G	C	C
DQ649097	•	C	•	•	G	•	T	A	G	C	C
DQ127906	•	C	•	•	G	•	T	A	G	C	C
DQ649098	•	C	•	•	G	•	T	A	G	C	C
DQ649099	•	C	•	•	G	•	T	A	G	C	C
DQ127908	•	C	•	•	G	•	T	A	G	C	C
DQ649094	•	C	•	•	G	•	T	A	G	C	C
DQ649095	•	C	•	•	G	•	T	A	G	C	C
DQ127907	•	C	C	A	G	G	T	A	G	C	C
DQ649100	•	C	•	•	G	•	T	A	G	C	C
DQ649101	•	C	•	•	G	•	T	A	G	C	C

* Nucleotide variation between samples from South Urals (Ass.No DQ011111 - DQ649101) and samples from N. America (Ass.No AY165708, Hebert et al., 2003). Numbers at the top of the columns refer to the part of AY165708 sequence. A dot indicates an identical base compared with sample AY165708. ** 3G>C nucleotide exchange led to amino acid change Gly>Ala.

Almost all of substitutions in the third nucleotide in codon did not influence the amino acid sequence. Only single substitution 3G>C in one of sequences from local population led to the amino acid substitution Gly/Ala. Nucleotide sequences defined by us and the coincident amino acid sequences deposited in the DDBJ/EMBL/GenBank under the accession numbers DQ011111, DQ127906 – DQ127909, DQ649094 – DQ649101.

To estimate the level of divergence past from the start of independent evolution in North American and European populations of Colorado potato beetle we compared nucleotide sequences of gene *cox*I fragment of Colorado potato beetle added coincident fragments of closely related species of Leptinotarsa: *L. haldemani* (USA, Arizona, Acc. No DQ459377), 2 samples of *L. juncta* (USA, Maryland, Acc. No AY532655; West Virginia, Acc.No AY532656) and 1 sample of *Zygogramma piceicollis* (Acc. No AY171413). As the reference sequence we used the coincident gene fragment of *Drosophila melanogaster* (Ass. No U37541).

Phylogenetic tree constructed on the computer-based comparison of these fragments is shown in Fig. 4. Most closely related between each other were the *L. decemlineata* individuals from South Urals, and joint sample from North America. Most solitary was the individual of *Z. piceicollis.*

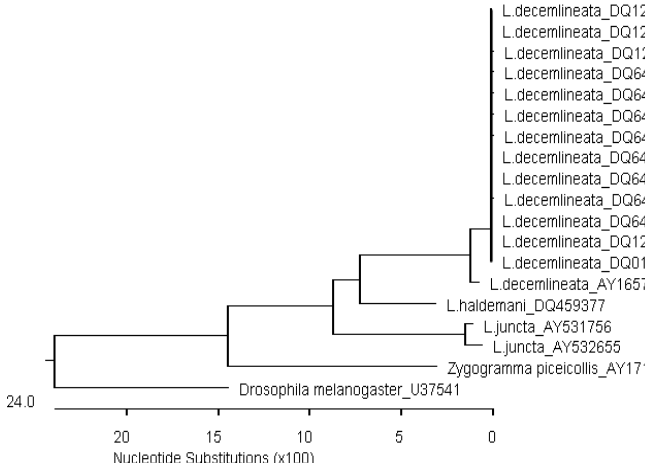

Fig. 4: Phylogenetics three in Genus *Leptinotarsa* and *Zygogramma* by aligments nucleotide sequenses of *cox*I part gene

Genus *Zygogramma* is considering being the ancestral for the genus *Leptinotarsa*, but it is insufficient to make such a conclusion on the base of examination of elytra tracery only (Tower, 1906). Using the additional morphological characteristics allowed considering the both of genus as closely related but developed independently from common ancestor (Ushatinskaya, 1981). Results obtained in our comparison of sequences of gene *cox*I confirmed the hypothesis about the community of *Zygogramma* and *Leptinotarsa* origin.

Dividing into the clusters of south-Urals and North-American individuals at this dendrogram evidences the hypothesis about the intensive sub-speciation processes in all the area of Colorado potato beetle.

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Research in Resistance Management

Insecticide Rotation as a Component of Thrips Resistance Management Programs

Introduction

The most studied and most economically important thrips species are: *Frankliniella occidentalis*, western flower thrips (WFT); *Thrips tabaci*, onion thrips; *Thrips palmi*, melon thrips; and *Scirtothrips dorsalis*, chilli thrips (Morse and Hoddle, 2006). Depending on the crop and thrips species, direct and/or indirect damage are possible. Direct damage commonly involves reduced quality from scarring caused by feeding or oviposition. Indirect damage includes reduced fruit set, crop stand, and crop growth. Several thrips species vector tospoviruses such as Tomato Spotted Wilt Virus and Iris Yellow Spot Virus, which can cause significant losses. Thrips are often pests of high value crops with low consumer tolerance for cosmetic damage. These crops also tend to have high production costs, so the potential for severe economic loss is high.

Chemical control is the most common tactic to manage thrips pests (Morse and Hoddle, 2006; Reitz, 2009). Spinosad (Success®, Entrust®) and spinetoram (Radiant®) are among the few insecticides highly effective against thrips. Given the significant risk of economic loss if thrips are not controlled, growers have a strong desire to use the most effective products. It has been difficult to persuade growers to implement insecticide resistance management tactics, such as employing IPM practices, including biological and cultural control, to minimize the number of insecticide applications and rotating insecticides with different modes of action.

Although few insecticides are highly effective against thrips, many more provide moderate levels of control and should be used in rotations for thrips resistance management. But growers must first be convinced that using less effective insecticides in rotation with highly effective insecticides will achieve acceptable control. The objectives of this study were to determine if certain insecticides are better rotational products for Radiant (spinetoram) based on WFT efficacy and conservation of thrips natural enemies, and determine if the attributes of individual products predict the performance of rotations that include these products.

Materials and Methods

Three similar field trials were conducted in 2009. The effects of Radiant and several other insecticides on populations of WFT and *Orius insidiosus* (minute pirate bug, MPB), a major thrips natural enemy, were assessed individually in a Quincy, FL trial on bell peppers. The effect of spray rotations with Radiant and these other insecticides on WFT populations was assessed in a Fresno, CA trial on onions. The effect of these rotations on MPB populations was assessed in a Plant City, FL trial on bell peppers.

Several insecticides and one tank mix that have shown promising WFT efficacy in trials reported in Arthropod Management Tests were selected for testing. The insecticides tested alone against WFT and MPB in the Quincy efficacy trial are shown in Table 1. The insecticides that were tested in rotation with Radiant in Fresno and Plant City are shown in Table 2. The specific product rotations are shown in Table 3.

Table 1: Insecticide products and application rates tested alone for WFT efficacy and MPB effects (bell pepper trial; Quincy, FL), 2009.

Product	Active ingredient	IRAC group	AI rate
Radiant® 120SC (+Dyne-Amic)	spinetoram	5	53 gai/ha (+0.25% v/v)
Movento 240SC (+Induce)	spirotetramat	23	88 gai/ha (+0.25% v/v)
Assail 30SG	acetamiprid	4A	84 gai/ha
Venom 20SG	dinotefuran	4A	200 gai/ha
Beleaf 50SG	flonicamid	9C	98 gai/ha
Requiem 25%EC	*Chenopodium* extract	unk	1.17 Lai/ha

unk = unknown

Table 2: Insecticide products and application rates tested in rotations for WFT efficacy (onion trial; Fresno, CA), and MPB effects (bell pepper trial; Plant City, FL), 2009.

Product	Active ingredient	IRAC group	AI rate
Radiant® 120SC (+Dyne-Amic)	spinetoram	5	61 gai/ha (+0.25% v/v)
Movento 240SC (+Induce)	spirotetramat	23	88 gai/ha (+0.25% v/v)
Assail 30SG	acetamiprid	4A	84 gai/ha
Venom 20SG	dinotefuran	4A	200 gai/ha
Beleaf 50SG	flonicamid	9C	98 gai/ha
Requiem 25%EC	*Chenopodium* extract	unk	1.75 Lai/ha
Ecozin Plus 12ME + Requiem 25%EC	azadirachtin + *Chenopodium* extract	18B+ unk	5.3 gai/ha+1.17 Lai/ha
Agri-Mek 18EC	abamectin	6	21 gai/ha
M-Pede® 49DSL	potassium salts of fatty acids	NC	0.98% ai v/v

unk = unknown, NC = not classified in an IRAC group.

Radiant 120SC and M-Pede 490SL are products of Dow AgroSciences LLC, Movento 240SC is a product of Bayer Crop Sciences, Assail 300SG is a product of United Phosphorous, Inc, Venom 200SG is a product of Valent USA Corp., Beleaf 500SG is a product of FMC Corp., Requiem 25%EC is a product of AgraQuest, Inc., Ecozin Plus 12ME is a product of Amvac Chemical Corp., Agri-Mek 18EC is a product of Syngenta Crop Protection, Inc.

Table 3: Insectide product rotations tested for WFT efficacy (onion trial; Fresno, CA), and MPB effects (bell pepper trial; Plant City, FL), 2009.

Rotation name	First spray	Second spray	Third spray	Fourth spray
Radiant/Movento	Radiant	Movento	Movento	Radiant
Radiant/Assail	Radiant	Assail	Assail	Radiant
Radiant/Venom	Radiant	Venom	Venom	Radiant
Radiant/Beleaf	Radiant	Beleaf	Beleaf	Radiant
Radiant/Requiem	Radiant	Requiem	Requiem	Radiant
Radiant/Eco+Req	Radiant	Ecozin Plus+ Requiem	Ecozin Plus+ Requiem	Radiant
Radiant/Agri-Mek	Radiant	Agri-Mek	Agri-Mek	Radiant
Radiant/M-Pede	Radiant	M-Pede	M-Pede	Radiant

Experimental design for all trials was a randomized complete block with four replications. Plots were 0.9-2.0 m (1 or 2 rows) wide by 9.1-12.2 m long, with untreated buffer rows on each side. Insecticide applications were made with backpack sprayers delivering a spray volume of 327-561 L/ha. Applications were made at 7 day intervals between 6 and 27 May 2009 in Quincy, 11 May and 1 June 2009 in Fresno, and 20 April and 11 May 2009 in Plant City.

At 2-3 days and 6-7 days after application, 10 pepper flowers or 5 onion plants were collected per plot. *Frankliniella* species were sampled after the first application and MPB were sampled after the first three applications in the Quincy trial. WFT and MPB were sampled after all four applications in the Fresno and Plant City trials. WFT and MPB were later extracted from the plant materials and counted. Analysis of variance was used to analyze insect count data (ARM 7 software, Gylling Data Management, Inc.; Brookings, SD). Means were separated using Fisher's LSD test ($\alpha = 0.05$).

To better understand the effectiveness of the insecticide rotations, basic population dynamics principles were applied to the Fresno trial. The growth of such populations can be described by the equation: $N_t = N_0 \cdot e^{r t}$ (where N_0 = initial population, N_t = population at time t, r = intrinsic growth rate, and t = time). The intrinsic growth rate can be calculated from the equation: $r = ln(\Delta N)/\Delta t$.

Results and Discussion

When tested alone, Radiant caused the greatest reduction in WFT numbers (Table 4), followed by Assail and Beleaf. Venom had the least effect on WFT. Assail and Venom caused the greatest reduction in MPB numbers, Movento had the least effect on MPB.

Table 4: Effect of several insecticides on WFT and MPB populations (bell pepper trial; Quincy, FL), 2009

Product	FT[1] larvae per 10 flowers		WFT adults per 10 flowers		Average FT[3] per 10 flowers	Average MPB[4] per 10 flowers
	2 DAA[2]	6 DAA	2 DAA	6 DAA		
Untreated	25.3 a	34.0 a	19.3 a	33.8 a	56.2	2.42
Radiant	1.5 b	0.8 f	4.0 d	4.0 e	5.2	1.36
Movento	10.0 b	6.5 def	7.8 cd	11.3 de	17.8	2.29
Assail	2.8 b	0.8 f	2.8 d	13.8 cde	10.1	0.32
Venom	6.3 b	16.0 bcd	13.5 abc	32.0 a	33.9	0.68
Beleaf	1.5 b	5.8 def	4.8 d	11.3 de	11.7	1.60
Requiem	10.5 b	20.3 bc	8.8 bcd	12.0 cde	25.8	1.98

Means within columns followed by the same letter are not significantly different, Fisher's LSD ($\alpha = 0.05$)

[1] FT = flower thrips. There was a mixed population of *F. occidentalis, F. bispinosa,* and *F. tritici* and the larvae of these species are not easily separated.

[2] DAA = days after application.

[3] Average number of larvae+adults across both sampling dates after a single application.

[4] Average number of adults+nymphs across 6 sampling dates (two samples after each of three applications).

The Radiant/Agri-Mek and Radiant/Venom rotations caused the greatest reduction in cumulative number of WFT adults (Table 5). Radiant rotated with Assail, Requiem, Requiem+Ecozin Plus, or M-Pede® caused reductions in WFT adult numbers statistically equivalent to the Radiant/Venom rotation. The Radiant/Agri-Mek and Radiant/Assail rotations caused the greatest reduction in cumulative number of WFT larvae (Table 6). Radiant rotated with Movento, Venom, or Beleaf caused reductions in WFT larval numbers statistically equivalent to the Radiant/Assail rotation.

Table 5: Effect of several insecticide rotations on WFT adult numbers (onion trial; Fresno, CA), 2009.

Rotation	Number of WFT adults per plant				Cumulative number of WFT adults
	2DAA1[1]	3DAA2	2DAA3	2DAA4	
Untreated	14.1 a	23.0 a	44.8 a	123.6 a	205.5 a
Radiant/Movento	0.8 b	11.3 b	40.4 abc	16.8 bcd	69.3 bc
Radiant/Assail	1.5 b	7.3 b	31.7 abcd	17.9 bcd	58.4 cd
Radiant/Venom	1.1 b	8.6 b	26.0 cd	11.1 cd	46.8 de
Radiant/Beleaf	0.9 b	13.1 b	43.3 ab	26.2 b	83.5 b
Radiant/Requiem	1.2 b	10.8 b	36.5 abc	15.5 bcd	64.0 cd
Radiant/Eco+Req	1.4 b	13.6 b	30.7 abcd	13.0 cd	58.7 cd
Radiant/Agri-Mek	1.4 b	6.5 b	18.9 d	8.2 d	35.0 e
Radiant/M-Pede	1.4 b	12.2 b	26.6 bcd	22.7 bc	62.9 cd

Means within columns followed by the same letter are not significantly different, Fisher's LSD ($\alpha = 0.05$)
[1] 2DAA1 = 2 days after the first application

Table 6: Effect of several insecticide rotations on WFT larval numbers (onion trial; Fresno, CA), 2009.

Rotation	Number of WFT larvae per plant				Cumulative number of WFT larvae
	2DAA1	3DAA2	2DAA3	2DAA4	
Untreated	72.6 a	118.0 a	127.1 a	305.1 a	624.0 a
Radiant/Movento	9.0 b	16.1 b	106.0 ab	36.3 b	168.0 bcd
Radiant/Assail	16.9 b	7.7 b	50.5 c	45.8 b	120.0 de
Radiant/Venom	11.1 b	12.7 b	43.4 cd	62.0 b	128.0 cd
Radiant/Beleaf	10.2 b	11.5 b	83.5 b	50.3 b	156.0 bcd
Radiant/Requiem	10.1 b	18.2 b	113.2 ab	63.3 b	204.0 b
Radiant/Eco+Req	15.8 b	18.1 b	110.7 ab	38.2 b	184.0 bc
Radiant/Agri-Mek	13.6 b	9.4 b	14.9 d	29.6 b	68.0 e
Radiant/M-Pede	14.0 b	21.6 b	104.5 ab	51.1 b	192.0 b

Means within columns followed by the same letter are not significantly different, Fisher's LSD ($\alpha = 0.05$)

Adult and larval WFT populations in the untreated plots exhibited density independent, exponential growth over the three week trial duration. Intrinsic growth rates for WFT adults (Table 7) and larvae (Table 8) were calculated for three periods: 2 days after the first application (Radiant applied to all treatments) to 3 days after the second application (rotation products first applied), 3 days after the second application to 2 days after the third application (rotation products applied a second time), and 2 days after the third application to 2 days after fourth application (Radiant applied to all treatments a second time). The growth rate for the first period reflects residual effects of Radiant and immediate effects of the rotation product. The growth rate for the second period reflects residual and immediate effects of the rotation product. The growth rate for the third period reflects residual effects of the rotation product and immediate effects of Radiant.

Table 7: Effect of several insecticide rotations on WFT adult intrinsic growth rate (onion trial; Fresno, CA), 2009.

Rotation	Intrinsic growth rate, WFT adults		
	2DAA1 to 3DAA2	3DAA2 to 2DAA3	2DAA3 to 2DAA4
Untreated	0.392	0.095	0.169
Radiant/Movento	0.216	0.182	-0.146
Radiant/Assail	0.075	0.210	-0.095
Radiant/Venom	0.068	0.158	-0.142
Radiant/Beleaf	0.078	0.171	-0.084
Radiant/Requiem	0.160	0.174	-0.143
Radiant/Eco+Req	0.052	0.116	-0.143
Radiant/Agri-Mek	0.010	0.152	-0.139
Radiant/M-Pede	0.053	0.111	-0.026

Table 8: Effect of several insecticide rotations on WFT larval intrinsic growth rate (onion trial; Fresno, CA), 2009.

Rotation	Intrinsic growth rate, WFT larvae		
	2DAA1 to 3DAA2	3DAA2 to 2DAA3	2DAA3 to 2DAA4
Untreated	0.061	0.011	0.146
Radiant/Movento	0.073	0.269	-0.179
Radiant/Assail	-0.098	0.269	-0.016
Radiant/Venom	0.017	0.176	0.059
Radiant/Beleaf	0.015	0.283	-0.084
Radiant/Requiem	0.074	0.261	-0.097
Radiant/Eco+Req	0.017	0.259	-0.177
Radiant/Agri-Mek	-0.046	0.066	0.114
Radiant/M-Pede	0.054	0.225	-0.119

Similar trends were observed for the intrinsic growth rates of adult and larval WFT populations. For adult WFT, all rotation products except Movento and Requiem achieved at least a 4-fold reduction in growth rate during the first period, with Agri-Mek achieving a 40-fold reduction. For larval WFT, all rotation products except Movento, Requiem, and M-Pede achieved at least a 3.5-fold reduction in growth rate, with Agri-Mek and Assail achieving negative growth rates. During the second period, the adult and larval growth rates for all rotation products were higher than the untreated plots. The treated plots may have been more attractive to immigrating adults, and the rotation products may not have adequate residual to control larvae hatching from eggs. Almost all rotations achieved negative adult and larval population growth rates in the third period where the second Radiant application was made.

