Resistant Pest Management Newsletter

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Resistance Management from Around the Globe

Susceptibility of *Anopheles arabiensis* (Diptera: Culicidae) adults to some commonly used agricultural insecticides in El Rahad Agricultural Corporation, Central Sudan

ABSTRACT
As with other insects, resistance to insecticides has become a limiting factor in the use of insecticides to control mosquitoes. Mosquito control depended on the use of insecticides for a long time (initially organochlorines, followed by organophosphates, carbamates and pyrethroids). This study aimed to determine the susceptibility of *Anopheles arabiensis* adult to the public health and agricultural insecticides, *viz*. DDT 4%, fenitrothion 1%, malathion 5%, bendiocarb 0.1%, propoxur 0.1%, deltamethrin 0.05% and *lambda*-cyhalothrin 0.05% at El Rahad Agricultural Corporation (RAC) area, Central Sudan, to provide base-line data about the *Anopheles* susceptibility, by determining their KDT_{50} and KDT_{95}. The WHO procedure was adopted. The number of knockdown (Kd) mosquitoes were recorded after 10, 15, 20, 30, 40, 50 and 60 min. of exposure. The percent mortalities after 24 hrs for each chemical were determined. The KDT50 and KDT95 were calculated with the 95% CL. These were as follows: for DDT 4% (34.84 and 74.41), in fenitrothion 1% (58.96 and 112.44), in malathion 5% (49.37 and 141.25), in bendiocarb 0.1% (97.20 and 292.06), in propoxur 0.1% (38.88 and 138.20), in deltamethrin 0.05% (58.30 and 147.82) and in *lambda*-cyhalothrin 0.05% (34.80 and 66.65) respectively. Some recommendations were listed: 1-The importance of annual monitoring and evaluation of insecticides commonly used in the area to evaluate the status of resistance. 2-Conducting bioassays for insecticides recommended but not commonly used. 3- Conducting bioassays for insecticides commonly used for other purposes and possibility for mosquitoes to come in contact with them. 4-Screening for new molecules with new modes of action.

Keywords: *Anopheles arabiensis*, DDT, fenitrothion, malathion, Bendiocarb, Propoxur, delta-methrin, lambda-cyhalothrin, knockdown time, Resistance, El Rahad Agricultural corporation Scheme, Central 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 Federal Ministry of Health (FMOH) in the year 2006 stated that malaria is a significant health problem in the Sudan, affecting 52% of outpatients and accounting for 9% of all hospitals deaths.

Estimating the burden of malaria is highly needed for evidence-based planning of malaria control. In Sudan, malaria has been the subject of a large amount of epidemiological, entomological and biomedical research (Abdalla *et al*., 2007).

The use of insecticides is the main strategy for controlling malaria vectors in the Sudan through indoor residual house spraying and, more recently, the use of insecticide-treated bed nets (ITNs) (WHO, 2005).

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* utilizes different types 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 from 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**).**

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. Insecticide resistance in *An. Arabiensis* has been reported in various African localities to commonly used insecticides in public health, like organochlorines (OCs) DDT, BHC, dieldrin, pyrethroids and other insecticides (Matambo, *et al*., 2007). Heimingway (1983) reported resistance to malathion (organophosphate, OP), DDT, dieldrin and permethrin (pyrethroids) insecticides in Sudan. Current surveys of *An. arabiensis* in the Sudan showed high levels of DDT, malathion and permethrin resistance in the Gezira and Central Sudan (Abdalla, *et al*., 2007 and El Gaddal, *et al*., 1985).

Several reports, workshops and scientists accused agricultural insecticide spraying, whether for the summer or the winter crops, as one of the major reasons behind the fast development of resistance in mosquitoes in general. Agricultural pesticides belong to organochlorines (OCs), e.g. endosulfan, Ops, *viz*. dimethoate, omethoate, malathion, chlorpyrifos, some carbamates, e.g. carbaryl , methomyl and aldicarb, and several pyrethroids, *e.g*. permethrin, deltamethrin, cypermethrin, alphamethrin, cyhalothrin, fenvalerate, etc.

Evidence of DDT resistance in *An. arabiensis* was clearly found in El Dwaim town, where DDT showed only 67.5% (±7) mortality rate. An. arabiensis was tolerated to DDT in Kosty, Kenana sugar cane and Assalaya sugar cane with mortality rates: 97% (±1), 93% (±1) and 95% (±2) respectively. The susceptibility of An. arabiensis to the pyrethroid insecticide Lambda cyhalothrin was 74% (±8) in Kosti town, 90% (±2) in Kenana, 94% (±2) in Assalaya, 88.75% (±3) in El Dwaim and 76% (±4) in Assalaya sugar cane area respectively (Ismail, 2009).

Current surveys of *An. arabiensis* in the Sudan showed high level of DDT and permethrin resistance in the central states of Sudan. The ministry of health, malaria vector control program relies mostly in the present studies, the investigation; the importance of the (kdr) mutation and potential enzyme-related in DDT resistance strain of *An. arabiensis* from Sennar state to the center of the Sudan was detected. Most colonies were exposed to insecticides from all four classes, approved for use in malaria vector control program (Abdella, 2007).