Most of the insecticide rotation treatments had relatively little impact on MPB population levels (Table 9). There were no clear trends across the four samples taken after each application. The only statistically significant differences between the rotation treatments and the untreated were higher MPB numbers in the Radiant/M-Pede rotation following the third application and fewer MPB in the Radiant/Assail rotation following the fourth application.

Table 9: Effect of several insecticide rotations on MPB numbers (bell pepper trial; Plant City, FL), 2009.

Rotation	MPB adults and nymphs per 10 flowers				Average number of MPB
	7DAA1	7DAA2	7DAA3	7DAA4	
Untreated	1.00 a	4.00 abc	5.75 b	3.25 a	2.88
Radiant/Movento	0.75 a	3.00 bc	5.00 b	2.50 ab	2.44
Radiant/Assail	1.00 a	6.75 a	5.25 b	1.25 b	2.09
Radiant/Venom	0.25 a	5.50 abc	5.75 b	2.25 ab	2.25
Radiant/Beleaf	0.25 a	4.75 abc	7.00 b	2.75 ab	2.66
Radiant/Requiem	0.75 a	6.25 a	5.75 b	2.00 ab	2.63
Radiant/Eco+Req	0.75 a	2.50 c	8.00 b	1.50 ab	2.50
Radiant/Agri-Mek	1.00 a	5.75 abc	7.25 b	2.00 ab	2.81
Radiant/M-Pede	0.25 a	4.50 abc	11.50 a	2.25 ab	3.34

Means within columns followed by the same letter are not significantly different, Fisher's LSD ($\alpha = 0.05$)

To determine if the WFT efficacy and MPB impact of the individual products predict the performance of the rotations, percent reduction of WFT (adults+larvae) and of MPB (adults+nymphs) were calculated for the rotations (Table 10). Reductions in WFT and MPB observed for the five insecticides tested alone were plotted against the reductions in WFT and MPB observed for the five rotations that included these products (Figure 1). There was no significant relationship between WFT efficacy of the products alone and WFT efficacy of the products in rotation with Radiant, all rotations achieved a 68 to 79% reduction in WFT. There was a positive relationship between MPB impact of the products alone and MPB impact of the products in rotation with Radiant. However, the rotations had much less effect on MPB, causing a maximum of 22 to 27% reduction, much less than the 72 to 87% reduction caused by some products individually.

Table 10: Summary of efficacy and rotation trial results, 2009.

Rotation	In rotation with Radiant			Rotation partner alone	
	Cumulative number of WFT[1]	Percent reduction in WFT	Percent reduction in MPB[2]	Percent control of WFT[3]	Percent reduction in MPB[3]
Untreated	828.3 a	0.0%	0.0%	0.0%	0.0%
Radiant/Movento	236.6 bcd	71.5%	15.2%	66.9%	5.2%
Radiant/Assail	179.3 cd	78.3%	27.2%	83.0%	86.7%
Radiant/Venom	176.0 d	78.7%	21.7%	42.4%	71.9%
Radiant/Beleaf	239.0 bc	71.0%	7.6%	80.3%	33.9%
Radiant/Requiem	268.5 b	67.6%	8.7%	54.5%	17.9%
Radiant/Eco+Req	241.2 bc	71.0%	13.0%		
Radiant/Agri-Mek	102.4 e	87.4%	2.2%		
Radiant/M-Pede	254.0 b	69.6%	-16.3%[4]		

Means within columns followed by the same letter are not significantly different, Fisher's LSD ($\alpha = 0.05$)

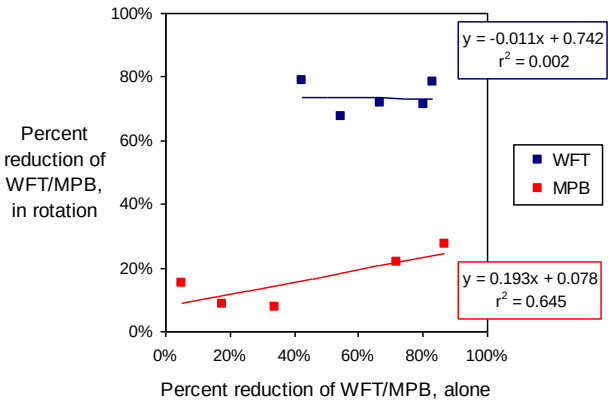
[1] WFT adults+larvae

[2] Based on data in Table 9.

[3] Based on data in Table 1.

[4] Negative value indicates an increase in MPB relative to the untreated.

Figure 1: Relationship between WFT efficacy/MPB activity of rotation partners alone and WFT control/MPB impact of rotations.



Conclusion

Although WFT efficacy of rotation products individually varied from moderate to high, WFT control was similar across the rotations tested. Radiant (spinetoram) rotated with Agri-Mek (abamectin) caused the greatest reduction in WFT numbers and had the greatest negative effect on intrinsic growth rate of WFT larvae. Rotations of Radiant with Assail (acetamiprid) or Venom (dinotefuran) were the next best options tested. Although effects of rotation products individually on MPB varied from low to high, the impact on MPB populations was similar across the rotations tested. Rotating insecticides highly active against thrips with moderately active insecticides, can achieve good levels of thrips control, and mitigate possible natural enemy effects.

Acknowledgments

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Duration of *Musca domestica* L. defensive reactions under the action of bitoxibacillin and N-acetyl-D-glucosamine

ABSTRACT
In this research the activity level of enzymes of phenoloxidase and antioxidant systems during the ontogenesis of *M. domestica* L. has been defined under the action of bitoxibacillin (BTB) and N-acetyl-D-glucosamine (NAGA). Similarity of the humoral reactions of *M. domestica* L. larvae to the action of BTB and NAGA, reproduced on the subsequent ontogenesis stages is revealed. The obtained data allows speaking about formation in a typhoid fly of the long-term immune answer to NAGA action, raising the general resistance of insects.

INTRODUCTION

Chitinous connections render the expressed biological activity concerning plant and animal organisms (Ravi Kumar, 2000; Pospieszny H., 2008). Perseptivity of use of chitinous derivatives in agriculture causes a great interest to studying of their influence on the systems providing resistance of economic significant insects to various factors of environment. Earlier we have shown that chitin monomer N-acetyl-D-glucosamine (NAGA) causes in *Musca domestica* L. and *Leptinotarsa decemlineata* Say. protective reactions of the phenoloxidase system, characteristic for anti-infectious answer at the bitoxibacillin (BTB) action (Gayfullina et al., 2007). The aim of present work is definition of duration of the protective reaction of *M. domestica* L. phenoloxidase and antioxidant systems under the action of NAGA and BTB.

MATERIALS AND METHODS

Insects. M. domestica L. larvae, pupas and imago were used as objects of research. Larvae and pupas were supported in the substratum of bran dissolved with water in glasses. Imago was contained in corfs 20x20x20 sm and fed with dried milk. In experiment three-daily larvae was placed on the substratum from the bran dissolved with 0.1 % water solution of NAGA or 0.01 % BTB – a biopreparation based on Gram-positive bacterium *Bacillus thuringiensis var. thuringiensis.* Activity of enzymes of phenoloxidase and antioxidant systems, and also phenoloxidase electrophoregrams was defined in haemolymph of six-daily larvae and three-daily imago, and also in internal contents of one-daily pupas in experience and control variants.

Enzyme activity in haemolymph. Phenoloxidase activity was determined in trys-HCl buffer (pH 7.5). Diphenoloxidase activity with respect to substratum L-digydroxiphenilalanin was measured on a spectrophotometer at 475 nm by optical density change during 5 min. Monophenoloxidase activity was evaluated by the velocity of L-tyrosine oxidation on a spectrophotometer at 475 nm by optical density change during 30 min. Peroxidase activity was determined at incubation in acetate buffer (pH 5.6) by velocity of benzidine oxidation on spectrophotometer at 540 nm by optical density change during 1 min. Catalase activity was determined by manganometric method. Activity of these enzymes was evaluated in protein concentration, which was measured by Bradford method (Skopes, 1985). Values of activity were normalized by control. Electrophoresis of the insect's protein preparation was carried out in 7.5% polyacrylamide gel with using vertical gel plates 115x115x1mm under the current strength of 30mA. On electrophoresis termination this gel was being coloured in incubative medium contained 0.4% L-digydroxiphenilalanin and 0.15% paraphenilendiamine under 37 ºC during 30 min (Raushenbach, 1997).

Additional BTB in the medium of the three-daily larvae has caused standard for the anti-infectious answer reaction of the protective enzyme systems of haemolymph – substantial increase of the activity level of diphenoloxidase, catalase and peroxidase, carrying out the nonspecific answer of insects humoral protection (fig. 1). Diphenoloxidase functions at development of infectious process are basically connected with participation in regulation of titer of biogenic amines thrown out in a haemolymph and being one of the central links of stress reaction of insects (Wright, 1987; Raushenbach, 1997). Increase of the catalase and peroxidase activity provides in this case neutralization of hydrogen peroxide, formed in considerable quantities in the oxidizing processes accompanying the anti-infectious answer. The fact is interesting that hyperactivity of phenoloxidase and antioxidizing enzymes was registered further at the stages of a pupa and imago. It is necessary to notice that the role of monophenoloxidase – the element of specific protection participating in processes of agglutination and recognition, internalization of insect cells and pathogens (Marmaras et al here starts to increase., 1996; Housseaw et al., 2001) - here starts to increase.

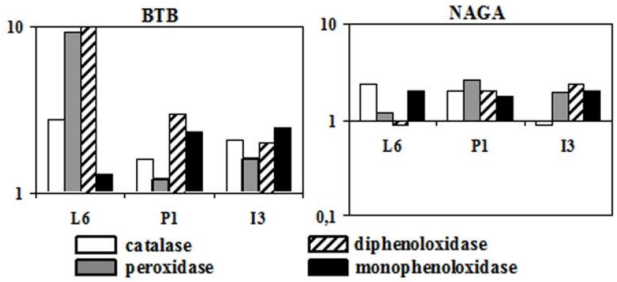

Fig. 1: Change of activity level of enzymes of phenoloxidase and antioxidant systems during ontogenesis of *M. domestica* L. under the action of

BTB and NAGA on larvae. L6 – six-daily larvae, P1 – one-day pupa, I3 – tree-daily imago. The data normalized under the control.

NAGA action on three-daily larvae also has caused authentic increase of activity level of penoloxidases and antioxidizing enzymes in the elder larvae**,** pupas and imago. However in this case sharp increases in enzymes activity were not observed that has been certainly connected with absence of pathogens and pathological process in an organism of insects. The increase of activity level of biochemical protective systems in ontogenesis of typhoid fly caused by NAGA action correlated with increase of viability of individuals after thermal stress (fig. 2). Besides, NAGA raised the metabolism level of insects as a whole, a physiological indicator of that was reduction of terms of larvae and pupa development and increase of pupa's weight.

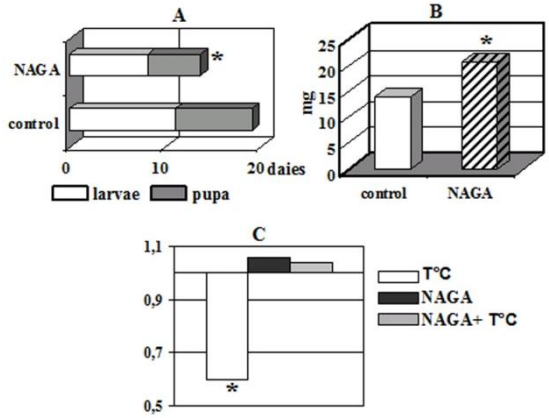

Fig. 2: Influence NAGA on the vital indicators of *M. domestica* L.. * - authentic difference from the control (P> 0.95). A – terms of development of larvae and pupa, B– weight of pupa, C – quantity of viable imago, the data normalized under the control.

BTB and NAGA action on the larvae induced a number of similar phenoloxidase isoforms, also stably reproduced on the subsequent stages of ontogenesis (fig. 3). Molecular forms with Rf 0.3 and Rf>1 attract the greatest attention. These phenoloxidase isoforms are specific to strictly certain stages of a typhoid fly development. The isoform with Rf 0.3 is characteristic for the pupal development stage, and the isoform with Rf>1 – for the moments of transition from the one ontogenesis stage to the next: at pupation, in recently pupated pupas and just flown out imago that confirms the assumption about similarity of mechanisms of morphogenetic and immune processes in insects (Natori et al., 1999). We suppose that the mechanism of formation of phenoloxidase new molecular forms at BTB and NAGA action consists in the change of glycosidation degree of phenoloxidases, not influencing on the substrate specificity of enzyme forms, but promoting the best performance of their functions at development of insects' protective reaction on infection. Thus, on the basis of the data received by us it is possible to assume that phenoloxidase system participates in formation of the long-term immune answer by selection of necessary glycosidated molecular forms of enzyme after contact with a pathogen at previous stages of ontogenesis.

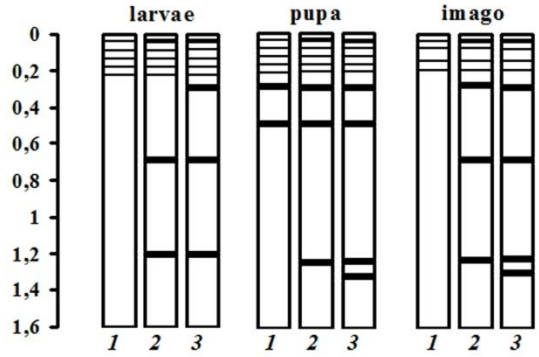

Fig. 3: Electrophoregrams of phenoloxidase in ontogenesis of *M. domestica* L. under the action of BTB and NAGA. 1 – control, 2 – BTB action, 3 – NAGA action.

CONCLUSION

So, obtained data shows again similarity of reactions of separate components of insect's humoral protection on the action of pathogen and NAGA. The induction of additional molecular forms of phenoloxidase and activity of catalase, peroxidase and diphenoloxidase, correlating with viability increase, can serve as an indicator of NAGA preadaptive action. We suppose that NAGA acts in a role of the signal molecule inducing the immune answer in pathogen absence and raising the general resistance of insects. Taking into consideration relatively short period of *M. domestica* L. life, reproduction of the immune answer induced in larvae under BTB and NAGA action at stages of a pupa and imago allows speaking about formation in insects of the long-term immune answer.

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MONITORING OF ACARICIDE RESISTANCE IN FIELD COLLECTED POPULATIONS OF *TETRANYCHUS URTICAE* KOCH (ACARI: TETRANYCHIDAE) ON OKRA

ABSTRACT

The field population of *T. urticae* collected from okra was maintained separately on the potted bhendi plants (Mahyco 10 hybrid) at least for 10 generations without exposing to any acaricides and were bioassayed with dicofol, fenazaquin and propargite from F_1 – Fn generations until the LC_{50} values were stabilized, so as to obtain a susceptible population. This susceptible population was compared with the populations from okra fields of different location *viz.*, Thondamuthur and Nachipalayam villages of Coimbatore district and Nasinoor village of Erode district for finding out the level of resistance development. The bioassays conducted with different generations of mites revealed that the LC_{50} value was concordant at F_9 and F_{10} generations for dicofol (1.48 and 1.43 ppm respectively), F_8 and F_9 generations for fenazaquin and propargite. Field populations from Thondamuthur, Nachipalayam and Nasinoor recorded the LC_{50} values of 7.75, 8.53, 6.80 ppm for dicofol, 9.65, 9.51 and 8.75 ppm for fenazaquin and, 36.02, 33.05 and 30.58 ppm for propargite. These populations recorded the resistant ratio (RR) of 5.42, 5.96 and 4.76 against dicofol, 1.54, 1.52 and 1.40 against fenazaquin and 1.62, 1.48 and 1.37 against propargite for the locations of Thondamuthur, Nachipalayam and Nasinoor respectively as against the laboratory reared susceptible strain. Higher resistance levels were detected for dicofol in all places which may be due to the frequent usage of this chemical against okra mite.

Keywords: Acaricide resistance, *Tetranychus urticae*, Resistance ratio

INTRODUCTION

The two spotted spider mite (TSSM), *Tetranychus urticae* Koch (Acari: Tetranychidae), is an extremely polyphagous herbivore, feeding on a wide range of host plant species including vegetables, throughout the world (Navajas, 1998). Damage due to *T. urticae* includes reduction in crop yield as well as aesthetic injuries, because of the webbings produced by the mites. Among the vegetables, okra is severely affected by *T. urticae* resulting in heavy yield loss. Continued and repeated usage of synthetic acaricides for the control of *T. urticae* resulted in the development of resistance against commercially available chemicals. Resistance to almost all the available groups of acaricides to *T. urticae* is known from different parts of the world (Devine *et al.*, 2001). This wide spread acaricide resistance has been a major obstacle in the cost effective integrated mite management programme (Cho *et al*., 1995). Therefore, focus should be oriented towards the establishment of baseline toxicity data for the frequently used acaricides and continued resistance monitoring is necessary to obtain adequate documentation of changes in population responses to old as well as new molecules. In India, very little work has been done on the acaricide resistance to *T. urticae* on okra. Hence, keeping this in view, the present study was undertaken to establish baseline toxicity data against the acaricides *viz*., dicofol, fenazaquin and propargite to *T. urticae* on okra.

MATERIALS AND METHODS

Investigations were carried out to fix the baseline toxicity data for the red spider mite, *T. urticae* against dicofol, fenazaquin and propargite. Monitoring of resistance in red spider mite (TSSM) for the above acaricides was also carried out on okra at Coimbatore and Erode districts of Tamil Nadu, India. The materials and methodologies adopted for the studies are described below.

Mass culturing of *T. urticae*

The two spotted spider mite (TSSM) required for the generation of baseline toxicity data were mass cultured in the Acarology glasshouse at Insectary, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore using potted plants of okra (Mahyco 10 hybrid) at 28 ± 1°C with 80 ± 5 % relative humidity. The population of mites collected

from okra fields of Nachipalayam village, Coimbatore district was maintained separately on the potted bhendi plants at the glass house at least for 10 generations without exposing to any pesticides. Utmost care was taken to maintain the population separately for carrying out the bioassay studies.

Acute Toxicity Studies

Leaf disc bioassay

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

Fresh okra leaf discs of 45 mm diameter, unexposed to acaricides were used for the study. The required acaricide concentrations of the formulated products *viz*., dicofol 18.5 EC, fenazaquin 10 EC and propargite 57 EC were prepared using distilled water. The leaf discs were dipped in the respective acaricide concentrations for five seconds and then shade dried on a filter paper in open air. Then the treated leaf discs were placed on the moistened cotton mat kept in a petridish (50 mm diameter). Adult females (30 nos.) from respective generations 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 for each concentration. Mortality of the adult 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) and the concentration mortality responses of various experiments were subjected to probit analysis (Finney, 1962) using a statistical package for Social Sciences (SPSS), Ver. 10.00 SPSS Inc., USA. Susceptibility index and the rate of resistance decline were also worked based on the LC_{50} and LC_{95} values of the F_1 and F_{10} generations for dicofol, fenazaquin and propargite.

Monitoring of acaricide resistance in field population

Field level monitoring of dicofol, fenazaquin and propargite resistance in *T. urticae* were assessed for the populations collected from Thondamuthur and Nachipalayam villages of Coimbatore district and Nasinoor village of Erode district as per the IRAC method IV in comparison with the susceptible population. The resistant ratio was worked out to monitor the level of resistance development.

RESULTS

Acute toxicity of acaricides to *T. urticae*

The LC_{50} and LC_{95} values for ten generations of *T. urticae* to dicofol, fenazaquin and propargite are presented in Table 1, 2 and 3. The LC_{50} of dicofol assessed for F_1 population was 6.66 ppm and LC_{95} value was 82.71 ppm (Table 1). The susceptibility of the population moderately increased from F_7 generation with an LC_{50} value of 2.20 ppm followed by F_8 to F_{10} generations. The LC_{95} value was also found to decrease form 82.71 ppm (F_1) to 10.43 ppm (F_{10}) (Table 1). Similarly the susceptibility of *T. urticae* to fenazaquin increased from F_8 generation with a LC_{50} value of 6.32 ppm and F_7 generation for propargite with a LC_{50} value of 22.45 ppm (Table 2 and 3).

Table 1: Acute toxicity of dicofol to *Tetranychus urticae*

Generations	χ^2*	Regression equation	LC_{50} (ppm)	95 % fiducial limit		LC_{95} (ppm)	95 % fiducial limit	
				LL	UL		LL	UL
F_1	3.511	Y = 3.760 + 1.503 x	6.66	4.59	9.66	82.71	27.68	247.19
F_2	3.175	Y = 3.781 + 1.573 x	5.94	4.15	8.50	65.90	24.65	176.17
F_3	1358	Y = 3.777 + 1.936 x	4.27	3.10	5.88	57.05	22.10	154.72
F_4	1.336	Y = 3.917 + 1.551 x	4.98	3.44	7.21	40.23	15.87	148.14
F_5	3.434	Y = 3.983 + 1.806 x	3.65	2.56	5.19	32.21	15.15	70.58
F_6	1.885	Y = 4.056 + 1.702 x	3.58	2.47	5.18	29.71	14.83	59.51
F_7	2.656	Y = 4.601 + 1.163 x	2.20	1.15	4.19	33.12	15.54	57.60
F_8	1.132	Y = 4.603 + 2.003 x	1.57	0.99	2.48	10.63	5.93	19.07
F_9	0.855	Y = 4.670 + 1.928 x	1.48	0.90	2.41	10.57	5.97	18.70
F_{10}	0.747	Y = 4.701 + 1.892 x	1.43	0.86	2.38	10.43	6.04	18.00

*All lines are significantly a good fit (P<0.05)

Table 2: Acute toxicity of fenazaquin to Tetranychus urticae

Generations	χ^2*	Regression equation	LC_{50} (ppm)	95 % fiducial limit		LC_{95} (ppm)	95 % fiducial limit	
				LL	UL		LL	UL
F_1	0.144	Y = -0.906 + 6.093 x	9.31	8.29	10.46	22.30	11.01	55.03
F_2	0.375	Y = 0.002 + 5.162 x	9.29	8.11	10.64	19.48	10.81	53.36
F_3	0.293	Y = -0.089 + 5.26 x	9.27	8.12	10.58	19.13	10.38	37.62
F_4	0.282	Y = -2.201+7.455 x	9.24	8.42	10.14	17.34	10.30	27.80
F_5	0.164	Y = 1.149 + 4.075 x	8.80	7.58	10.22	16.80	9.90	28.52
F_6	0.413	Y = 0.650 + 4.67 x	8.51	7.51	9.64	16.34	9.73	26.32
F_7	0.240	Y = 0.379 + 5.113 x	8.01	7.13	8.99	16.34	9.04	25.33
F_8	0.225	Y = 1.428 + 4.459 x	6.32	4.88	8.17	15.36	8.71	25.07
F_9	0.221	Y = 1.4339 + 4.446 x	6.31	4.88	8.18	15.34	8.66	24.91
F_{10}	0.305	Y = 1.618 + 4.233 x	6.27	4.76	8.27	15.28	8.44	24.79

*All lines are significantly a good fit (P<0.05)

Table 3: Acute toxicity of propargite to *Tetranychus urticae*

Generations	χ^{2*}	Regression equation	LC_{50} (ppm)	95 % fiducial limit		LC_{95} (ppm)	95 % fid
				LL	UL		LL
F_1	0.187	Y = -1.358 + 4.260 x	32.26	28.18	36.94	85.59	41.91
F_2	0.201	Y = -2.343 + 4.867 x	31.07	26.80	36.01	84.20	43.92
F_3	0.367	Y = -0.504 + 3.773 x	27.17	24.68	34.43	77.39	40.34
F_4	0.437	Y = -0.242 + 3.579 x	29.15	24.56	33.67	78.48	43.65
F_5	0.448	Y = 0.264 + 3.301 x	28.76	22.75	32.46	75.59	45.26
F_6	0.569	Y = 0.346 + 3.334 x	24.85	20.66	29.90	70.25	38.06
F_7	0.588	Y = 0.126 + 3.762 x	22.45	18.22	27.67	70.25	45.80
F_8	2.563	Y = 0.512 + 3.320 x	22.35	18.20	27.54	69.06	37.98
F_9	2.665	Y = 0.360 + 3.440 x	22.30	18.19	27.34	67.06	36.98
F_{10}	2.660	Y = 0.212 + 3.557 x	22.28	18.18	27.15	64.72	35.74

*All lines are significantly a good fit (P<0.05)

The susceptibility index (SI) of F_{10} generation over F_1 was 4.66, 1.48 and 1.45 based on LC_{50} and 7.93, 1.46 and 1.15 based on LC_{95} respectively, for dicofol, fenazaquin and propargite (Table 4). The rate of resistance decline (R) was found to be negative indicating that susceptibility increased with the subsequent generations. The R value was -0.0668, -0.0172 and -0.0161 and hence, the number of generations required for a 10 – fold decrease in LC_{50} was calculated as 14.92, 58.14 and 61.21 respectively, for dicofol, fenazaquin and propargite (Table 4).

Table 4: Susceptibility index (SI) and rate of resistance decline of *T. urticae* to dicofol, propargite and fenazaquin

Sl.No.	Acaricides	Susceptibility Index		Rate of resistance decline	
		LC_{50}/ LC_{50}	LC_{95}/ LC_{95}	R	G
1.	Dicofol	4.66	7.93	-0.0668	14.92
2.	Fenazaquin	1.48	1.46	-0.0172	58.14
3.	Propargite	1.45	1.15	-0.0161	61.21

Monitoring of acaricide resistance in field population

Field level monitoring of dicofol, fenazaquin and propargite resistance in *T. urticae* were assessed for the populations collected from Thondamuthur and Nachipalayam villages of Coimbatore district and Nasinoor village of Erode district in comparison with the susceptible population. Populations from Nachipalayam area recorded highest LC_{50} values of 8.53, 9.65 and 36.02 ppm respectively, for dicofol, fenazaquin and propargite (Table 5a, 5b and 5c). The resistant ratio (RR) indicated that the level of resistance development is higher for dicofol with a RR value of 5.42, 5.96 and 4.76 respectively, for the populations from Thondamuthur, Nachipalayam villages of Coimbatore and Nasinoor village of Erode districts (Table 6).

Table 5a: Acute toxicity of dicofol to *Tetranychus urticae*

Locations	χ^{2*}	Regression equation	LC_{50} (ppm)	95 % fiducial limit	
				LL	UL
Thondamuthur	1.23	Y = 3.67 + 1.49 x	7.75	5.27	11.38
Nachipalayam	2.46	Y = 3.54 + 1.55 x	8.53	5.90	12.33
Nasinoor	2.12	Y = 3.58 + 1.69 x	6.80	4.88	9.61

*All lines are significantly a good fit (P<0.05)

Table 5b: Acute toxicity of fenazaquin to *Tetranychus urticae*

Locations	χ^{2*}	Regression equation	LC_{50} (ppm)	95 % fiducial limit	
				LL	UL
Thondamuthur	0.41	Y = -2.25 + 7.41 x	9.51	8.58	10.53
Nachipalayam	0.61	Y = -3.63 + 8.76 x	9.65	8.76	10.63
Nasinoor	0.95	Y = -5.31 + 10.98 x	8.75	8.27	9.26

*All lines are significantly a good fit (P<0.05)

Table 5c: Acute toxicity of propargite to *Tetranychus urticae*

Locations	χ^{2*}	Regression equation	LC_{50} (ppm)	95 % fiducial limit	
				LL	UL
Thondamuthur	0.081	Y = -1.87+4.519 x	33.05	28.44	38.42
Nachipalayam	0.425	Y = -1.177+3.966 x	36.02	29.62	43.80
Nasinoor	1.083	Y = -3.352+5.636 x	30.58	27.36	34.19

*All lines are significantly a good fit (P<0.05)

Table 6: Resistance level of *T.urticae* against dicofol, propargite and fenazaquin

Sl.No.	Location	Resistance Ratio at LC_{50}		
		Dicofol	Fenazaquin	Propargite
1.	Thondamuthur	5.42	1.52	1.48
2.	Nachipalayam	5.96	1.54	1.62
3.	Nasinoor	4.76	1.40	1.37

DISCUSSION

Present investigation reveals the first report on the susceptibility index and baseline toxicity of dicofol, fenazaquin and propargite to *T. urticae* on okra.

The baseline toxicity data established can be used for further monitoring of resistance in *T. urticae* populations from different geographical locations of Tamil Nadu against dicofol, fenazaquin and propargite. Populations of *T. urticae* often resist organophosphates, carbamates, dicofol and tetradifon (Croft and Van de Baan, 1988) and resistance to other acaricides, such as fenbutatin oxide and abamectin is also widely reported (Beers *et al*., 1998). Cross resistance between compounds has also been noted and is often suggested to be the result of common metabolic detoxification-based mechanisms (Fergussion *et al*., 1991). Development of bifenthrin resistance was also reported by Herron *et al.* (2001)

and fenpyroximate by Naun *et al*. (2001) owing to its high reproductive potential, Shorter life cycle, Numerous generations and exposure to more insecticide applications in a single season. In India, there are no published literatures on the establishment of base line toxicity data for dicofol, fenazaquin and propargite to *T. urticae* and hence, the results obtained in the present investigations would be useful for further studies.

Monitoring of acaricide resistance in field population

The resistance level in *T. urticae* to dicofol, fenazaquin and propargite assessed on okra was reported based on one time survey. Indiscriminate usage of dicofol might have created higher selection pressure and hence, the resistance ratio was higher compared to fenazaquin and propargite. However, the present investigation has also disclosed the possibility of development of initial resistance in *T. urticae* to fenazaquin and propargite which needs to be confirmed by further studies. Karthik *et al*. (2009) documented the first report of resistance development in *T. urticae* to wettable sulphur from India. However, present investigation is the first documented report of resistance monitoring in *T. urticae* against dicofol, fenazaquin and propargite in India.

To facilitate effective resistance management, baseline susceptibility data are generated and resistance monitoring continues over an ongoing basis in *T.urticae* on okra. Further investigations on the mechanisms conferring acaricide resistance may pave way for the development of resistance management strategies. Also, if it were possible to predict likely mechanisms to new pesticides before they evolve in the field; it might be feasible to have programmes that manage susceptibility (Mckenzie and Batterham, 1998).

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RELATIVE TOXICITY OF THREE SYNTHETIC PYRETHROIDS AND ENDOSULFAN TO CABBAGE BUTTERFLY, *PIERIS BRASSICAE* LINN.

ABSTRACT

Relative toxicity of three synthetic pyrethroids and endosulfan was evaluated against 1 to 2 days and 8 to 9 days old larvae of *Pieris brassicae* using dry film technique. The order of toxicity to 1-2 day old larvae with LC_{50} values in parentheses was: delthamethrin (0.0000239) > cypermethrin (0.0000329) > fenvalerate (0.00002578) > endosulfan (0.0007329). The former three insecticides were respectively, 29.31, 21.93 and 2.79 times more toxic than endosulfan. The order of toxicity against 8-9 days old larvae was: deltamethrin > cypermethrin > fenvalerate > endosulfan. The synthetic pyrethroids were 22.99, 21.45 and 3.49 times more toxic than endosulfan respectively. The LC_{50} values of deltamethrin, cypermethrin, fanvalerate and endosulfan against 8-9 days old larvae were 0.00001157, 0.00001240, 0.00007566 and 0.00026763 per cent , respectively. Among synthetic pyrethroids, deltamethrin and cypermethrin were found superior to others regarding protection against larvae of *P. brassicae*. The studies indicated that the population of *P. brassicae* to the insecticides tested was quite susceptible.

INTRODUCTION

Cabbage (*Brassicae oleracea* var. *capitata*) is widely grown both in hills as well in plains (Ahmad *et al.,*2007). High incidence of cabbage butterfly, *Pieris brassicae* linn. is one of the limiting constrains (Rai *et al.,*1985, Mishra and Ram, 1997). Several insecticides have been tested for the effective management of this pest, but synthetic pyrethroids are the most potent insecticides against a wide variety of crop pests (Elliot *et al.,* 1978). So, there is a need to determine the relative toxicity of three synthetic pyrethroids *viz.*, cypermethrin, deltamethrin and fanvalerate along with endosulfan by bioassay method using *P. brassicae* larvae.

MATERIAL AND METHODS

The stock solutions of known strength of the insecticides were prepared in acetone from the technical grades for the determination of LC_{50} values. The desired insecticidal concentration were applied in the form of a dry film, deposited on the inner surface of the tube (20 x 25 cm dia) by following Gupta and Rawlin's (1966) technique. Thin and uniform film of insecticide was prepared by taking one ml of insecticide solution in a test tube and rotated with the help of a hand rotating machine till dryness.

Toxicity of insecticidal film was determined against the laboratory reared 1-2 day and 8-9 day old larvae of *Pieris brassicae* reared on natural diet (cabbage leaves collected from the untreated field). Then, 1 to 2 old such larvae were released into each tube which served as one replicate. Three replications of each insecticidal concentration were maintained. Simultaneously, a control set with dry film of acetone only was also run. Simultaneously, an another set of experiment was carried out for LC_{50} values where 8-9 days old larvae were used as test insect. In this case one larva in each tube was kept and such ten tubes constituted one replication. The tubes were then kept in an incubator maintained at $28^0 \pm 2^0$C. After 24 hr, mortality counts were made and moribund larvae were counted as dead. The mortality in control if any was corrected by using Abbot's formula (1925). The dosages mortality data so obtained were subjected to Probit analysis (Finney, 1971) to find out LC_{50} values of a particular insecticide as unity.

RESULT AND DISCUSSION

On the basis of LC_{50} values, it is evident from Table 1 that deltamethrin was the most toxic insecticides and endosulfan the least to *P. brassicae*. The order of toxicity with respect to LC_{50} values is as deltamethrin > cypermethrin > fenvalerate > endosulfan. By comparing the LC_{50} values, it indicates that all the synthetic pyrethroids tested were more toxic than endosulfan. Vaissayre and Renou (1978) reported LC_{50} value ranging from 5.44 to 6.19 mg/larvae for three synthetic pyrethroids (deltamethrin, fenvalerate and cypermethrin) as compared to 28.68 mg/larvae for DDT against the larvae of *H. armigera* as determined by topical application method. Baruah and Chauhan (1996) reported the order of toxicity against *H. armigera* as deltamethrin> cypermethrin> fenvalerate>endosulfan.

Table 1: Relative toxicity of some insecticide to 1-2 day old larvae of *P. brassicae*

Insecticide	Heterogeneity* χ^2 (3)	Regression equation (Y)	LC_{50}(%)	Fiducial limits LC_{50} ±	Relative toxicity
Cypermethrin	0.330	0.32986+3.49835x	0.00000329	0.000000108	21.93
Deltamethrin	0.634	1.67266+2.39919x	0.00000239	0.000000116	29.31
Fenvalerate	1.485	1.65573+2.36826x	0.00002578	0.000001190	2.79
Endosulfan	1.157	0.45555+2.43617x	0.00007329	0.000001007	1.00

* In none of the cases, thedata were found to be significantly heterogenous at P =0.05%
Y= Probit kill
X= Log concentration

Table 2: Relative toxicity of some insecticide to 8-9 day old larvae of *P. brassicae*

Insecticide	Heterogeneity* χ^2 (3)	Regression equation (Y)	LC_{50}(%)	Fiducial limits LC_{50} ±	Relative toxicity
Cypermethrin	1.644	0.14911+2.45772x	0.00001240	0.000000114	21.45
Deltamethrin	0.826	0.19737+2.51673x	0.00001157	0.000000113	23.99
Fenvalerate	0.640	0.16007+2.57555x	0.00007566	0.000001172	3.49
Endosulfan	0.531	1.53741+2.42533x	0.00026763	0.000001184	1.00

* In none of the cases, thedata were found to be significantly heterogenous at P =0.05%
Y= Probit kill
X= Log concentration

Comparison of LC_{50} values of different insecticides revealed that deltamethrin, cypermethrin and fenvalerate were 29.31, 21.93 and 2.79 times more toxic than endosulfan. The relative toxicity observed during the present study is in conformity with that of Baruah and Chauhan (1996) who reported that deltamethrin, cypermethrin and fenvalerate were 34.93, 20.13 and 5.28 times more toxic than endosulfan. On the basis of LC_{50} values, it is clear from Table 2 that deltamethrin is the most toxic insecticide and endosulfan the least to 8-9 day old larvae of P. brassice. The order of toxicity with respect to LC_{50} values is as deltamethrin > cypermethrin > fenvalerate > endosulfan.