Insecticide susceptibility bioassay carried by Abdella (2007) the results showed 100% mortality on bendiocarb 54%-78% on permethrin, 55%-66% on DDT and 76%-78% on malathion. These finding have serious implication for malaria control program in Gezira state.

Susceptibility of *An. arabiensis* in Eastern Sudan to insecticide DDT, malathion and fenitrothion was 97.8%, 96.3% and 100 respectively. *An. arabiensis* is the sole malaria vector in the Eastern Sudan and is perennial rather than seasonal (Himeidan *et al.*, 2004).

A colony of *An. arabiensis* from the Sennar region of Sudan was selected for resistance to DDT. Adults from the F-16 generation of the resistant strain were exposed to all four classes of insecticides approved for use in malaria vector control and showed high levels of resistance to them all (24 hours mortalities: malathion 16.7%, bendiocarb 33.3%, DDT 12.1%, dieldrin 0%, deltamethrin 24.0% and permethrin 0%). Comparisons between the unselected base colony and the DDT-resistant strain showed elevated glutathione-Stransferase ($P < 0.05$) in both sexes and elevated esterases ($P < 0.05$) in males only. The Leu-Phe mutation in the sodium channel gene was detected by polymerase chain reaction and sequencing, but showed no correlation with the resistant phenotype. These results do not provide any explanation as to why this colony exhibits such widespread resistance and further studies are needed to determine the precise mechanisms involved. The implications for malaria vector control in central Sudan are serious and resistance management through the rotational use of different classes of insecticides is recommended (Matambo *et al.*, 2007).

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 compared 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 *An. arabiensis* mosquitoes in the Rahad Agricultural Corporation (RAC) area, the study aims at

determining the DDT 4%, fenitrothion 1%, malathion 5%, bendiocarb 0.1%, propoxur 0.1%, deltamethrin 0.05% and *lambda*-cyhalothrin 0.05% The present study investigates the susceptibility of *An. arabiensis* adults to the recommended public health insecticides and their counterparts (DDT 4%, fenitrothion 1%, malathion 5%, bendiocarb 0.1%, propoxur 0.1%, deltamethrin 0.05% and *lambda*-cyhalothrin 0.05%) that are used in the agricultural sector, viz. in the Rahad Agricultural Corporation (RAC). The study aims at determining the knockdown (KDT_{50} and KDT_{95}) to *An. arabiensis* and evaluates the level of resistance to these insecticides.

MATERIALS AND METHODS

The Study Area

EL Rahad agricultural areas, which are found at the East part of the Sudan, include a big agricultural scheme surrounded by many channels. It is covered by many types of vegetation which make a suitable habitat for mosquito's breeding sites. This study was carried out at (12.5°-14.5° N and 32.9°-35.4° E). The state is delimited from the North-east direction by the River Nile state, from the West by Khartoum and Gezira state, from the East by the Ethiopia boarders and from the South by the Blue Nile state.

The climate of the area is tropical continental with 600 mm of annual rain fall. There are three seasons per year; a dry winter from November to February with an average temperature of 27°C, a hot dry summer from March to May with an average temperature 32° C and a cooler rainy season from June to October with an average temperature 26° C. In general the land is flat, but in many places it is interrupted by the seasonal khors (Elnaiem, *et. al.*, 1997).

An. arabiensis existed throughout the year with two peaks, a major one at the end of the rainy season (September) during the irrigated season of the scheme. The second peak is most likely due to presence of breeding sites that were formed from the puddles of irrigation canals around the area. Thus *An. arabiensis* has become perennial instead of seasonal because of irrigation and agricultural practices. Similar findings were reported from an irrigated area in the Gezira scheme in central Sudan (Elgaddal *et al*., 1985).

Sampling

Sampling Sites

The study was carried out by collection of mosquitoes' aquatic stages from: village 10, village 13, village 18, village 19, village 20 and some channels and testing them.

Sample Design

Mosquito larva collection was carried out weekly March to April 2010.

Methodology

Insecticide Susceptibility/Resistance Tests

One- to three-day-old female mosquitoes were tested with different insecticides for their susceptibility, using the WHO standard protocol (WHO, 1998) under optimum conditions (temperature 26—29 ◦C and 70—80% relative humidity). The results were recorded for either dead or alive mosquitoes during 24 hours post-exposure. The test kits and the impregnated papers were obtained from the WHO Eastern Mediterranean Regional Office (EMRO) in Cairo, Egypt. Each test had five replicates on papers impregnated with the above-mentioned insecticides and a control tube with oil-treated paper, i.e. without insecticide for public health insecticides, and for their counter part of agricultural insecticides the impregnation was completed in the BNNICD and the insecticide procured from the ARC while the impregnated papers were brought from University of Gezira department of Pesticides and Toxicology. A total of 25 mosquitoes were used per tube. Knockdown was recorded after 1 hour and final mortality was recorded after 24 hours post exposure, during which time a 10% sugar solution was made available to survivors. Dead and surviving mosquitoes were stored separately in clearly labeled 1.5 ml tubes containing silica gel.