From the comparison of LC_{50} values, it is clear that all the synthetic pyrethroids tested are more toxic than endosulfan. Elliot *et al.*, (1974) stated that pyrethroids were considerably more active any other available compound. Ahmad and Sharma (1985) reported the order of toxicity as decis > ripcord > ambush > cymbush > sumicidin > endosulfan against maize stem borer *Chilo partellus.* Similar order of toxicity, *i.e.* deltamethrin > cypermethrin > fenvalerate > permethrin > BHC, against *S. litura* was reported by Gupta *et.al.* (1985).

Comparison of relative toxicity of different insecticide with endosulfan as standard indicates that the deltamethrin, cypermethrin and fenvalerate are 22.99, 21.45 and 3.49 times more toxic than endosulfan. The present findings are in conformity with that of Peter and Sundararajan (1990) who reported that deltamethrin was the most toxic insecticide against 4th instar larvae of *H.armigera* by topical application method. It was 28.4 times more toxic than endosulfan. The pyrethroids were more toxic than the organophosphorous and organochlorine insecticides.

In the present investigation, younger larvae are more susceptible than the older ones and the LC_{50} values for synthetic pyrethroids increased approximately by a factor of 3.4 times to 8-9 day old larvae of *P. brassicae* as compared to 1-2 day old larvae. The present findings are in conformity with that of Kohli (1982) who also noted that younger larvae were generally more susceptible and the LD_{50} for synthetic pyrethroid increased approximately by a factor of 2 per instar.

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Influence of increasing agridiversity through maize border cropping on sucking pests in Bt-cotton

ABSTRACT
Sucking insect pests pose a major threat to the successful cultivation of transgenic Bt cotton. Besides insecticides, habitat management aims at favoring natural enemies and can be included in transgenic cotton sucking pest management. The present study showed that maize border cropping can be included but after further investigations on time and method of sowing.

INTRODUCTION

Transgenic cotton hybrids, since their introduction, have been successful in reducing the massive reliance on insecticides for bollworm management. After targeting bollworms successfully, Bt cotton still incurs yield losses due to sucking pests (jassids, whiteflies, thrips, mealy bug, aphids and stainers) which pose a threat to Bt cotton sustainability. Broad spectrum insecticides used earlier, against bollworms, are no longer required and the whole scenario has changed in the favor of both sap feeders and natural enemies. Results from non-target studies of the potential impact of Bt cotton have been mixed. Studies by Sisterson *et al* (2004), Naranjo and Ellsworth (2003) and Moar *et al* (2003) found no significant differences in arthropod abundance between Bt and non-Bt cotton plots. But Men *et al* (2002) reported that the Bt cotton decreased the diversities of natural enemy sub-communities among the arthropod communities. Habitat management, an ecological approach, aims at favoring natural enemies and can be included in transgenic cotton sucking pest management (Landis *et al* 2000). Habitat can be altered to increase effectiveness of natural enemies through enhanced survival, fecundity, food supplements etc. Increasing agro ecosystem diversity by mixing crop species encouraged predators of sucking pests reported earlier on non-Bt cotton (Lingappa *et al* 2001). The present study was undertaken to seek information on the role of habitat diversity (maize border crop) in Bt cotton on the abundance of sucking insects and predators.

MATERIALS AND METHODS

The field experiment was carried out during 2008 at PAU Regional Station, Faridkot. Transgenic cotton RCH 134 was sown on 8thMay with plant spacing of 67.5x90 cm in randomized block design with four treatments (T1: *Bt* without seed treatment with imidacloprid ; T2: *Bt* without seed treatment + one maize(PMH-1) border row; T3: *Bt* with seed treatment; T4: *Bt* with seed treatment + one maize border row) each replicated five times. The data was recorded on sap feeders i.e. jassids, whitefly and thrips from three leaves per plant and natural enemies from five plants per plot. Mealy bug was recorded in terms of grade (0-no mealy bug, 1-scattered appearance, 2-one branch fully infested, 3-infestation on more than one branch, 4-heavy infestation in total plant).

RESULTS AND DISCUSSION

The data in Table 1 and 2 revealed that the seed treatment had no significant impact on the numbers of sucking insects as the numbers varied non significantly among the treatments at all observation dates. Maize border rows also did not contribute much to the predator population as evident from the observations taken at 63, 106 and 130 days after sowing. Results of our studies are consistent with findings of Virk *et al* (2004) in which maize contributed the least amongst the trap crops (maize, sorghum, pearl millet and marigold) to seed cotton yield and parasitization efficiency of *T. chilonis*. Studies by Sharma *et al* (2009) indicated that maize interlaced with cowpea acted as a source of predators to cotton crop and attributed to the abundance of floral nectar, alternate prey shelter, mating and oviposition sites harbored in the border crop compared with monoculture cotton having lesser biodiversity. In a similar study involving okra (Malvaceae), Nderitu *et al* (2008) found that the okra plots bordered by maize had the highest mean aphid population among the border crops i.e. maize, sorghum, pigeon pea and millet. However, maize bordered plots recorded the highest number of parasitized aphids in the study period.

Table 1: Incidence of sucking insects and predators at days after sowing

63 DAS					
Treatment	Jassid/ 3 leaves	Whitefly/ 3 leaves	Thrip/ 3 leaves	Mealy bug (grade)	Predators*/ plant
T1	1.27 (1.50)	1.00 (1.41)	5.93 (2.62)	0.33 (1.14)	1.58 (1.60)
T2	2.07 (1.75)	1.73 (1.60)	6.47 (2.73)	0.00 (1.00)	1.98 (1.72)
T3	2.40 (1.84)	1.13 (1.46)	6.93 (2.81)	0.67 (1.28)	1.71 (1.65)
T4	1.53 (1.58)	2.00 (1.72)	6.07 (2.65)	0.67 (1.28)	1.89 (1.70)
CV	7.26	19.87	8.31	19.53	3.13
CD (p=0.05)	0.24	NS	NS	NS	NS

106 DAS					
Treatment	Jassid/ 3 leaves	Whitefly/ 3 leaves	Thrip/ 3 leaves	Mealy bug (grade)	Predators*/ plant
T1	1.67 (1.63)	5.63 (2.53)	0.00 (1.00)	0.67 (1.28)	1.70 (1.64)
T2	1.42 (1.55)	3.02 (1.99)	0.00 (1.00)	1.00 (1.41)	1.66 (1.63)
T3	1.63 (1.62)	4.50 (2.35)	0.00 (1.00)	1.00 (1.41)	1.61 (1.61)
T4	2.75 (1.93)	3.68 (2.16)	0.00 (1.00)	0.67 (1.28)	1.87 (1.69)
CV	5.55	14.75	...	13.58	4.61
CD (p=0.05)	0.18	NS	...	NS	NS

130 DAS					
Treatment	Jassid/ 3 leaves	Whitefly/ 3 leaves	Thrip/ 3 leaves	Mealy bug (grade)	Predators*/ plant
T1	2.33 (1.82)	2.20 (1.78)	0.20 (1.09)	0.33 (1.14)	0.98 (1.40)
T2	2.40 (1.84)	2.93 (1.97)	0.40 (1.17)	0.00 (1.00)	1.07 (1.43)
T3	2.67 (1.91)	2.53 (1.87)	0.67 (1.03)	0.67 (1.28)	1.38 (1.54)
T4	2.13 (1.77)	2.80 (1.94)	0.67 (1.03)	0.33 (1.14)	0.00 (1.00)
CV	8.92	10.79	8.05	17.16	8.00
CD (p=0.05)	NS	NS	NS	NS	0.21

Figures in parentheses are transformed values
DAS: Days after sowing
T1: *Bt* without seed treatment
T2: *Bt* without seed treatment + maize border crop
T3: *Bt* with seed treatment
T4: *Bt* with seed treatment + maize border crop
*Predators: Coccinellids, Spiders, Syrphids, Chrysopa, Zealous etc.
Jassid/plant (3 leaves- top, middle, bottom)
Whitefly/ plant (3 leaves- 3rd, 5th, 7th leaf)
Thrips/plant (3 leaves- top, middle, bottom)
Mealybug: grade (0,1,2,3,4)

Table 2: Assessment of influence of seed treatment /border crop in Bt cotton sucking pests management: overall mean and yield

Treatments	Overall mean							Yield# (Kg/ plot)
				Mealy bug				
	Jassid/ 3 leaves	Whitefly/ 3 leaves	Thrip/ 3 leaves	Grade	Infestation (%)	Intensity of infested plants	Predators */ plant	
T1	2.0 (1.77 4)	3.4 (2.07 9)	2.3 (1.69 3)	0. (1.67 29)	40.0	1.0	1.4 (1.57 6)	29. (30.605 5)
T2	2.3 (1.82 1)	3.9 (2.20 0)	2.6 (1.65 9)	0. (1.63 26)	38.0	1.0	1.6 (1.64 2)	25. (27.279 1)
T3	2.2 (1.88 0)	3.7 (2.12 5)	3.0 (1.70 3)	0. (1.69 29)	40.0	1.0	1.7 (1.60 4)	30. (32.040 8)
T4	2.4 (1.86 4)	3.7 (2.10 5)	3.1 (1.72 9)	0. (1.57 25)	34.0	1.0	1.5 (1.56 8)	27. (29.599 3)
CV	5.84	6.44	6.68	9.45	--	--	5.94	14.57
CD (p=0.05)	NS	NS	0.10	NS	--	--	NS	NS

Results of the current studies indicated that maize, being fast growing crop, should establish slightly before the cotton. Flowering in maize should be at the time so that predators are able to establish themselves in sufficient numbers prior to insect pests. Pollen generating plants e.g. maize probably contribute to the density of the predators besides affecting behavior and activity by providing carbohydrate food sources such as nectar to the adult wasps. Also, staggered planting would be contributing more as compared to single sowing on the basis that pollen and nectar would be available for the longer duration to the natural enemies. These factors need to be studied elaborately if border or inter cropping should be included in IPM system for sucking pests' management in Bt cotton.

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Monitoring for Pyrethroid resistance in pink bollworm *Pectinophora gossypiella* (Saunders) field strains

Abstract
Field experiments were conducted in two Governorates in Egypt (El-Behera and Alexandria) to study the situation of pyrethroid resistance in pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), through two cotton seasons (2006 and 2007) using the attracticide resistance monitoring technique (ARMT) and the results were collected in both early and late season.

The toxicity parameter (LC_{50}) for the susceptible strain indicated that deltamethrin was the most toxic in the two durations (6 hr and 12 hr assessments) with LC_{50} of 0.8 and 0.19 ppm, respectively; followed by lambda-cyhalothrin (LC_{50} values = 1.11 and 0.38 ppm, respectively) and then the least toxic was cypermethrin with LC_{50} values of 1.5 and 0.5 ppm, respectively.

The resistance levels were varied between El-Behera population and Alexandria population and also varied between the two inspections (6 hr and 12 hr). On the other hand, the resistance values were differed from the early season assessment to the late season assessment and from the beginning of this investigation to the end. The starting resistance levels for El-Behera strain in the early 2006 cotton season at the 6 hr assessment were 79.8, 28.71 and 30.26 folds against cypermethrin, deltamethrin and lambda-cyhalothrin; respectively, while these values reached 110.34, 40.5 and 41.26 folds, respectively by the late 2007 cotton season. Also this event was occurred in Alexandria strain. Regarding the calculated resistance values which derived from 12 hr assessment they showed different values than that of 6 hr assessment for both strains. For El-Behera strain these values became 36.92, 32.36 and 34.42 folds against cypermethrin, deltamethrin and lambda-cyhalothrin; respectively; while the corresponding resistance values of Alexandria strain reached 87.22, 48.36 and 41.31folds, respectively.
Key words: Pink bollworm, *Pectinophora gossypiella*, resistance, Pyrethroids.

INTRODUCTION

Pink bollworm (PBW) *Pectinophora gossypiella* (Saunders) is a worldwide pest of cotton and in some regions of the world is the key cotton pest. It has caused loss in yield and costs of insect control; substantial indirect losses occur as result of the destruction of beneficial insects and the development of insecticides resistance in cotton. It has been extremely difficult to control using pesticides but considerable success has been achieved using alternative control tactics.

Synthetic pyrethroids were introduced to replace the resistance-prone and environmentally unsuitable organochlorines, cyclodienes and organophosphates. They have high contact activity and are particularly effective against lepidopterous larvae. They possess an inherently high activity and could be applied at extremely low doses (McCaffery 1999). Most but not all of pyrethroids have a very low mammalian toxicity, low environmental impact and were immobile in the soil (Elliott 1989). They are used to control a wide range of insect pests of agriculture and horticultural crops and for use in control of insect vectors of disease. Pyrethroids have also made substantial inroads into public health, industrial, amenity and household outlets, as well as grain and food stores. Since their introduction in the mid-1970s the pyrethroids have proved a powerful insecticide control tool which has in some cases threatened their longer term viability through rapid development of pest resistance (Dent 2000).

Resistance to one or more pesticides has been documented in more than 447 species of insects and mites (Roush and McKenzie 1987). Resistance to insecticides is one of the most serious problems facing agriculture today and it's an increasingly urgent worldwide problem. Agricultural productivity is jeopardized because the widespread resistance in crop and livestock pests. Many previous studies revealed the high resistance of pink bollworm to insecticides in the cotton fields (Georghiou, 1983). In Egypt, insecticides have been widely used against cotton pests. However, although synthetic insecticides were the most efficient and widely used against bollworms but the onset of resistance development to them in bollworms have been detected and documented (Miller, 1990, Al-Beltagy *et al*., 1996, Shekeban, 2008 and El-Arami 2008).

For resistance management tactics to be effective, resistance must be detected in its early stage (Rouch & Miller 1986) and early detection necessitates using one or more techniques that being accurate, easy, rapid and inexpensive, which would aid production, consultants and extension personal in making informed decisions on adequate control measures (Mink & Boethel 1992). The attracticide method was developed in summer of 1985 and was full implementation in 1986 and 1987 as an effective and rapid method to monitor insecticide resistance in pink bollworm adult in cotton fields to a wide range of insecticides (Miller, 1986 and Haynes *et al* ,1986 and 1987). Monitoring insecticide resistance in field population moth of bollworms is in great importance in resistance management programs (Tabashnik and Cushing, 1987).

The present investigation aims to study the situation of resistance to pyrethroids in pink bollworm in two Governorates in Egypt.

MATERIALS AND METHODS

The used insecticides, the pheromone source and the sticky adhesive were provided by the Ministry of Agriculture, Egypt.

1- INSECTICIDES USED: Three pyrethroid insecticides were used:

1.1. Cypermethrin : Syper. EC 10%, 0.6 liter / Fadden
1.2. Deltamethrin : Demthrin. EC 2.5%, 0.75 liter / Fadden
1.3. Lambda-cyhalothrin: Katron. EC 2.5%, 0.8 liter / Fadden

2- PHEROMONE USED

Pink rubber septum containing 1 mg Gossyplure**.**

3- OTHER CHEMICALS USED

Stickum® (sticky adhesive) and acetone.

4- INSECTS USED: Pink bollworm (PBW) *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae).

4.1. The laboratory strain: Male moth population of PBW was supplied by the Bollworm Research Department, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt, where it has been reared for more than 10 years in conditioned laboratory without exposure to insecticides. The rearing procedure was adopted as that recorded by Abdel Hafizes *et al* (1982).

4.2. Field strains: Male moth populations of PBW from both Kafr El-Dawar District cotton fields, El-Behera Governorate, and Faculty of Agriculture Research Farm, Alexandria Governorate, Egypt were used locally in the present study.

5 - ARMT PROCEDURE:-

The attracticide resistance monitoring technique (ARMT) is that method where delta traps were used with sticky adhesive coated cards containing the insecticide concentrations placed in the trap bottom. Rubber septa with 1mg Gossyplure acted as the pheromone source. This technique provided stable LC_{50}'s with low control mortality. This technique was used as described by Miller (1986) and modified by Shekeban (2000).

6- Statistical analysis

6.1. Regression equation and confidence limits:

Regression equation, $LC_{50,}$ LC_{95} and confidence limits were calculated according to probit analysis computer program (Finney 1971).

6.2. Resistance Ratio (R.R.):

Resistance ratio (RR) values were measured according to the following equation:

$$\text{Resistance Ratio (R.R.)} = \frac{LC_{99} \text{ of the field strain}}{LC_{99} \text{ of the susceptible strain}} \quad \text{(Fold)}$$

RESULTS AND DISCUSSION

1- Toxicity Parameters and Resistance Ratios at 6 hr. assessment.

Data in table (1) presented the regression equation, the LC_{50}s, the LC_{95}s and the resistance ratios of the tested pyrethroids at the early 2006 cotton season from the six hours assessment.

Table 1: Toxicity Parameters and Resistance Ratios of the tested pyrethroids at the 6 hr. assessment at early 2006 cotton season

strain	insecticides	Regression Equation	LC_{50}	LC_{95}	RR
Susceptible Strain	Deltamethrin	Y= 0.095+0.9X	0.80 (1.0 - 0.6)	37.1	-
	Lambda-cyhalothrin	Y= -0.044+1.X	1.11 (1.4 - 0.9)	46.0	-
	Cypermethrin	Y = -0.17+0.99X	1.50 (1.9 - 1.2)	67.2	-
El-Behera Strain	Deltamethrin	Y = -1.6 + 1.2X	22.97 (28.4 - 18.6)	537.72	28.71
	Lambda-cyhalothrin	Y = -2.5 + 1.7X	33.59 (39.4 - 28.6)	354.98	30.26
	Cypermethrin	Y = -2.9 + 1.4X	119.70 (143.6 – 99.8)	1795.16	79.8
Alexandria Strain	Deltamethrin	Y =-1.7 + 1.7X	10.83 (13.2 - 8.8)	105.07	13.53
	Lambda-cyhalothrin	Y = -2.3+ 1.9X	17.51 (20.4 - 14.9)	131.62	15.77
	Cypermethrin	Y =-2.3 + 1.5X	30.68 (38.7 - 24.1)	369.31	20.45

Regarding the LC_{50} values, deltamethrin was the most toxic against the three tested populations followed by lambda-cyhalothrin and then the least toxic was cypermethrin. For the susceptible strain, the LC_{50} values were 0.8, 1.11 and 1.5 ppm for deltamethrin, lambda-cyhalothrin and cypermethrin, respectively; while the corresponding LC_{95} values were 37.1, 46 and 67.2 ppm, respectively. The LC_{50} values against El-Behera strain were 22.97, 33.59 and 119.7 ppm, while the LC_{95} values were 537.72, 354.98 and 1795.16 ppm for deltamethrin, lambda-cyhalothrin and cypermethrin, respectively. The LC_{50} values of the three pyrethroids, deltamethrin, lambda-cyhalothrin and cypermethrin, against Alexandria strain were 10.83, 17.51 and 30.68 ppm, and their LC_{95}s were 105.07, 131.62 and 369.31 ppm, respectively.

Comparing the LC_{50} values of the two field strains with that of the susceptible strain showed that the resistance levels were differed between the two field strains and they were 28.71, 30.26 and 79.8 folds for El-Behera strain and 13.53, 15.77 and 20.45 folds for Alexandria strain against deltamethrin, lambda-cyhalothrin and cypermethrin, respectively.

Table (2) show the obtained data after the 6 hr assessment at the late 2006 cotton season which take the same way where the LC_{50} values indicated that deltamethrin was the most toxic followed by lambda-cyhalothrin and cypermethrin was the least toxic one against the two strains. For El-Behera strain the LC_{50} of deltamethrin was 27.08 and the LC_{95} was 438.42 ppm, the LC_{50} of lambda-cyhalothrin was 34.76 and the LC_{95}

was 326.47 ppm and the LC_{50} of cypermethrin was 141.82 and the corresponding LC_{95} was 2512.01 ppm. The resistance ratios were 33.85, 31.13 and 94.54 fold for deltamethrin, lambda-cyhalothrin and cypermethrin, respectively.

Table 2: Toxicity Parameters and Resistance Ratios of the tested pyrethroids at the 6 hr. assessment at late 2006 cotton season

Strain	Insecticides	Regression Equation	LC_{50}	LC_{95}	RR
Susceptible Strain	Deltamethrin	Y= 0.095+0.9X	0.80 (1.0 - 0.6)	37.1	-
	Lambda-cyhalothrin	Y= -0.044+1.X	1.11 (1.4 - 0.9)	46.0	-
	Cypermethrin	Y = -0.17+0.99X	1.50 (1.9 - 1.2)	67.2	-
El-Behera Strain	Deltamethrin	Y = -1.9+ 1.4X	27.08 (32.6 - 22.5)	438.42	33.85
	Lambda-cyhalothrin	Y = -2.6 + 1.7X	34.76 (40.4 - 29.8)	326.47	31.13
	Cypermethrin	Y = -2.8 + 1.3X	141.82 (173.34 - 116.15)	2512.01	94.54
Alexandria Strain	Deltamethrin	Y = -1.8 + 1.7X	11.41 (13.8 - 9.4)	102.99	14.26
	Lambda-cyhalothrin	Y = -2.3 + 1.8X	18.41 (21.5 - 15.7)	146.13	16.58
	Cypermethrin	Y = -3.1 + 1.9X	48.97 (57.5 - 41.6)	377.23	32.64

The obtained data for Alexandria strain showed LC_{50} values of 11.41, 18.41 and 48.97 ppm and LC_{95} values of 102.99, 146.13 and 377.23 ppm for deltamethrin, lambda-cyhalothrin and cypermethrin, respectively. Whereas the calculated resistance ratios were 14.26 fold for deltamethrin, 16.58 fold for lambda-cyhalothrin and 32.64 for cypermethrin. The recorded resistance values reported that El-Behera strain was more resistant than Alexandria strain. On the other hand, cypermethrin has received the highest resistance values in both two strains.

Regression equation, LC_{50} values, LC_{95} values and the resistance ratios of the tested pyrethroids for the three strains in early 2007 cotton season after 6 hr of treatment were calculated and then tabulated in table (3). The LC_{50} values of the three pyrethroids (deltamethrin, lambda-cyhalothrin and cypermethrin) for El-Behera strain were 29.1, 40.4 and 151.6 ppm; respectively, while the corresponding LC_{95} values were 309.7, 394.1 and 1527.7 ppm, respectively. For Alexandria field strain, deltamethrin proved to be the most potent pyrethroid with LC_{50} of 11.67 ppm and LC_{95} of 164.5 ppm followed by lambda-cyhalothrin with LC_{50} of 21.86 ppm and LC_{95} of 218.8 ppm, while the LC_{50} of the least toxic, cypermethrin, was 59.37 ppm and its LC95 = 650.38 ppm. On the other hand, the results showed clear differences between the two tested strains in the resistance ratios and also some differences between the three tested pyrethroids in the same strain. For El-Behera strain the resistance ratios against deltamethrin, lambda-cyhalothrin and cypermethrin were 36.37, 36.39 and 101.06 fold; respectively, whereas these values were 14.58, 19.69 and 39.58 fold, respectively for Alexandria strain.

Table 3: Toxicity Parameters and Resistance Ratios of the tested pyrethroids at the 6 hr. assessment at early 2007 cotton season

Strain	Insecticides	Regression Equation	LC_{50}	LC_{95}	RR
Susceptible Strain	Deltamethrin	Y= 0.095+0.9X	0.80 (1.0 - 0.6)	37.1	-
	Lambda-cyhalothrin	Y= -0.044+1.X	1.11 (1.4 - 0.9)	46.0	-
	Cypermethrin	Y = -0.17+0.99X	1.50 (1.9 - 1.2)	67.2	-
El-Behera Strain	Deltamethrin	Y =-2.4 + 1.6X	29.1 (34.1 - 24.9)	309.7	36.37
	Lambda-cyhalothrin	Y =-2.7 + 1.7X	40.4 (47.2 - 34.6)	394.1	36.39
	Cypermethrin	Y =-3.4 + 1.6X	151.6 (184.9 - 118.3)	1527.7	101.06
Alexandria Strain	Deltamethrin	Y = -1.5 + 1.4 X	11.67 (14.5 - 9.3)	164.50	14.58
	Lambda-cyhalothrin	Y = -2.2 + 1.6 X	21.86 (25.7 - 18.6)	218.80	19.69
	Cypermethrin	Y = -2.2 + 1.4 X	59.37 (50.6 - 32.1)	650.38	39.58

In table (4), the same LC_{50}s and LC_{95}s of the tested pyrethroids were installed for the susceptible strain. For El-Behera strain the LC_{50}, LC_{95} and the resistance value of deltamethrin were 32.4, 364.14 ppm and 40.5 fold; respectively, while they were 13.99, 188.04 ppm and 17.48 fold for Alexandria strain. Lambda-cyhalothrin LC_{50}, LC_{95} and the resistance value for El-Behera strain were 45.8, 425.83 ppm and 41.26 fold, whereas they were 36.19, 197.38 ppm and 32.6 fold, respectively for Alexandria strain. Cypermethrin, the least toxic with the highest resistance ratios, has LC_{50} of 165.51 ppm, LC_{95} of 1807.33 ppm and resistance value of 110.34 fold for El-Behera strain, where they were 66.48 ppm, 745.92 ppm and 44.32 fold, respectively for Alexandria strain.

Table 4: Toxicity Parameters and Resistance Ratios of the tested pyrethroids at the 6 hr. assessment at late 2007 cotton season

Strain	Insecticides	Regression Equation	LC_{50}	LC_{95}	RR
Susceptible Strain	Deltamethrin	Y= 0.095+0.9X	0.80 (1.0 - 0.6)	37.1	-
	Lambda-cyhalothrin	Y= -0.044+1.X	1.11 (1.4 - 0.9)	46.0	-
	Cypermethrin	Y = -0.17+0.99X	1.50 (1.9 - 1.2)	67.2	-
El-Behera Strain	Deltamethrin	Y = -2.4 + 1.6 X	32.40 (38.0 - 27.6)	364.13	40.5
	Lambda-cyhalothrin	Y = -2.8 + 1.7 X	45.80 (53.5 - 39.2)	425.83	41.26
	Cypermethrin	Y = -3.5 + 1.6 X	165.51 (197.4 - 138.9)	1807.33	110.34
Alexandria Strain	Deltamethrin	Y = -1.7 + 1.5X	13.99 (17.1 - 11.4)	188.04	17.48
	Lambda-cyhalothrin	Y = -3.5 + 2.2X	36.19 (40.8 - 32.1)	197.38	32.6
	Cypermethrin	Y = -2.9 + 1.6X	66.48 (78.5 - 56.3)	745.92	44.32

2- Toxicity Parameters and Resistance Ratios after 12 hr. of treatment.

The toxicity parameters (LC_{50}, LC_{95}) of deltamethrin, lambda-cyhalothrin and cypermethrin against the susceptible strain at 12 hr assessment were obtained and tabulated in tables 5-8 of this section as follow: the LC_{50}s were 0.19, 0.38 and 0.5 ppm, while the LC_{95}s were 7.3, 15.5 and 18.4 ppm, respectively. These results proved that deltamethrin was the most toxic followed by lambda-cyhalothrin where cypermethrin was the least toxic. These toxicity data were in harmony with those obtained by several investigators (i.e. El-Bassiony, 2001, Shekeban *et al* 2002 and Shekeban 2007). The obtained date were met with those reported by Shekeban (1989) and Marei *et al* (1991) when reported that, deltamethrin exhibited the highest toxicity among the other tested pyrethroids against the 4th instar larvae of cotton leafworm (CLW).

Data in table (5) presented the results of 12 hr assessment for the early 2006 cotton season. These data showed that the LC_{50}s of deltamethrin, lambda-cyhalothrin and cypermethrin against El-Behera strain were 1.19, 7.93 and 6.62 ppm; respectively, while the LC_{95}s were 32.93, 78.46 and 148.54 ppm, respectively. On the other hand El-Behera strain recorded resistance ratios of 6.26, 20.86 and 13.24 fold against the three previous mentioned pyrethroids, respectively. For Alexandria strain data showed that deltamethrin was the most toxic with LC_{50} value of 4.15 ppm and LC_{95} value of 41.59 ppm followed by lambda-cyhalothrin with LC_{50} value of 9.01 ppm and LC_{95} value of 97.51 ppm, while the LC_{50} of the lowest toxic one, cypermethrin, was 19.14 ppm and the LC_{95} was 153.39 ppm. The recorded resistance ratios were 21.84, 23.71 and 38.28 fold against deltamethrin, lambda-cyhalothrin and cypermethrin, respectively.

Table 5: Toxicity Parameters and Resistance Ratios of the tested pyrethroids at the 12 hr. assessment at early 2006 cotton season

Strain	Insecticides	Regression Equation	LC_{50}	LC_{95}	RR
Susceptible Strain	Deltamethrin	Y = 0.76+1.03X	0.19 (0.243 - 0.136)	7.3	-
	Lambda-cyhalothrin	Y = 0.4 +1.02X	0.38 (0.482 - 0.285)	15.5	-
	Cypermethrin	Y = 0.32+1.05X	0.50 (0.635 - 0.386	18.4	-
El-Behera Strain	Deltamethrin	Y = -0.08+1X	1.19 (2.99 - 0.45)	32.93	6.26
	Lambda-cyhalothrin	Y = -1.5 + 1.7X	7.93 (10.1 - 6.2)	78.46	20.86
	Cypermethrin	Y = -0.9 + 1.2X	6.62 (12.8 - 3.3)	148.54	13.24
Alexandria Strain	Deltamethrin	Y = -1.1 + 1.6X	4.15 (5.9 - 2.9)	41.59	21.84
	Lambda-cyhalothrin	Y = -1.7 + 1.5X	9.01 (10.4 - 6.0)	97.51	23.71
	Cypermethrin	Y = -2.3+ 1.8X	19.14 (24.8 - 14.7)	153.39	38.28

Table (6) showed that, the obtained data for El-Behera strain after the 12 hr assessment at the late 2006 cotton season recorded LC_{50}, LC_{95} values and resistance ratio for deltamethrin equal to 1.78, 81.04 ppm and 9.36 fold, respectively. In the same manner they were 10.10, 102.72 ppm and 26.57 fold, respectively for lambda-cyhalothrin and 13.88, 297.21 ppm and 27.76 fold for cypermethrin, respectively. For Alexandria strain the LC_{50} values were 4.32, 8.9 and 22.83 ppm for deltamethrin, lambda-cyhalothrin and cypermethrin, respectively. The corresponding LC_{95} values were 39.2, 91.0 and 236.23 ppm, while the resistance ratios were 23.73, 23.42 and 45.66 fold.

Table 6: Toxicity Parameters and Resistance Ratios of the tested pyrethroids at the 12 hr. assessment at late 2006 cotton season

Strain	Insecticides	Regression Equation	LC_{50}	LC_{95}	RR
Susceptible Strain	Deltamethrin	Y = 0.76+1.03X	0.19 (0.243 - 0.136)	7.3	-
	Lambda-cyhalothrin	Y = 0.4 +1.02X	0.38 (0.482 - 0.285)	15.5	-
	Cypermethrin	Y = 0.32+1.05X	0.50 (0.635 - 0.386	18.4	-
El-Behera Strain	Deltamethrin	Y = -0.3 + 0.99X	1.78 (3.9 - 0.8)	81.04	9.36
	Lambda-cyhalothrin	Y = -1.6 + 1.6X	10.10 (12.5 - 8.0)	102.72	26.57
	Cypermethrin	Y = -1.4 + 1.2X	13.88 (21.4 - 8.8)	297.21	27.76
Alexandria Strain	Deltamethrin	Y = -0.98 + 1.7X	4.32 (5.7 - 2.7)	39.20	23.73
	Lambda-cyhalothrin	Y = -1.5+ 1.6X	8.90 (11.2 - 6.9)	91.00	23.42
	Cypermethrin	Y = -2.2 + 1.6X	22.83 (29.5- 17.6)	236.23	45.66

The presented data in table (7) discuss the toxicity parameters that observed after 12 hr of treatment at the early 2007 cotton season. The LC_{50}s of deltamethrin, lambda-cyhalothrin and cypermethrin against El-Behera strain were 5.44, 10.03 and 15.85 ppm; respectively, while in parallel the LC_{95}s were 64.35, 143.53 and 304.97 ppm. The new recorded resistance ratios were 28.63, 26.39 and 31.7 fold against deltamethrin, lambda-cyhalothrin and cypermethrin, respectively. Also, the data for Alexandria strain showed LC_{50} and LC_{95} of deltamethrin equal to 5.78 and 98.58 ppm, also of lambda-cyhalothrin equal to 10.24 and 185.7 while they were 26.52 and 264.59 ppm for cypermethrin, respectively. The recorded resistance ratios were 30.42, 26.94 and 53.04 fold against the three examined pyrethroids, respectively.

Table 7: Toxicity Parameters and Resistance Ratios of the tested pyrethroids at the 12 hr. assessment at early 2007 cotton season

Strain	Insecticides	Regression Equation	LC_{50}	LC_{95}	RR
Susceptible Strain	Deltamethrin	Y = 0.76+1.03X	0.19 (0.243 - 0.136)	7.3	-
	Lambda-cyhalothrin	Y = 0.4 +1.02X	0.38 (0.482 - 0.285)	15.5	-
	Cypermethrin	Y = 0.32+1.05X	0.50 (0.635 - 0.386	18.4	-
El-Behera Strain	Deltamethrin	Y=-1.2 + 1.5X	5.44 (7.4 - 3.9)	64.35	28.63
	Lambda-cyhalothrin	Y = -1.4 + 1.4X	10.03 (12.9 - 7.8)	143.53	26.39
	Cypermethrin	Y = -2.6 + 1.7X	15.85 (41.2 - 13.7)	304.97	31.7
Alexandria Strain	Deltamethrin	Y = -1.1 + 1.3X	5.78 (8.1 4.1)	98.58	30.42
	Lambda-cyhalothrin	Y = -1.3 + 1.3X	10.24 (13.4 - 7.8)	185.70	26.94
	Cypermethrin	Y = -2.3 + 1.7X	26.52 (33.4 - 21.0)	264.59	53.04

The installed data in table (8) showed the LC_{50}, LC_{95} and the resistance value of deltamethrin for El-Behera strain as 6.15 ppm, 117 ppm and 32.36 fold; respectively, while they were 9.19 ppm, 140.39 ppm and 48.36 fold for Alexandria strain. Lambda-cyhalothrin had LC_{50}, LC_{95} and the resistance value for El-Behera strain = 13.08 ppm, 174.63 ppm and 34.42 fold; respectively, whereas they were 15.7 ppm, 119.07 ppm and 41.31 fold, respectively for Alexandria strain. Cypermethrin LC_{50}, LC_{95} and resistance value for El-Behera strain were 17.96 ppm, 791.33 ppm and 35.92 fold, where they were 43.61 ppm, 469.03 ppm and 87.22 fold, respectively for Alexandria strain.

Table 8: Toxicity Parameters and Resistance Ratios of the tested pyrethroids at the 12 hr. assessment at late 2007 cotton season

Strain	Insecticides	Regression Equation	LC_{50}	LC_{95}	RR
Susceptible Strain	Deltamethrin	Y = 0.76+1.03X	0.19 (0.243 - 0.136)	7.3	-
	Lambda-cyhalothrin	Y = 0.4 +1.02X	0.38 (0.482 - 0.285)	15.5	-
	Cypermethrin	Y = 0.32+1.05X	0.50 (0.635 - 0.386	18.4	-
El-Behera Strain	Deltamethrin	Y = -1.0 + 1.3X	6.15 (8.6 - 4.3)	117.00	32.36
	Lambda-cyhalothrin	Y = -1.5 + 1.4X	13.08 (14.9 - 9.3)	174.63	34.42
	Cypermethrin	Y = -1.3+ 1.0X	17.96 (28.19 - 11.3)	791.33	35.92
Alexandria Strain	Deltamethrin	Y = -1.3 + 1.4 X	9.19 (11.9 - 7.1)	140.39	48.36
	Lambda-cyhalothrin	Y = -2.2 + 1.9 X	15.70 (18.4 - 13.4)	119.07	41.31
	Cypermethrin	Y = -2.6 + 1.6 X	43.61 (52.6 - 36.1)	469.03	87.22

On this criterion, deltamethrin was highly toxic against both field and laboratory male moth populations of PBW than the other tested pyrethroids. This finding is in good agreement with that of Shekeban *et al*, (2003a, b). The previous toxicity results pointed out to high increase in the LC_{50} values of the tested pyrethroids against PBW male moths field population compared to that of laboratory one and these data are in a harmony with the results of many other authors (i.e.,. Al-Beltagy *et al*, 2001 a, b; Shekeban, 2002 a, b).