Specimen's Collection, Identification and Rearing

Anopheline larvae were collected from different breeding sites in the study area using standard larval collection kits including: dippers, screened netting, glass and plastic pipettes, plastic buckets, iron dishes and sorted out from other aquatic organisms. Larvae were kept in plastic bottles and bowls covered by cherish so as to protect them from the heat. After cleaning and isolating of predators in the field, we transferred them in the same day of collection to the insectary of BNNICD.

In the insectary larvae were reared and were being fed. When larvae became adults we put them in cages with fine meshes (156 meshes/ inch) to rest. I used (125) adult *Anopheles arabiensis* mosquitoes per test as exposure and another (25) adult *Anopheles arabiensis* mosquitoes per test as control.

Chemical Used in the Study

In this present study we chose certain chemicals used in the public health sector and recommended from WHO:

Class	Insecticides	Concentration %	Date of expiry
Organochlorines	DDT	4	January 2014
Organophosphates	fenitrothion	1	December 2010
	malathion	5	January 2011
Carbamates	bendiocarb	0.1	December 2011
	propoxur	0.1	January 2012

These same insecticide classes are widely used to control agricultural pests in Africa and this can pose additional selection pressure on mosquitoes when insecticide contaminated ground water permeates their larval habitats (Hilary *et al*, 2009).

On the other side we also tested an agricultural insecticides recommended.

Class	Insecticides	Concentration %	Date of expiry
Pyrethroid	deltamethrin	0.05	October 2010
Carbamate	*lambda*-cyhalothrin	0.05	October 2010

The WHO tubes for testing susceptibility of adult mosquitoes

The WHO tube test kit consists of two plastic tubes (125 mm in length, 44 mm in diameter), with each tube fitted at one end with a 16-mesh screen. One tube (exposure tube) is marked with a red dot, the other (holding tube) with a green dot. The holding tube is screwed to a slide unit with a 20 mm hole into which an aspirator will fit for introducing mosquitoes into the holding tube. The exposure tube is then screwed to the other side of the slide unit. Sliding the partition in this unit opens an aperture between the tubes so that the mosquitoes can be gently blown into the exposure tube to start the treatment and then blown back to the holding tube after the timed exposure (generally one hour). The filter-papers are held in position against the walls of the tubes by four spring wire clips: two steel clips for attaching the plain paper to the walls of the holding tube and two copper clips for attaching the insecticidal paper inside the exposure tube (WHO, 2006).

Data analysis

Data was subjected to probit statistical analysis to determine KDT_{50}, KDT_{95}.

RESULTS

Susceptibility tests

An. arabiensis females were collected (ca. 1050) and tested. A total of 42 replicates were used, each one containing 25 *An. arabiensis*, for all the agricultural and public health insecticides tested. All the agricultural insecticides were procured from the Agricultural Research Corporation. The number of knocked down mosquitoes were counted during the exposure time (one hour), and the overall mortality rates were recorded for each insecticide after 24 hours and analyzed using SPSS software program; probit analysis.

The WHO criteria for determining resistance/susceptibility were applied: 98—100% mortality indicates susceptibility; <80% mortality suggests resistance; and 80—97% mortality requires confirmation of resistance (WHO, 1998).

Assessment of resistance to seven insecticides to *Anopheles arabiensis* and detection of knockdown resistance (kdr), carried out in El Rahad area. The result observed from the study showed that *Anopheles arabiensis* is resistant to DDT 4% (88%± 3), deltamethrin 0.05% (72.8%± 7), malathion 5% (59.2%± 2) and propoxur (54.4%± 10), while it is highly resistant to bendiocarb (12.8%± 2). It is found tolerant to fentrothion 0.1% (96.8%± 2) although it was found susceptible to *lambda*-cyhalothrin 0.05% (99.2%± 0.8) according to WHO criteria (Table 1, Fig. 1). During bioassay, control showed no mortality.

Table (1): Insecticides Percentage of mortality rates after 24 hours post exposure for the *An. arabiensis* 2010

No	Insecticides	No tested (Replicates)	No Killed (24 hrs)	% mortality (Mean)	(± SE)
1-	DDT	150 (6)	110	88 %	(± 3)
2-	Fenitrothion	150 (6)	121	96.8 %	(± 2)
3-	Malathion	150 (6)	74	59.2 %	(± 9)
4-	Bendiocarb	150 (6)	16	12.8 %	(± 2)
5-	Propoxur	150 (6)	68	54.4 %	(± 10)
6-	Deltamethrin	150 (6)	91	72.8 %	(± 7)
7-	Lambdacyhalothrin	150 (6)	124	99.2 %	(± 0.8)

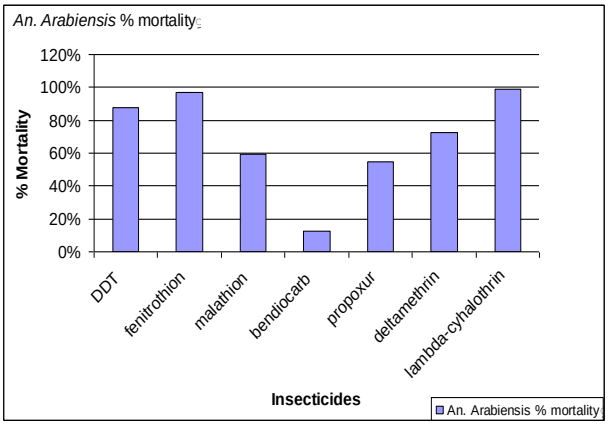

Figure (1): *An. Arabiensis* Percentage mortality, after 24 hours exposure to the tested chemicals.