The intensive and unwise use of insecticides in controlling cotton bollworms leads to the development of resistance in pink bollworm to approximately the conventionally used groups. The obtained data declared that, the evolution of resistance to the tested pyrethroids from early to late season and from season to another in spite of the single application with one of the recommended pyrethroids for pink bollworm control per season may be as a result of the cross-resistance mechanism. These findings are in agreement with the results obtained by Shekeban 2000, El-Bassiony 2001and El-Arami 2008.

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ESTERASE IZOFORMS IN HOUSEFLY (*MUSCA DOMESTICA* L.) SELECTED BY INSECTICIDES OF DIFFERENT CHEMICAL CLASSES

Abstract: The esterase izoforms in the housefly selected by insecticides from three chemical classes: organophosphates, pyrethroids and derivate of benzylphenylurea were researched in this work. Clear differences were discovered in izoferment spectrum of nonspecific esterases in all 6 strains moreover not always these changes correlated with class of selectant.

INTRODUCTION

Still in the forties the XX-th centuries by methods of classical biochemistry it has been established that a number of enzymes have some forms different on molecular weight, solubility, an optimum pH and other properties. The additional data about existence of enzymes in the various forms has been received after use in biochemical researches of a method electrophoresis. Multiple forms (izoferments) are revealed at many enzymes of insects. They are connected with performance of many functions in an organism of insects, including protective function as take part in insecticides detoxification. Esterase izoferments in sensitive and resistant insects often differs. For instance, three from ten izoferments in resistant to several insecticides cockroach *Blatella germanica* possessed the more high activity in contrast with sensitive individuals (Prabhakaran, Kamble, 1996). New izoferment induced in gnat of the genus *Simulium*, is resistant to DDT и pyrethroids (Montagna et al., 2003). The spectrum of esterase izoferments noticeably differed in resistance to profenofos persons the tobacco budworm *Heliothis virescens* (Harold, Ottea, 2000).

In given work, electrophoretic spectrum of esterases of sensitive and 6 selected houseflies' strains were also studied.

MATERIAL AND METHODS

The objects of the studies were imago of one sensitive strain and six selected strains of housefly. For selection were used following insecticides: from class OP - phosmet (phtalophos, 20% e.k.), phoxim (volaton, 50% e.k.); from class pyrethroids - deltamethrin (decis, 2.5% e.k.), fenvalerat (sumicidin, 20% e.k.), ethophenprox (trebon, 30% e.k.); from derivates of benzylphenylurea - chlorfluazuron (eim, 12% e.k.). The determination of resistance index (RI) to selectants is conducted, as it is described earlier (Sokolyanskaya, 2007).

The electrophoresis of esterases was conducted in polyacrylamid gel (PAAG), system N1 (Maurer, 1971). Fractionating of esterases was made by bringing on column PAAG 100 μl of gomogenate, containing protein in concentration 3 mg/ml, 20 μl 40% sucrose and 5 μl bromfhenol blue. The mode of the electrophoresis: 2 mA on tubule - 15 minutes; 4 mA on tubule - 60-70 minutes. For staining of esterase, isoforms gels were placed in incubation medium, consisting of 10 ml 0,2 M sodium -phosphate buffer, pH=7,0; 0,5% α- naphthyl acetate (0,2 ml) and 3 mg fast blue RR (in calculation on 1 gel).

The protein concentration was defined by method Louri (Lowry et al., 1951).

RESULTS AND DISCUSSION

In flies of the sensitive strain it was a success to reveal 10 izoferments with α-naftilacetat activity. Two izoforms with Rf 0.26 and 0.57 possessed by strong activity as regards substratum, three izoferments with Rf 0.35; 0.47; 0.6 - an average activity and four izoferments with Rf 0.16; 0.51; 0.75; 0.8 - a weak activity.

Already at the beginning of selection, in sixth generation, there were discovered clear differences in izoferment spectrum of nonspecific esterases in all 6 strains, moreover not always these changes correlated with class of selectant (fig. 1). Izoform with Rf 0.26 nearly in all selected strains saved the high activity, with the exclusion of strain, selected chlorfluazuron - in this strain on initial stage of the selection activity of given izoform vastly decreased. In all selected strains izoform with Rf 0.57 has reduced its activity as compared with sensitive strain. In ditto time the most light izoform, on the contrary, has enlarged its activity in all strains, except strain R-e. In this strain less light faction with Rf 0.75 possessed by greater activity. This izoform in all rest selected strains also enlarged its activity as compared with sensitive flies, but not so vastly, and in strain R-tr it reduced. Besides of, in chlorfluazuron selected strain two izoforms induced; with Rf 0.55 with weak esterase activity and with Rf 0.67 with average activity. In ethophenprox selected flies, on the contrary, 2 weak heavy esterase fractions were not revealed, but the active fraction with Rf 0.42 has induced and the total number izoferments was 8. The last izoform induced also in strain, selected fenvalerat (with strong activity), and delthamethrin and phosmet (with weak activity). In fenvalerat selected strain also two heavy fractions were not revealed, but at the expense of the inductions two new izoforms with Rf 0.42 and 0.62 total number of the plural forms complied with such in sensitive strain. The delthamethrin selected flies had the same number of izoforms but their spectrum too several differed from sensitive strain: in them izoform with Rf 0.16 were saved and new with Rf 0.42 induced. In phosmet

selected strain the total number izoforms was 10, but, unlike sensitive strain, the fraction with Rf 0.16 was absent and new with Rf 0.42 induced. In phoxim selected strain at the expense of the absence izoform with Rf 0.16 general their number was 9.

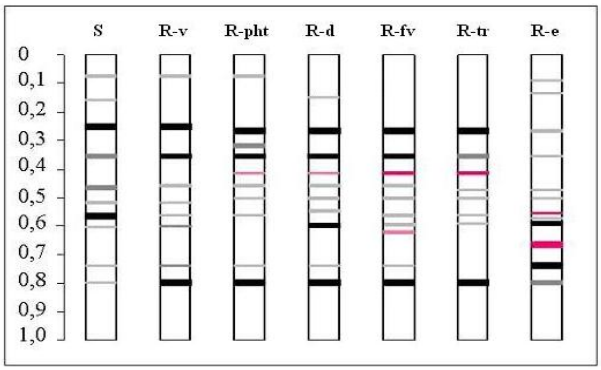

Fig. 1. Izoferment spectrum of nonspesific esterases in sensitive and resistant strains in 6th .generation.

To 30th generation the amount of izoforms in all selected strain did not change, with the exclusion of strain R-v, in which else two fractions: with Rf 0.42 and 0.59 induced, but instead of fraction 0.09 appeared the fraction 0.16 (fig. 2).

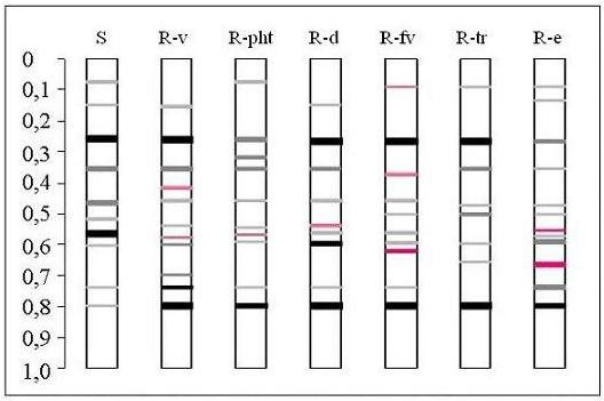

Fig. 2. Izoferment spectrum of nonspesific esterases in sensitive and resistant strains in 30th generation.

In all flies izoform 0.42 disappeared, but new: izoforms induced – in phosmet selected strains - 0.59, in delthamethrin selected - 0.55, in fenvalerat selected flies - 0.09 and instead of fraction 0.35 appeared the fraction 0.38. In ethophenprox selected flies also newly the heavy fraction with Rf 0.09 with weak activity appeared. The chlorfluazuron selected strain – the single, saved izoferment composition in process of the selection, but also here there are small changes: the activity of the zones 0.8 and 0.26 has increased, the activity of the zone 0.75 has weakened.

The similar results were received and by other researchers on different insect species. For instance, in resistant to several insecticides cockroach *Blatella germanica* three from ten izoferments possessed the more high activity as compared with sensitive individuals (Riskallah, 1983). The intensity of the zone one of the izoferments (E6) in resistant to malathion mosquitoes *Aedes aegypti* was well above, than in sensitive insects (Tan et al., 1988). New izoferment were induced in gnats of the genus *Simulium*, resistant to DDT and pyrethroids (Ishaaya, Casida, 1981). The spectrum of izoferments markedly differed in the mosquito population *Culex pipiens*, resistant to malathion and temephos (Prabhakaran, Kamble, 1996), as well as in resistant to prophenphos individuals tobacco budworm *H. virescens* (Field et al., 1984).

The comparison of the izoferment esterase spectrum with the general esterase activity allows to draw a conclusion that exactly light fractions of these ferments (with Rf 0.8 for OP and pyrethroids and with Rf 0.8 and 0.75 for SHI chlorfluazuron) take over first blow of the action of xenobiotics and try to reflect it, enlarging its activity. Our data differs from those, received in diamondback moth *Plutella xylostella* (Maa, Liao, 2000; Shankara, Sannaveerappanavar, 2009) at which slow moving (heavy) fractions took part in resistance forming to insecticides. Probably, it explains by species specificity of the insects.

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Insecticidal resistance status of newer insecticides vis –a- vis conventional insecticides against American bollworm, *Helicoverpa armigera* Hubner, (Lepidoptera: Noctuidae)

ABSTRACT
The American bollworm *Helicoverpa armigera* (Hubner) is one of the most destructive pests of cotton, legumes and more than 100 other plant species. In the present study, the status of newer insecticides vis –a- vis conventional insecticides in Khandwa population of *H. armigera* was estimated using the standard topical application procedure. The mean resistance frequencies of cypermethrin 0.1µg/µl, fenvelerate 0.2µg/µl, endosulfan 10µg/µl, quinolphos 0.75µg/µl, chloropyriphos 1.0µg/µl, methomyl 1.2µg/µl and spinosad ranged from 65.34 to 93.75, 69.38 to 95.14, 3.04 to 46.88, 8.13 to 50.35, 16.38 to 63.55, 4.13 to 50.70 and 0.00 to 21.53 per cent respectively. Of all the insecticides tested spinosad is the most effective, resistance to spinosad was the lowest among the insecticides.

Key wards: Cotton, *Helicoverpa armigera*, insecticide resistance, newer insecticide, conventional insecticides

INTRODUCTION

American bollworm *Helicoverpa armigera* (Hubner) is one of the major pests of cotton, legumes and more than 100 other plant species (Bhatnagar *et al*; 1982). During the 1980's *H. armigera* emerged as a major pest of cotton besides other crops in India. The crop losses due to *H. armigera* are commonly more than half the yield, and annual losses to cotton and pulses alone have been estimated at $ 290-350 million (King, 1994). This pest has developed the resistance to all the major insecticide classes and its becoming increasingly difficult to control their population in India, (Armes *et al.*, 1992; Kranthi *et al*., 1997 and Mc caffery *et al*., 1989).

Insecticides have adverse effects on natural predators and parasites of bollworms, beneficial insects, human health and micro organism such as earthworm, blue green algae and nitrogen fixing bacteria. Over use of insecticides also leads to environmental pollution (soil and water), increase in cost of cultivation, resurgence of minor pests and development of resistance in insects against insecticides. Since *H. armigera* has a strong propensity to develop resistance against different group insecticides, there is a need to study the status of newer molecules vis-à-vis with conventional insecticides. Such kind of studies will be helpful in using different group of molecules with care and preserving the usefulness of insecticides in pest management programme, and in further delaying the development of resistance in the meantime. Keeping these things in view, the present studies were contemplated to determine the relative toxicity of synthetic molecules with diversified modes of action against this pest in laboratory to provide organised guidance for the selection of insecticides that can be incorporated in pest management programmes.

MATERIALS AND METHODS

The present study was conducted at Regional Agriculture Research Station, B.M. College of Agriculture, Khandwa (Madhya Pradesh), India during 2004-05 and 2005-06.

Insecticides

The discriminating doses of insecticides belonging to different groups i.e. cypermethrin 0.1µg/µl, fenvelerate 0.2µg/µl (Pyrethroids), endosulfan 10µg/µl (cyclodines), quinolphos 0.75µg/µl, chloropyriphos 1.0µg/µl (organophosphates), methomyl 1.2µg/µl (Carbamates) and spinosad 1.5µg/µl (Spinosyns) were used for the present study.

Rearing and Bioassay of *H. Armigera*

The eggs of *H. armigera* was collected from field sites from cotton plants by breaking off plant parts and transporting them in cloth bags placed in an ice box to the laboratory. In the laboratory, eggs were removed from the plant parts using a hairbrush moistened with an aqueous solution of 0.1 percent sodium hypochloride. The eggs were placed on the side wall @ two eggs per cell in a 12- well LINBRO tissue culture rearing tray containing chickpea based semi- synthetic diet. A semi- permeable film was stretched over each tray before closing the lid. Once the larvae attain 30-40mg weight (third-fourth instar), these was randomly assigned to topical application treatments. The technical insecticide in acetone was applied as 1.0 µl drop (by Hamilton repeating dispenser), to the thoracic dorsum of test insect. A larva was kept individually in 12-well LINBRO tissue culture plates containing semi synthetic diet, at 25 ± 2^0c under a 12:12 light: dark regime for six days (144hrs) and larval mortality was assessed after every 24hrs.

The weekly standard errors for the discriminating dose data were calculated by the following formula:
Weekly pooled binomial standard error

$$= \sqrt{p- (100-p)/n-1)}$$

Where, p= % larvae surviving in discriminating dose

n= total no. of larvae tested

The percent survival (resistance) was also calculated by using formula as given below-

Percent Survivorship (resistance)

= (1- number of dead larvae/number of larvae dosed) X 100

RESULTS AND DISCUSSION

The fluctuation of resistance frequencies of *H. armigera* were reported (Table-1, 2 and 3) over standard meteorological week (from 36th to 50th standard week) for different discriminating doses of insecticides.

Table-1: Seasonal per cent resistance in *Helicoverpa armigera* to different insecticides (2004-05)

SMW	Cypermethrin 0.1µg/µl	Fenevalerate 0.2µg/µl	Endosulfan 10µg/µl	Chloropyriphos 1.0µg/µl	Quinalphos 0.75µg/µl	Methomyl 1.2µg/µl	Spinosad 1.5µg/µl
36	66.67 ± 6.88*	68.75 ± 6.76	2.08 ± 2.08	6.25 ± 3.53	18.75 ± 5.69	6.25 ± 3.53	0.00 ± 0.00
38	66.67 ± 6.88	68.75 ± 6.76	6.25 ± 3.53	8.33 ± 4.03	22.92 ± 6.13	10.42 ± 4.46	0.00 ± 0.00
39	68.75 ± 6.76	70.83 ± 6.63	8.33 ± 4.03	8.33 ± 4.03	25.00 ± 6.32	14.58 ± 5.15	4.17 ± 2.92
40	73.33 ± 5.76	76.67 ± 5.51	16.67 ± 4.85	11.67 ± 4.18	45.00 ± 6.48	16.67 ± 4.85	8.33 ± 3.60
42	79.17 ± 5.92	77.08 ± 6.13	20.83 ± 5.92	29.17 ± 6.63	45.83 ± 7.27	22.92 ± 6.13	8.33 ± 4.03
43	82.00 ± 5.49	80.00 ± 5.71	28.00 ± 6.41	40.00 ± 7.00	50.00 ± 7.14	28.00 ± 6.41	12.00 ± 4.64
44	82.00 ± 5.49	82.00 ± 5.49	28.00 ± 6.41	44.00 ± 7.09	58.00 ± 7.05	28.00 ± 6.41	16.00 ± 5.24
45	83.33 ± 5.44	85.42 ± 5.15	37.50 ± 7.06	45.83 ± 7.27	58.33 ± 7.19	31.25 ± 6.76	16.67 ± 5.44
47	89.58 ± 4.46	91.67 ± 4.03	45.83 ± 7.27	50.00 ± 7.29	58.33 ± 7.19	35.42 ± 6.98	20.83 ± 5.92
48	92.00 ± 3.88	94.00 ± 3.39	48.00 ± 7.14	50.00 ± 7.14	64.00 ± 6.86	40.00 ± 7.00	22.00 ± 5.92
49	91.67 ± 4.67	94.44 ± 3.87	50.00 ± 8.45	52.78 ± 5.92	66.67 ± 7.97	44.44 ± 8.40	22.22 ± 7.03
50	91.67 ± 4.67	94.44 ± 3.87	50.00 ± 8.45	52.78 ± 5.92	66.67 ± 7.97	47.22 ± 8.44	22.22 ± 7.03

* Weekly Binomial Standard Error

Table-2: Seasonal per cent resistance in *Helicoverpa armigera* to different insecticides (2005-06)