Knockdown bioassay

Knockdown susceptibility status of *Anopheles arabiensis* to insecticides; the adult mosquitoes were exposed to the insecticides recommended by WHO using adult susceptibility test according to the standard procedure. The number of mosquito's knockdown after 10, 15, 20, 30, 40, 50 and 60 minutes were recorded. Knockdown effects of the tested insecticides to the *Anopheles arabiensis* population collected from El Rahad presented in table (2). The results indicated that the *Anopheles arabiensis* was found to be resistant to all insecticides tested. The susceptibility bioassay results showed the knockdown times KDT_{50} and KDT_{95} for all the insecticides used; calculated using log-time and probit-mortality regression models.

Table (2): KDT_{50} and KDT_{95} for *An. arabiensis* collected from El Rahad area 2010 that expose to the tested insecticides:

No	Insecticides	No tested (Rep.)	KD (1hr) % mortality	KDT50 minute (95% CL)	KDT95 minute (95% CL)	Test significance
1-	DDT	150 (6)	87.2 %	33.58 (27.48-41.09)	79.06 (61.76-101.07)	X^2=9.7 df=4 P= 0.04
2-	fenitrothion	150 (6)	52.0 %	78.63 (50.45-62.89)	358.28 (75.41-299.1)	X^2=13 df=4 P= 0.01
3-	malathion	150 (6)	53.2 %	49.91 (31.66-79.13)	157.82 (85.56-716.31)	X^2=23.48 df=4 P= 0.00
4-	bendiocarb	150 (6)	21.6 %	106.08 (77.21-174.38)	423.03 (166.32-1327.79)	X^2=6.47 df=4 P= 0.14
5-	propoxur	150 (6)	71.2 %	39.81 (33.97-43.15)	140.17 (109.48-202.37)	X^2=7.83 df=4 P= 0.001
6-	deltamethrin	150 (6)	51.2 %	64.62 (53.85-66.86)	191.25 (108.15-299.06)	X^2=5.21 df=4 P= 0.36
7-	*lambda*-cyhalothrin	150 (6)	90.4 %	35.38 (32.78-36.67)	67.46 (61.55-73.82)	X^2=5.24 df=4 P= 0.00

During the exposed time of mosquitoes to insecticides numbers of mosquitoes knocked down were recorded after 10, 15, 20, 30, 40, 50 and 60 minutes of exposure. The study observed that in DDT 4% the percent mortalities after 24 hrs were 0%, 0%, 16%, 42.4%, 61.6%, 78.4% and 87.2% respectively. In fenitrothion 1% the percent mortalities after 24 hrs were 0.8%, 4.8%, 9.6%, 8.8%, 16%, 42.4% and 52% respectively. In malathion 5% the percent mortalities after 24 hrs were 0%, 0%, 5.6%, 34.4%, 43.2%, 48% and 53.6% respectively. In Bendiocarb 0.1% the percent mortalities after 24 hrs were 0.04%, 0.05%, 0.02%, 6.4%, 12.8%, 24% and 21.6% respectively. In Propoxur 0.1% the percent mortalities after 24 hrs were 5.6%, 4.4%, 26.4%, 37.6%, 50.4%, 63.2% and 71.2% respectively. In deltamethrin 0.05% the percent mortalities were 0%, 1.6%, 4%, 7.2%, 25.6%, 35.2% and 51.2% respectively. In *lambda*-cyhalothrin 0.05% the percent mortalities were 0%, 1.6%, 4%, 43.2%, 64.8%, 80% and 90.4% respectively.

The quick knockdown time minutes were obtained by DDT (KDT_{50}= 33.58) and *lambda*-cyhalothrin (KDT_{50}= 35.38) to *Anopheles arabiensis* followed by propoxur (KDT_{50}= 39.81), malathion (KDT_{50}= 49.91), deltamethrin (KDT_{50}= 64.62), fenitrithion (KDT_{50}= 78.63), while the latest knockdown obtained by bendiocarb (KDT_{50} = 106.08) (Table 2 and Fig. 2, 3, 4,

5, 6, 7 and 8). With regard to the KDT_{95} seeming that the quick knockdown time minutes were obtained by *lambda*-cyhalothrin (KDT_{95}= 67.49) and DDT (KDT_{95}= 79.06) which indicate that the population more homogenous to *lambda*-cyhalothrin and DDT than other insecticides (Table 2 and Fig. 2, 3, 4, 5, 6, 7 and 8).

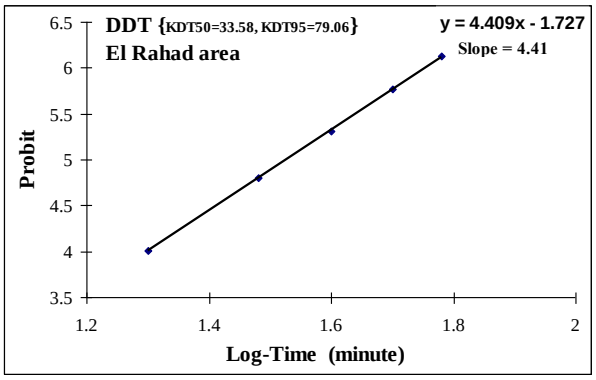

Figure (2): Regression line of log time knockdown of DDT to *Anopheles arabiensis* collected from El Rahad area after 60 minute of exposure

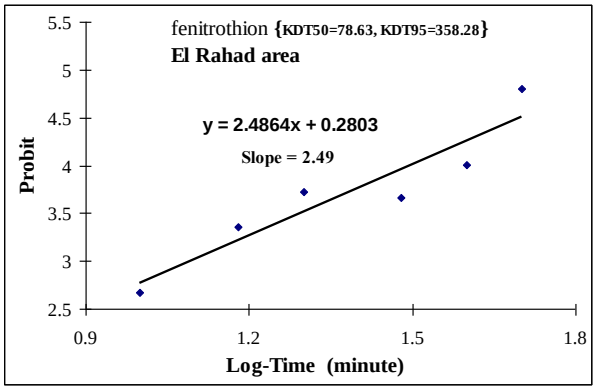

Figure (3): Regression line of log time knockdown of fenitrothion to *Anopheles arabiensis* collected from El Rahad area after 60 minute of exposure

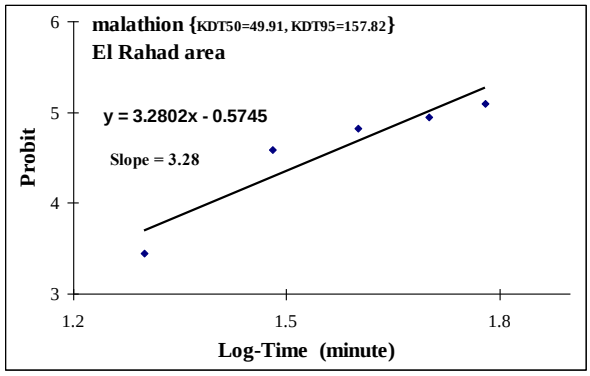

Figure (4): Regression line of log time knockdown of malathion to *Anopheles arabiensis* collected from El Rahad area after 60 minute of exposure**.**

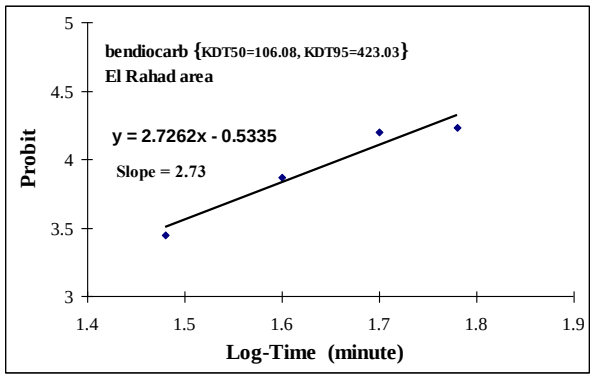

Figure (5): Regression line of log time knockdown of bendiocarb to *Anopheles arabiensis* collected from El Rahad area after 60 minute of exposure**.**

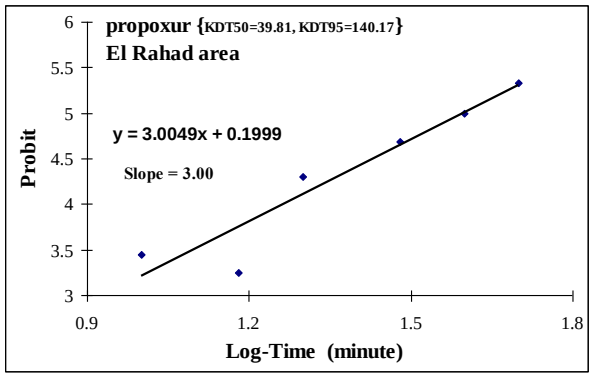

Figure (6): Regression line of log time knockdown of propoxur to *Anopheles arabiensis* collected from El Rahad area after 60 minute of exposure**.**

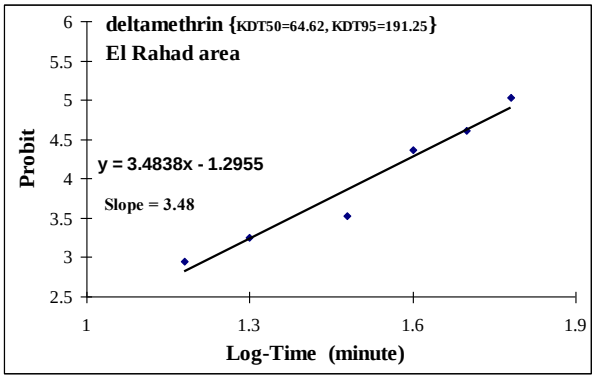

Figure (7): Regression line of log time knockdown of deltamethrin to *Anopheles arabiensis* collected from El Rahad area after 60 minute of exposure.