SMW	Cypermethrin 0.1µg/µl	Fenevalerate 0.2µg/µl	Endosulfan 10µg/µl	Chloropyriphos 1.0µg/µl	Quinalphos 0.75µg/µl	Methomyl 1.2µg/µl	Spinosad 1.5µg/µl
36	64.00 ± 6.86*	70.00 ± 6.55	4.00 ± 2.80	10.00 ± 4.29	14.00 ± 4.96	2.00 ± 2.00	0.00 ± 0.00
38	66.00 ± 6.77	72.00 ± 6.41	10.00 ± 4.29	12.00 ± 4.64	18.00 ± 5.49	6.00 ± 3.39	0.00 ± 0.00
39	70.83 ± 6.63	75.00 ± 6.32	12.50 ± 4.82	12.50 ± 4.82	18.75 ± 5.69	12.50 ± 4.82	2.08 ± 2.08
40	72.00 ± 6.41	74.00 ± 6.27	14.00 ± 4.96	18.00 ± 5.49	22.00 ± 5.92	16.00 ± 5.24	6.00 ± 3.39
42	77.08 ± 6.13	79.17 ± 5.92	16.67 ± 5.44	27.08 ± 6.48	27.08 ± 6.48	20.83 ± 5.92	6.25 ± 3.53
43	77.08 ± 6.13	83.33 ± 5.44	25.00 ± 6.32	27.08 ± 6.48	35.42 ± 6.98	29.17 ± 6.63	12.50 ± 4.82
44	86.11 ± 5.85	83.33 ± 6.30	27.78 ± 7.57	33.33 ± 7.97	44.44 ± 8.40	36.11 ± 8.12	13.89 ± 5.85
45	89.58 ± 4.46	87.50 ± 4.82	28.08 ± 6.48	35.41 ± 6.98	50.00 ± 6.84	37.50 ± 7.06	16.67 ± 5.44
47	92.00 ± 3.88	94.00 ± 3.39	34.00 ± 6.77	44.00 ± 7.09	52.00 ± 7.14	42.00 ± 7.05	18.00 ± 5.49
48	91.67 ± 4.67	94.44 ± 3.87	36.11 ± 8.12	44.44 ± 8.40	55.56 ± 7.10	41.67 ± 8.33	19.44 ± 6.69
49	95.83 ± 2.92	95.83 ± 2.92	39.58 ± 7.13	45.83 ± 7.27	56.25 ± 7.24	47.92 ± 7.29	18.75 ± 5.69
50	93.75 ± 3.53	95.83 ± 2.92	43.75 ± 7.24	47.92 ± 7.29	60.42 ± 7.13	54.17 ± 7.27	20.83 ± 5.92

*Weekly Binomial Standard Error

Table-3: Average seasonal per cent resistance in *Helicoverpa armigera* to different insecticides (04-05 and 05-06)

SMW	Cypermethrin 0.1µg/µl	Fenevalerate 0.2µg/µl	Endosulfan 10µg/µl	Chloropyriphos 1.0µg/µl	Quinalphos 0.75µg/µl	Methomyl 1.2µg/µl	Spinosad 1.5µg/µl
36	65.34 ± 6.87*	69.38 ± 6.66	3.04 ± 2.44	8.13 ± 3.91	16.38 ± 5.33	4.13 ± 2.77	0.00 ± 0.00
38	66.34 ± 6.83	70.38 ± 6.59	8.13 ± 3.91	10.17 ± 4.34	20.46 ± 5.81	8.21 ± 3.93	0.00 ± 0.00
39	69.79 ± 6.70	72.92 ± 6.48	10.42 ± 4.43	10.42 ± 4.43	21.88 ± 6.01	13.54 ± 4.99	3.13 ± 2.50
40	72.67 ± 6.09	75.34 ± 5.89	15.34 ± 4.91	14.84 ± 4.84	33.50 ± 6.20	16.34 ± 5.05	7.17 ± 3.50
42	78.13 ± 6.03	78.13 ± 6.03	18.75 ± 5.68	28.13 ± 6.56	36.46 ± 6.88	21.88 ± 6.03	7.29 ± 3.78
43	79.54 ± 5.81	81.67 ± 5.58	26.50 ± 6.37	33.54 ± 6.74	42.71 ± 7.06	28.59 ± 6.52	12.25 ± 4.73
44	84.06 ± 5.67	82.67 ± 5.90	27.89 ± 6.99	38.67 ± 7.53	51.22 ± 7.73	32.06 ± 7.27	14.95 ± 5.55
45	86.46 ± 4.95	86.46 ± 4.99	32.79 ± 6.77	40.62 ± 7.13	54.17 ± 7.02	34.38 ± 6.91	16.67 ± 5.44
47	90.79 ± 4.17	92.84 ± 3.71	39.92 ± 7.02	47.00 ± 7.19	55.17 ± 7.17	38.71 ± 7.02	19.42 ± 5.71
48	91.84 ± 4.28	94.22 ± 3.63	42.06 ± 7.63	47.22 ± 7.77	59.78 ± 6.98	40.84 ± 7.67	20.72 ± 6.31
49	93.75 ± 3.80	95.14 ± 3.40	44.79 ± 7.79	49.31 ± 6.60	61.46 ± 7.61	46.18 ± 7.85	20.49 ± 6.36
50	92.71 ± 4.10	95.14 ± 3.40	46.88 ± 7.85	50.35 ± 6.61	63.55 ± 7.55	50.70 ± 7.86	21.53 ± 6.48

* Weekly Binomial Standard Error

Resistance in *H. armigera* to cypermethrin:
The resistance frequency exhibited against cypermethrin 0.1µg/µl discriminating dose ranged from 66.67 per cent to 92.0 per cent on cotton during 2004-05 from 36th to 48th standard week. However, the resistance frequency showed during 2005-06 season to cypermethrin0.1µg/µl ranged from 64.0 to 95.83 per cent from 36th to 49th standard week. The higher 92.0 per cent (48th SMW) and 95.83 per cent (49th SMW) resistance was recorded during 2004-05 and 2005-06 respectively. Findings of both the seasons revealed that the resistance frequency in *H. armigera* to cypermethrin0.1µg/µl ranged from 65.34 to 93.75 per cent. The higher 93.75 per cent resistance was recorded during 49th standard week. The lower 65.34 per cent resistance was observed 36th standard week.

Resistance in *H. armigera* to fenvelerate:
The per cent resistance of *H. armigera* larvae at 0.2 µg discriminating dose of fenvalerate was ranged from 68.75 to 94.44 per cent and 70.0 to 95.83 per cent during 2004-05 and 2005-06 respectively. The peak 94.44 per cent (2004-05) and 95.85 per cent (2005-06) resistance was found in 49th and 50th standard week during both the season. Based on two year (2004-05 & 2005-06) data, resistance frequencies ranged from 69.38 to 95.14 per cent. The higher (95.14 per cent) resistance was recorded in 49th and 50th standard week while, lower (69.38%) resistance was observed in 36th standard week.

Resistance in *H. armigera* to endosulfan:
Resistance to cyclodine insecticide endosulfan 10 µg was ranged from 2.08 to 50.0 per cent and 4.0 to 43.75 per cent from 36th to 50th standard week during 2004-05 and 2005-06 crop seasons respectively. The lowest (2.08 per cent) & (4.0 per cent) resistance was found beginning of the season (36th standard week) during 2004-05 and 2005-06 respectively, resistance was gradually increased during the season. Based on two years mean data, resistance to endosulfan in *H. armigera* ranged from 3.04 to 46.88 per cent. The higher (46.88 per cent) resistance was recorded in 50th standard week. While, lower resistance frequency (3.04 %) was observed in 36th standard week.

Resistance in *H. armigera* to chloropyriphos:
For chloropyriphos at 1.0 µg discriminating dose the percent resistance frequency of *H. armigera* larvae, ranged from 6.25 to 52.78 during 2004-05 and from 10.0 to 47.92 during 2005-06. Based on two year data, resistance frequencies to chloropyriphos in *H. armigera* ranged from 8.13 to 50.35 per cent. The higher (50.35 per cent) resistance was recorded in 50th standard week. However, lower (8.13%) resistance was observed in 36th standard week.

Resistance in *H. armigera* to quinalphos:
Insecticidal resistance frequency in *H. armigera* to quinolphos 0.75 µg discriminating dose indicated that resistance frequency ranged from 18.75 to 66.67 per cent during 2004-05 and 14.0 to 60.42 per cent during 2005-06 seasons. Based on mean of two year, resistance to quinalphos was ranged from 16.38 to 63.55 per cent. The higher (63.55 per cent) resistance was observed in 50th standard week while, lower (16.38 percent) resistance was recorded in 36th standard week.

Between organophosphates groups of insecticides tested, the percent resistance for Chloropyriphos was towards lower side as compared to quinalphos.

Resistance in *H. armigera* to methomyl:
Resistance frequency observed in *H. armigera* to 1.2 µg of methomyl (a carbamate group of insecticides) was ranged from 6.25 to 47.22 percent during 2004-05 and from 2.0 to 54.17 per cent during 2005-06 seasons. The average resistance data of both seasons to methomyl indicated that resistance ranged from 4.13 to 50.70 per cent in *H. armigera*. The higher (50.70 per cent) resistance was observed in 50th standard week while, lower (4.13 percent) resistances was recorded in 36th standard week.

Resistance in *H. armigera* to spinosad:
The resistance frequency to new generation insecticide, spinosad 1.5 µg was ranged from 0.0 to 22.22 per cent and 0.0 to 20.83 per cent in *Helicoverpa armigera* larvae during 2004-05 and 2005-06 respectively. Based on average data of two year, resistance ranged from 0.00 to 21.53 per cent to spinosad in *H. armigera*. The higher (21.53 per cent) resistance was recorded in 50th standard week while, lower (3.13 per cent) resistance

was found in 39th standard week. There was no resistance observed during 36th and 38th standard week. The average resistance showed by the *H. armigera* larvae towards spinosad was found to be lower than rest of the insecticides tested over both the season.

In the present investigation on resistance of seven insecticides (newer and conventional) belonging to five groups indicated that the resistance was lower in Spinosad which belongs to spinosyns, a new and unique class of insect control agent. Spinosad is comprised primarily two macrocyclic lactones; spinosyn A and D, secondary metabolites produced by the actinomycete, *Saccharopolyspora spinosa*, under natural fermentation conditions. The mode of action of spinosad is two fold; the primary target side is the nicotinic acetycholine receptor, but the GABA receptor is also affected to some degree. Toxicity of spinosad may be attributed to their novel mode of action. During both the studies years no resistance was observed of the insecticide up to 38th SMW. Break in resistance was observed from 39th SMW and it reached at its peak in 50th SMW when 22.22 and 20.83 per cent larvae developed resistance during 2004-05 and 2005-06 respectively. The low level of resistance of spinosad on *H. armigera* also reported by Choudhary *et al*., (2004), Dayakar and Venkateswarlu (2004), Suryawanshi *et al*., (2004), Yadav and Choudhary (2006).

The break up of resistance in cyclodiene compound Endosulfan was the next to spinosad. The Endosulfan being used for years to gather against *H. armigera* by the farmers, even than its resistance break in 36th SMW during both the years and reached its peak in 50th SMW, when 50.0 percent larvae during 2004-05 and 43.75 per cent larvae 2005-06 showed resistance. Present findings are in agreement with Patel and Koshiya (1999), Dayakar and Venkatesverlu (2004), Gill (2005) and Yadav and Choudhary (2006) that lower resistance of Endosulfan against *H. armigera*.

Resistance to methomyl, a carbamate compound in *H. armigera* from south west Madhya Pradesh, was break in 36th SMW during both the years and reached its peak in 50th SMW. Thiodicarb and methomyl are the most widely used carbamates against *H. armigera*. The significant resistance to methomyl was recorded by Kranthi *et al.* (2001b), Gunning *et al.* (1996), Wu *et al.* (1996), and Armes *et al.* (1996). Dayakar and Venkatesvelu (2004) also reported 16.0 to 64.0 per cent resistance to methomyl against *H. armigera.* Suryawanshi *et al.* (2004) reported 62.26 per cent and Yadav and Choudhary (2006)35.48 and 38.89 per cent resistance.

The organophosphate compound Chloropyriphos and quinalphos are the most widely used insecticides in cotton pest management in Madhaya Pradesh apart from pyrethroids and Endosulfan and hence the resistance development to these insecticides was not uncommon. As quinalphos is the commonly used insecticide on cotton than Chloropyriphos, the per cent resistance was more in quinalphos compared to Chloropyriphos. Present findings are in accordance with Dayakar and Venketasuverlu (2004), Yadav and Choudhary (2006) 36.54 to 37.50 per cent and 60.61 to 64.39 per cent resistance to Chloropyriphos and quinalphos. Similar results of quinalphos resistance were also reported by Armes *et al.* (1996), Velu *et al.* (2004).

Resistance to pyrethroids was exceptionally high in parts of central India. Armes *et al.* (1996) reported that the most highly resistance population of *H. armigera* were generally found in the central and southern regions of India. They stated that it was primarily from these regions that reports of inadequate control were most frequent and concomitant numbers of insecticide application were high. The present study indicated that both pyrethroids cypermethrin and fenvalerate resistance frequencies were high throughout the two cropping season (2004-05 & 2005-06). In Madhya Pradesh high level of resistance in population of *H. armigera* was found against cypermethrin and fenvalerater by Choudhary *et al.* (2004) and Yadav and Choudhary (2006). Prior to this study the high level of resistance to cypermethrin and fenvalerate was recorded by Armes *et al*. (1996), Suryawanshi *et al.*(2004), Tikar *et al.*(2004). High levels of cypermetrhin and fenvalerate resistance frequencies in central India were also reported by Kranthi *et al.*(1997), Kranthi *et al.* (2001a, 2002), Arora *et al.*(2003). Rama Subramanian and Ragupathy (2004), Velu *et al.* (2004) also reported above 90 per cent to fenvalarate in test insect.

The highest percent of insecticides being used against *H. armigera* on cotton are between August and November. It was anticipated that the most rapid increase in resistance frequencies would occur, between September and December, as moths resulting from insecticide selected larvae mated and oviposited.
Except Spinosad, resistance was observed in all the other insecticides from the beginning of the pest population on the crop. A gradual increase in the per cent resistance was recorded in all the insecticides which reached on its peak in 48th and 49th SMW in synthetic pyrethroids (Cypermethrin & Fenvalerate), 49th and 50th SMW in cyclodiene (Endosulfan) and organophosphate (Chloropyriphos & Quinalphos) and 50th SMW in carbamate (Methomyl). *H. armigera* develop resistance against synthetic pyrethroids more

rapidly than other group of insecticides. Present findings indicated that Spinosad is superior in respect of resistance and may be included in IPM to replace the use of most resistant insecticides to the pest.

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Abstracts in Resistance Management

Pyrethroid resistance in Chagas disease vectors: The case of *Triatoma infestans* cuticle. Review

The development of insecticide resistance in the blood-sucker insect *Triatoma infestans* (Hemiptera, Reduviidae), the major vector of Chagas disease in South America, is threatening the effectiveness of current control programs in the region. Among recognized mechanisms of resistance, increased detoxification and/or reduced sensitivity of the target site have been frequently reported as the major cause of resistance in many insect pests (Hemingway and Ranson, 2000*;* Hardstone *et al.*, 2008). To a much lesser extent, insecticide penetration has been investigated; reduced penetration was reported to participate in combination with the other mechanisms (Motoyama *et al*., 1990; Delorme *et al*., 1988). In a model analyzing the interaction between different resistant genes, reduced penetration was proposed to contribute multiplicatively combined with any other resistance factors (Raymond et al., 1989). Increasing deltamethrin resistance ratios (RR) have been reported in various *T. infestans* populations in the Argentina-Bolivia border (RR ~100). Although nerve insensitivity related to the kdr gene has yet to be explored, attempts to explain the underlying resistance mechanism showed that the small differences in detoxifying enzyme levels detected between resistant and susceptible strains did not explain the resistance values observed (Santo Orihuela et al., 2008). We will briefly review compelling evidences of the participation of the bug cuticle in pyrethroid resistance, and will also discuss the potential of alternative biocontrol methods.

THE INSECT CUTICLE

The first barrier against insecticide penetration is the insect cuticle or integument formed by composite deposition layers, named epicuticle and procuticle, and the innner epidermal cell tissue layer, involved in synthesis and secretion of the major cuticle components. The insect epicuticle is the more external and thinner layer (0.1–3 µm width); its outermost surface is covered by thin deposits of lipids (from 10-various hundreds nm width). The major roles attributed to this waxy layer are preventing water loss, and participating in chemical communication; they have also been proposed to alter chemicals and insecticide penetration (Blomquist, 2003; Juárez, 1994). In *T. infestans*, the epicuticle lipids are mostly hydrocarbons, and diverse combinations of free and esterified fatty acids and fatty alcohols (Juárez and Calderón Fernández, 2007). The widest deposition zone is the procuticle with chitin and proteins as bulk components, a high variability in sclerotization degree, and also containing variable amounts of lipids embedded in this matrix (Vincent, 2002; Noble-Nesbitt, 1991). Removal of lipids, or inhibition of cuticular lipid synthesis in *T. infestans*, was shown to raise detrimental effects on insect development and water barrier properties (Juárez, 1994). Recently, the amount of surface hydrocarbon and cuticle thickness were shown to be linked to insecticide resistance. Capillary gas chromatography (CGC) coupled to mass spectrometry (MS) analyses revealed that pyrethroid-resistant bugs have significantly larger amounts of surface hydrocarbons (56.2 ± 6.4% higher) than susceptible specimens. Also, a significantly thicker cuticle was detected by scanning electron microscopy (32.1 ± 5.9 µm and 17.8 ±5.4 µm for pyrethroid-resistant and pyrethroid-susceptible, respectively) (Pedrini et al., 2009). To our knowledge, this was the first evidence that modifications in the cuticle structure are related to insecticide penetration resistance. Concurrently, overexpression of cuticular genes that were not involved in pyrethroid detoxification has been recently reported (Awolola *et al.*, 2009).

CUTICLE - INSECTICIDE INTERACTION

The mode of entry of contact pesticides is through the insect cuticle. Penetration resistance mechanism presumes some alteration occurs at the cuticle to restrict effective insecticide concentration at the target site. In susceptible *T. infestans*, insecticide penetration was shown to be uniform through the entire surface of the soft nymph cuticle, although restricted to the intersegmental junctions in the highly sclerotized adult cuticles (Fontán and Zerba, 1987). Furthermore, penetration was enhanced in lipid-free cuticles of *T.*

infestans nymphs; also, insects deprived of their protective lipid cover showed mortality values more than 2-fold higher than control insects topically treated with fenitrothion (Juárez, 1994). Deltamethrin penetration was compared between resistant (Py-R) and susceptible (Py-S) strains after topical application. Internal amount of deltamethrin in Py-S bugs was > 2-fold that detected in Py-R bugs ($P<0.05$) (Fig. 1).