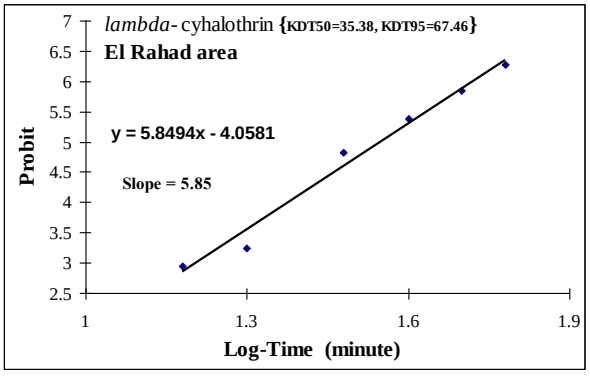

Figure (8): Regression line of log time knockdown of *lambda*-cyhalothrin to *Anopheles arabiensis* collected from El Rahad area after 60 minute of exposure

DISCUSSION

This entomological study was carried out in an agricultural area in eastern Sudan. *An. arabiensis* was the main vector (99.9%) found in the area; only 0.1% was *An. pharoensis* and no other species were detected. This agrees with a previous study from the nearby area (Gedaref in the eastern Sudan where *An. arabiensis* was the main vector, besides 2 other species, *An. pharoensis* and *An. funestus* (Hamad *et al*., 2002). *Anopheles arabiensis* and *An. gambiae* are the only species of the *An. gambiae* complex reported in Sudan (Petrarca *et al*., 2000; Zahar, 1985). Previous studies carried out in central Sudan based on morphology and cytogenetics identified only *An. arabiensis* were found (El Gaddal *et al*., 1985; Petrarca *et al*., 2000).

In Africa, resistance to pyrethroid insecticide in malaria vector mosquitoes may become a major problem for malaria interventions because pyrethroids are the mainstay of vector control strategies (WHO, 2000). According to the WHO criteria for characterizing insecticide susceptibility; *Anopheles species* was found to be resistance to DDT 4% 88% (±3) and evidence of DDT resistance in *An. arabiensis* was clearly found in other many semi areas like El Dwaim, where DDT showed only 67.5% (±7) mortality rate. Current surveys of *An. arabiensis* in the Sudan showed high level of DDT and permethrin resistance in the central states of Sudan. Also the insecticide susceptibility bioassay result showed 55.4-99.1% mortality on DDT 4% in Gezira and Sennar states (Hiba *et al*, 2007). Resistance to the organochlorines DDT and the now obsolete dieldrin was first reported in African malaria vectors in the 1950s and 1960s (Hilary *et al*, 2009).

Anopheles sp were found to be tolerant to fenitrothion 0.1% (96.8%) at the same time it found also tolerant in an irrigated area of eastern Sudan (96.3%) (Hamad *et al*., 2002). This result was agreed with central Sennar (96.6%) march 2005 (Dukeen, 2006).

The use of malathion for IRS in central Sudan was stopped in 1978 as a result of physiological resistance (El Gaddal *et al*., 1985). Insecticides from the organophosphate group are still widely used for agricultural purposes in the Gezira Agricultural Scheme (GAS, unpublished data) and presumably play an important role in selecting for resistance to this group of insecticides at this study *Anopheles sp* was found to be resistance to malathion 5% 59.2% (±9) and the semi result (56.3%) obtained from Khartoum state 2005 (Dukeen, 2006). On the other hand the results obtained from Gezira and Sennar states (Hiba *et al*, 2007) showed that the insecticide susceptibility bioassay result showed 76-100% mortality on malathion 5%. *Anopheles arabiensis* showed high resistant to bendiocarb (12.8%) and also it found resistance in Sennar state (33.3%). *An. arabiensis* was found resistance to propoxur (54.4%) in the study area and also it recorded low percentage of mortality in other areas like (47.5%) in Tabat and Wad Raiah in Gezira state in February 2005 (Dukeen, 2006).

Anopheles arabiensis was found resistance to deltamethrin 0.05% (72.8%), in Sennar *Anopheles arabiensis* was found highly resistance (24.0%),. while in El Maygooma, Khartoum state found at the same range (52-96%) (Dukeen, 2006). *An. arabiensis* was found susceptible to *lambda*-cyhalothrin 0.05% (99.2%) at El Rahad area, while in other studies it was found resistance in Kosti town (74%±8), in Kenana (90%±2), in Assalaya village (94%±2), in El Dwaim (88.75%±3) and in Assalaya sugar cane area (76%±4) (Ismail, 2009).

The KdT_{50} and KdT_{95} for *An. Arabiensis* collected from El Rahad area 2010 the most effective insecticides among the tested were *lambda*-cyhalothrin and DDT, followed by propoxur; then medium fenitrithion,

malathion and deltamethrin and the weakest is bendiocarb. The fastest in knockdown is DDT, followed by *lambda*-cyhalothrin and propoxur; the rest of the tested insecticides take from 64-106min. Thus indicates that resistances may happen because the lack of operational research in the area, proper application of the insecticides and the accurate using of techniques related with dosages and procedures. The highest values of the slope are recorded by *lambda*-cyhalothrin followed by DDT and the rest are from 2.5-3.5. The high values refer to the more homogeneity of the population of *An. Arabiensis* to the tested insecticides.

Possible factors influencing the frequency of resistant individuals observed in the study area were discussed. The results of this study highlight the importance of standardized longitudinal insecticide resistance monitoring and the urgent need for studies to monitor the impact of this resistance on malaria vector control activities.

These results come in line with Gedaref state ministry of health reports which, indicate that this area is the highest endemic area with malaria victims comparing with other areas of the State, this reflects that the intensive use of different insecticides in agricultural and public health sectors in this area could be the reason of resurgence of the malaria vectors and selection malaria vector resistant.

In conclusion, the findings on insecticide resistance or susceptibility have serious implications for the vector and pests control programs. A resistance management strategy through the rotational use of insecticides should be applied, but issues of cost and residual life would have to be taken into consideration. As it stands; resistance data collected during this study indicate vector control program failure, and whether this is due to insecticide resistance in *An*. *arabiensis*, poor spray coverage, incorrect dosages applied to the walls or breakdown of the insecticide this needs to be urgently addressed. The results of this study will enable informed selection of insecticides for vector control programs as well as provide baseline information essential in the monitoring of the development of insecticide resistance. It is important to note that the establishment of the El Rahad agricultural corporation in the area has resulted in a serious abundance of *An. arabiensis* throughout the year. More studies are needed in order to assess the role of malaria vector control and other pests.

REFERENCES

Abdalla, H. M. A. (2007) Insecticide resistance in malaria vectors in central Sudan, A dissertation, Faculty of Science, University of Witwatersrand, Johannesburg

Abdalla S.I, Malik E. M, Ali K.M., (2007). The Burden of malaria in Sudan .*Malaria Journal*, (6):97

Abdalla H., T. S. Matambo, L.L. Koekemoer, A.P. Mnzavae, R.H. Hunt and M. Coetzee, (2008). Insecticide susceptibility and vector status of natural populations of Anopheles arabiensis from Sudan. Royal Society of Tropical Medicine and Hygiene. (102), 263-271.

Clive Shif (2002). Integrated Approach to Malaria Control in "CLINICAL MICROBIOLOGY REVIEWS", p. 278–293-Copyright © 2002, American Society for Microbiology.

Coetzee, M., Craig, M. and Le Sueur, D. (2000). Distribution of African malaria mosquitoes belonging to the *Anopheles gambiae* complex. *Parasitology Today*, 16 (2), 74-77.

Dukeen M. Y. H., (2006). Summery Results of Insecticides' Susceptibility Tests Conducted in Sudan during the period (2004 – 06). FMoh. Khartoum –Sudan. June, 1st 2006.

El Gaddal A. A., Haridi M. A., Hassan F. T. And Hussein H., (1985). Malaria control in the Gezira-Managil Irrigated Scheme of the Sudan. *Journal of tropical medicine and hygiene*, (88):153-159.

Elnaiem D. A., Hassan H. K. and Ward R. D., (1998). Phlebotomine sand-flies in a focus of visceral leishmaniasis in a boarder area of eastern Sudan. *Routledge, part of the taylor and france group* . (91): 307-318.

El Gaddal, A. A. (1991).1985 The experience of the Blue Nile Health Project in the control of malaria and other water associated diseases. In: American Association for Advancement of Science: Malaria and development in Africa.

Gillies, M. T. (2001). Vector behaviour and bionomics, p.453–485. *In* W. Wernsdorfer and I. McGregor (ed.), Malaria. Churchill Livingstone, Edinburgh,United Kingdom

Hamad A. A., Nugud A.E.H.D., Arnot D.E., Giha H.A., Abdel-Muhsin A. M.A., Satti G.M.H.; Theander T.G., Creasey A.M.; Babiker H.A. and Elnaiem D.E.A., (2002). A marked seasonality of malaria transmission in two rural sites in eastern Sudan. *Acta trop*ica, (83): 71-82.

Hemingway, J.,(1983). Biochemical studies on Malathion resistance in *An.arabiensis* from Sudan. *Transaction of royal society tropical medicine and hygiene*. (77): 477-480.

Hilary R., Hiba A., Athanase B., Wamdaogo M. G., Clement K.-H., Elise Y. K., Fale N. S., Frederic S. and Maureen C., (2009).Insecticide resistance in *Anopheles gambiae*: data from the first year of a multi-country study highlight the extent of the problem. *Malaria Journal*, 8:299

Himeidan Y.E., Dukeen M. Y., El-Rayah , Adam I., (2004). *Anopheles arabiensis*: abundance and insecticide resistance in an irrigated area of eastern

Sudan. *East Mediterranean journal*. 10(1-2):167-74.