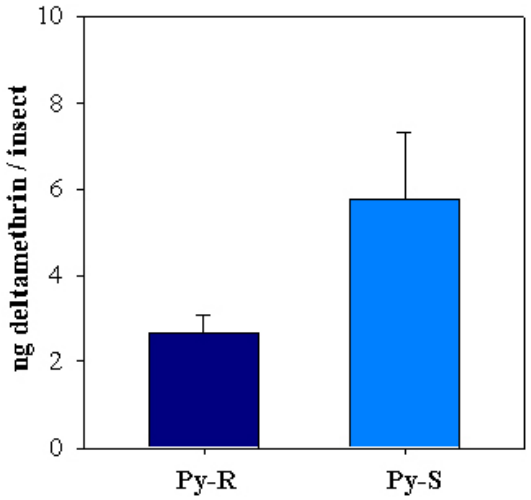

Figure 1: Deltamethrin penetration in *T. infestans* 4th instar nymphs.
Py-R: Resistant specimens, Py-S: Susceptible specimens
Prior to deltamethrin application, piperonil butoxide (PBO) was topically applied in order to prevent enzyme detoxification. Four hours later, deltamethrin was extracted from surface and internal tissues, with hexane and acetone, respectively; quantization was performed by CGC-MS analysis.

CUTICLE- ENTOMOPATHOGENIC FUNGI INTERACTION

The biochemical interaction between the entomopathogenic fungi *Beauveria bassiana* (Ascomycota: Hypocreales) and their host surface were studied by examining fungal ability to degrade the epicuticle layer; hydrocarbons were shown to be the preferred carbon source (Napolitano and Juárez, 1997). In addition to the innate fungal capability to utilize cuticle hydrocarbons, a whole set of fungal hydrocarbon-degrading enzymes can be further induced after growth in a hydrocarbon-enriched medium (Pedrini *et al*., 2007**)**. The entomopathogenic fungi ability to kill susceptible triatomine bugs has been proven in various laboratories (Lecuona *et al*., 2001). Furthermore, *B. bassiana* was pathogenic to *T. infestans*, regardless bug susceptibility to pyrethroids (Fig. 2). In addition, fungi adapted to degrade *T. infestans* cuticular hydrocarbons showed an enhanced ability to kill both susceptible and resistant bugs, thus providing further evidence of its unique potential to counter insecticide resistance (Pedrini et al., 2009).

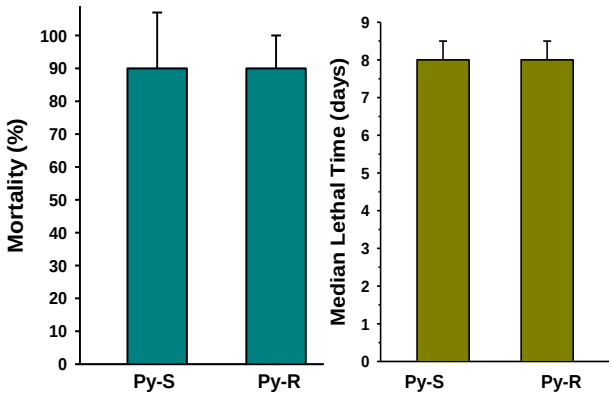

Figure 2: *B. bassiana* virulence parameters.
Py-R: pyrethroid-resistant; Py-S: pyrethroid-susceptible.
Parameters were estimated after exposure of fifth instar nymphs to a commercial *B. bassiana* formulation (1×10^8 conidia/ml for 6 seconds).

CONTROL OF PYRETHROID-RESISTANT *T. INFESTANS* WITH ENTOMOPATHOGENIC FUNGI

Among alternative control tools to control *T. infestans* resistant populations, we investigated the potential of *B. bassiana* in the field. Successful control trials were performed in rural villages located in the Argentina-Bolivia border and infested with pyrethroid-resistant bugs (Pedrini et al., 2009). A "trap and kill" device, based on manipulating *T. infestans* behavior, was designed in order to help contact with a virulent strain of *B. bassiana*. The device contained an attractant CO_2 source combined with a powder formulation of the *B. bassiana* strain GHA (Laverlam International, Butte, MT). Traps were both set on the walls and floor of houses. Two weeks after treatment, dead and alive insects were collected, and evaluated for fungal infection. After one intervention, roughly 50% of the collected bugs were killed by fungal infection. The causative agent of Chagas disease, the parasite *Trypanosoma cruzi*, is mostly acquired through contact with infected blood-feeding triatomine bugs. Based on available *T. infestans* population models, the impact of the bioinsecticide performance in reducing the risk of acquiring the parasite infection was estimated to drop more than 2-fold. According to this model, successive bioinsecticide applications will further reduce the number of infective bites per human per night, and thus are expected to significantly reduce the infection risk. Given that pyrethroid- resistant insects were unable to counter entomopathogenic fungal infection; these results might help to provide a safe and efficient alternative to overcome bug pyrethroid-resistance.

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Eretmocerus mundus Mercet (Hymenoptera; Aphelinidae) resistance to selected insecticides

Key words: *Eretmocerus mundus*, *Bemisia tabaci*, resistance, insecticides, imidacloprid, endosulfan, deltamethrin.

The sweet potato whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is an important pest with great capacity for virus spreading on vegetable and ornamental crops in the Mediterranean Basin. In this area, *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae) is one of the most effective natural enemies to control this pest (Greenberg *et al*., 2002; Stansly *et al*., 2005). High survival and efficiency of natural enemies to control pests when insecticide treatments are applied is one important challenge facing biological control. High rates of *E. mundus* natural parasitism has been observed on vegetable crops subjected to periodically insecticide treatments (Rodríguez-Rodríguez *et al*., 1994; González-Zamora *et al*., 1997; Stansly *et al*., 2005) which may indicate that natural populations of *E. mundus* could have resistance to certain insecticides.

The resistance levels to imidacloprid, endosulfan and deltamethrin were determined in *E. mundus* adults. A parasitoid population collected from an ornamental greenhouse was bioassayed and compared with a reference susceptible population. The LC_{50} resistance factor was 18.7 for imidacloprid, 2.6 for endosulfan and 3.5 for deltamethrin showing that the parasitoid can develop resistance to insecticides with diverse modes of action. After more than two years without pressure of insecticide treatments, adults of *E. mundus* lost the resistance to imidacloprid, whereas the resistance to endosulfan and deltamethrin was maintained.

When the susceptibility of adults and pupae of the parasitoid against different insecticide doses was

compared with the reference susceptible population, both developmental stages were equally susceptible to imidacloprid. In contrast, *E. mundus* adults were more susceptible than pupae to endosulfan and deltamethrin. These results may indicate that *E. mundus* pupae would have a high capacity to survive when these insecticides are applied to the crop.

Additionally, five populations of *E. mundus* were collected from commercial tomato and cucumber greenhouses, and insecticide resistance levels of adults and pupae were determined in comparison with the reference population. Both, pupae and adults, of all tested populations were resistant to deltamethrin, whereas only two were resistant to imidacloprid. Resistance to both insecticides was found simultaneously in two populations of *E. mundus*, indicating that the ability of this parasite to be multiresistant to insecticides with different mode of action is also present in vegetable crops. The capacity of *E. mundus* to be insecticide resistant may produce higher survival of the parasitoid when is necessary to complement biological control with insecticide treatments. Therefore, the presence of resistant *E. mundus* in commercial crops could be a major advantage regarding the implementation of conservative biological control programs in the area.

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THE PROBLEM OF THE INSECT AND MITE RESISTANCE TO INSECTOACARICIDES; RESISTANCE TO ORGANOCHLORINES AND ORGANOPHOSPHATES

Systematic use of insecticides and acaricides in agriculture to protect crops from pests has led to many populations of herbivores evolving, enabling their ability to transfer doses of toxins that are lethal to most other individuals of the same species. This ability, called resistance, is the most relevant for insects and mites. To date, resistance is awarded for almost all of harmful species, which is a regular struggle. Resistance leads to increased doses of the drug and the multiplicity of treatments and as a consequence - to the economic losses and environmental contamination. Often, after 4-6 years of application a pesticide becomes ineffective, and is replaced by another drug. However, resistance develops over time and to this new compound.

Number of species of arthropods that have resistant populations has been steadily increasing (Fig. 1). There are currently more than 700 species (Thacker, 2002), resistant to one or more insecticides.

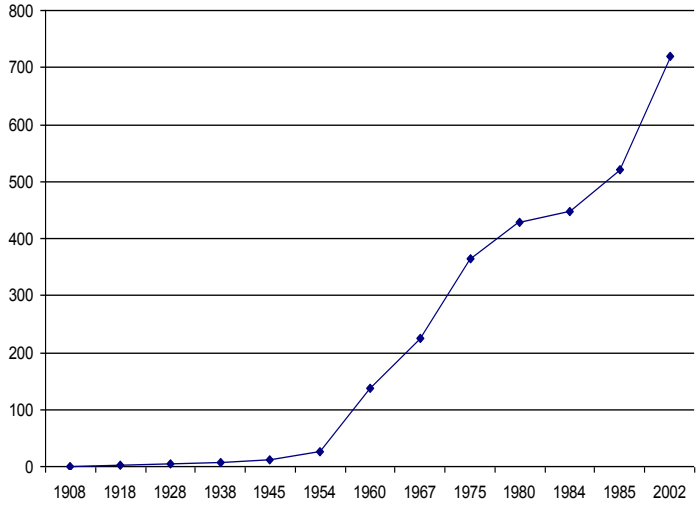

Figure 1: Dynamics of increase of the resistant species of arthropods in the world.

Almost all resistant species (98%) have agricultural or veterinary importance. By the beginning of the twentieth century in Russia the list included 46 species of pest arthropods - 40 insects and 6 mites (Sukhoruchenko, 2001).

Resistance in all organisms develops with varying speeds. In some species it develops quickly, in others slowly, still others may not occur, despite repeated treatment. For example, despite long-term treatments by DDT in a wheaten belt of the USA no evidence of resistance was found in a corn moth, whereas resistance in houseflies developed in many areas for 2-3 years in the application of the drug. Even within a species in different populations resistance may differ in speed of development. For example, Colorado potato beetle on Long Island showed a much greater propensity for resistance in comparison with the mainland population (Forgash, 1984).

It is obvious that the speed of development of resistance depends on the number of generations of the species in the year and, as a consequence the multiplicity of treatments. For example, the tick - a pest of fruit trees - *Panonychus ulmi*, which has a year to 10 generations, quickly developed resistance to many groups of insecticides. In the other fruit tick - *Bryobia rabri*, having a year only 2 generations, resistance was not detected (Georghiou, 1981).

In polifag, pest resistance develops more slowly than in monophagous. This may be due to two factors: firstly, the processing of gluttony being a minority of the population and the selection so it is less effective, and secondly, its possible influx of untreated-sensitive migrants. Perhaps this explains the resistance of cattle ticks in South America, first appearing in species, settled on a single host and only later - in having two or three hosts (Wharton, Roulston, 1970).

As an example of the negative influence of isolation, lack of flow of individuals, we can note the high resistance at house flies in California, found in populations inhabiting poultry houses, which are specially curtain to prevent migration of flies. Ironically, the protection from flies arriving from the outside probably contributed to a more high-level resistance (Georghiou, 1981).

The most frequently resistance is encountered in Diptera (35% of the total number of species), which reflects the strong pressure of selection. Significant quantities of resistant species are also marked in such agricultural important units, as lepidoptera (15%), beetles (15%), homoptera (10%), as well as in ticks (13%). Resistant species include major pests, as chemical control conducted with them mainly. With respect to chemical groups, resistance to ciclodienes detected in 62% of the species, to DDT - in 52%, to organophosphates (OP) - at 47%. Lower percentages were observed in a relatively recently used carbamate and pyrethroids (Georghiou, 1986). Later there was evidence of resistance to juvenile hormone analogues and chitin synthesis inhibitors (Cerf, Georghiou, 1978; Kotze, Sales, 2001), as well as the avermectins (Clark et al., 1995) and neonicotinoids (Horowitz et al., 2004).

The first object that had developed resistance to organochlorine compounds (DDT) was housefly *Musca domestica*. It was in 1946. In 1950 resistance in some populations of flies were also affected in Russia (Derbeneva-Ukhova, Morozova, 1950). Currently flies resistant to DDT are found almost everywhere. Resistance to organochlorine compounds in Russia showed malaria mosquitoes (Drobozina et al, 1972) and cotton moth (Zverev, 1980). In 1966, after 15 years application of aldrin and heptachlor in the vicinity of Edmonton (Canada) populations, the cabbage fly *Hylemya brassica* became resistant to these organochlorine compounds and have not lost it after 5 years of cessation treatments (McDonald, Swailes, 1975). The same kind of flies in France in one district showed high levels of resistance to aldrin (RI= 226), in other areas resistance was low (RI = 2-12), and resistance to dieldrin was practically unstable (Henneguon, Aude, 1975).

In 1975-76 in Czechoslovakia in spider mites *Tetranychus urticae*, collected in 35 greenhouses, resistance marked to tetradifone (RI =1,6-19,6) and thiometone (RI=1,5-208,5). Ticks collected on the plantations of hops were high-resistant to thiometone (Hunkova et al, 1983). The larvae of click beetles *Agnyphus variabilis* in New South Wales after prolonged use of lindane developed 30-100-fold resistance to this drug, as well as 500-fold for dieldrin and heptachlor (Gunning, Forrester, 1984). In several countries, there were pest stocks populations - weevils, grain grinder, mealworm beetles, sawtoothed grain beetle resistant to lindane (Roslavtseva, Didenko, 1981). Essential negative property of organochlorine pesticides tspecially DDT is their sharply expressed ability to persistence in soil, water and air. Therefore application these drugs in the majority of the countries is limited or it is forbidden it is absolutely forbidden.

When organophosphates replaced organochlorine pesticides problem of resistance has not ceased to be relevant. Among the arthropods of veterinary and medical significance housefly also the first acquired resistance to these compounds. In Denmark depending on the composition of the drug resistance to organophosphorus compounds (OP) in houseflies manifested in the years 1955-72. (Keiding, 1977). In

Czechoslovakia resistance index (RI) of houseflies to chlorophos reached 1000, to fenitrotion - 28, yodofenphos - 100 (Rupes et al, 1983). In the USSR high resistance to trichlorfon was noted after 10-14 years of its use in the cities Dushanbe, Minsk, Mytischi, Tashkent (RI> 100) (Gvozdev and others, 1976).

Studies carried out in Malta (Harris et al, 1976), showed the presence of resistance in natural populations of *M. domestica* to malathion and the increase their tolerance to chlorophos and fenchlorphos. For the year the resistance of houseflies to OP (diazinon, fenitrotione, dihlorphos) significantly increased on an island in Tokyo Bay (Yasutomi, 1975), to dichlorphos – on pastures in Nebraska (Boxler, Campbell, 1983). In Australia after 7 years of organophosphates treatments against larvae of the fly *Lucilia cuptina* populations resistant to diazinon were found in 1965 (Shanahon, Roxburgh, 1974). Resistance to stiriphos (up to 68h) was founded in populations of the horn fly *Haematobia irritans* in central and southern Georgia, USA (Sheppard, 1983), to chlorpyriphos in mosquito larvae *Culex quinguefascitus* in Tanzania (Curtis et al, 1984), to several organochlorine compounds in the greenhouse whitefly in France (Henneguon, Auge, 1981), to carbophos and trichlorfon in hothouse whitefly in Georgia (Lekveyshvili, 1984), to dihlorphos, protiophos and fentoate in *Plutella xylostella* in Japan (Myiato et al, 1982), to dichlorvos, prothiophos, tsianophos, isocsatione, dimethoate and dimetylvinphos in cabbage moth in Japan (Koshihara, 1988), to dicrotophos, metamidiphos, azinphos the Egyptian cotton cutworm *Spodoptera littoralis* in Egypt (El-Dahan, Saad, 1981), to chlorpyriphos-methyl and chlorpyriphos-ethyl in *Cydia pomonella* Switzerland (Charmilot, Pasquier, 2002).

Resistance to organophosphorus compounds is awarded in many beetles - pests stocks (grain). In saw-toothed grain beetle *O. surinamensis* in granaries of Queensland (Australia) recorded strained resistant to fenitrothion (PI=60), dinitrothion, malathion, pirimiphos-bromide, metacriphos (Heather, Wilson, 1985). In Bangladesh resistance to phosphine showed in *Sitophilus oryzae*, *Cryptolestes* sp. and *Rhyzopertiha dominica* (Halliday et al, 1983). In the US granaries populations of *Tribolium castaneum*, *R. dominica*, *Sitophilus* sp, *Cryptolestes* sp. resistant to malathion, fentoat were found (Haliscak, Perman, 1983). Resistance to OP was widely distributed among the secondary pests of apple: spider mites, leafhoppers, aphids, moths, snappers (Croft, 1988). In some cotton growing areas of Brazil mite *Tetranychus urticae* has a resistance to monocrotophos (Chiavegato et al, 1983). The other mite species, *T. kanzawai*, collected with vegetables and wild plants in Japan, showed a high level of resistance to several organophosphates (Kuwahara, 1984). Several greenhouse and field populations of *T. urticae* in Bulgaria have drug resistance on the basis of dimethoate and mevinphos, and partly difenprophos (Darakchieva, 1982).

Resistance to organophosphorus compounds was observed in hops, cotton, cereal and ordinary peach aphids. In England resistant populations of peach aphid first were discovered in the greenhouses and then in the field of sugar beet. In the western and southern Europe, Australia resistant individuals of this species were found at the beginning on a peach, and then - on potatoes, sugar beets and peppers (Perrin, 1983). In the cotton-growing areas of Tajikistan aphid *Aphis gossypii* and *Acyrthosiphon gossypii* showed 4-10-fold resistance to rogor (Ivanova, 1975).

Resistance to OP notes and for some is useful to arthropods. For example, in the field fozalon, fenitrothion, chlorpyriphos, azinphos-methyl and demeton-S-methyl in conventional doses were harmless to the predatory mite *Typhlodromus pyri* (Cranham et al, 1983) to allow the use of these compounds against pests of *Panonychus ulmi*.

Despite numerous examples of the development of resistance drugs of this class may still be used for pest control, because to some of them resistance forms very slowly. For example, in selected by phosmet and phocsim houseflies resistance to selectants developed slowly enough, for 30 selections RI has increased only in 2-4 time (Sokolyanskaya, 2007). Now organophosphorus compounds still are applied in agriculture, but even more often they are superseded by more modern insecticides.

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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, September 13, 2010
Monday, March 14, 2011

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

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