Ismail S. M.,(2009).Insecticide resistance in *Anopheles arabiensis* patton in White Nile state during the dry season.

Matambo, T.S., Abdalla, H., Brooke, B.D., Koekemoer, L.L., Mnzava, A., Hunt, R.H.,and Coetzee, M. (2007). Insecticide resistance in the malarial mosquito Anopheles arabiensis and association with the kdr mutation. Journal of Medical and Veterinary Entomology, (21), 97–102.

Petrarca, V.; Nugud A. D.; Ahmed, M. A.; Haridi, A. M.; Di Deco, M. A,; Coluzzi, M. (2000). Cytogenetics of the *Anopheles gambiae* complex in Sudan, with special reference to *Anopheles arabiensies*: relationship with East and West Africa populations: *Medical and Vterinary Entomology* 14 (2):149-164.

Roberts, D.; Manguin, S.; and Mouchet, J. (2000). DDT house spraying and re-emerging malaria. Lancet 356:330–332.

Weill, M.; Lutfalla, G.; Mogensen, K.; Chandre, F.; Berthomieu, A.; Berticat, C.; Pasteur, N.; Philips, A.; Fort, P.; Raymond, M. (2003). Comparative genomics: Insecticide resistance in mosquito vectors. Nature 423 (6936), 136–137.

WHO, (1975) Manual on practical entomology in malaria. Part II: methods and techniques. Geneva,

WHO, (1998). Test procedure for insecticide resistance monitoring in malaria vectors, Bio efficacy. WHO/ CDS/ CPC/ MAL/ 98.12, WHO, Geneva.

WHO, (2001). Draft guidelines on monitoring of insecticide resistance and mapping of malaria vectors in the eastern Mediterranean Region.

WHO, (2005). Atlas of insecticide resistance in malaria vectors of the WHO African region, Harare October 2005 WHO. ANVR (*African Network for Vector Resistance*).

WHO, (2006). Guidelines for testing mosquito adulticide for indoor residual spraying and treatment of mosquito nets. WHO/CDS/NTD/WHOPES/GCDPP/2006.3.

WHO, (2001). criteria and meaning of tests for determining the susceptibility or resistance of insect to insecticides VBC/81.6.

WHO, (2000). WHO expert committee on malaria. *World Health Organ Technical Report Ser*, (892): 1-71.

Zahar, A.R., (1985). Vector bionomics in the epidemiology and control of malaria. Part I, section III, (D) East Africa. World Health Organization, Geneva, WHO/VBC/85.3.

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Fungicidal activity of fluopyram for suppression of *Alternaria* species pathogenic on California pistachio

ABSTRACT
Fluopyram, a new succinate dehydrogenase inhibiting fungicide (SDHI), was recently registered for use in pistachio for the control of Alternaria late blight (ALB). Lack of disease control due to boscalid (the first SDHI labeled on pistachio) resistance has been documented in several California pistachio orchards. Because widespread resistance to boscalid is prevalent in pistachio, the sensitivity of 23 boscalid-resistant mutants and 18 boscalid-sensitive isolates of *Alternaria alternata* were tested for their sensitivity to fluopyram. The EC_{50} values of fluopyram did not show a clear distinction between resistant and sensitive isolates and showed a wide range of EC_{50} values. The EC_{50} ranged from 0.0813 µg ml^{-1} to values higher than 100 µg ml^{-1}. Twenty-three of the 41 isolates (56%) showed an EC_{50} value below 1 µg ml^{-1}. Only 4 isolates had an EC_{50} value above 10 µg ml^{-1}. The other 14 isolates showed an EC_{50} value between 1 µg ml^{-1} and 10 µg ml^{-1}. Based on the sensitivity distribution, a discriminatory concentration of 5 µg ml^{-1} was selected for monitoring sensitivity to fluopyram. A total of 125 isolates of *A. alternata* were collected in 2010 from commercial pistachio orchards and tested for resistance against boscalid and fluopyram in PDA amended with 10 and 5 ppm of each fungicide, respectively. 72 isolates showed resistance or reduced sensitivity to boscalid and 17 to fluopyram. The occurrence of known mutations in *AaSDH* genes was investigated using previously developed CAPS assays. This analysis showed that 50, 18, and 1 isolates had substitutions in subunits AaSDHB (H277Y, 48 mutants; H277R, 2 mutants), AaSDHC (H134R) and AaSDHD (H133R), respectively. Results from this study also indicated the existence of *A. alternata* boscalid resistant mutants showing a lack of sensitivity to fluopyram with uncharacterized mutations. Further DNA sequence analysis of *AaSDH* genes from these isolates could reveal mutations associated with these phenotypes. In light of these findings, concurrent use of fluopyram and boscalid for the control of ALB should be avoided.

INTRODUCTION

Alternaria late blight (ALB) caused by *Alternaria alternata*, *A. tenuissima*, and *A. arborescens* is an annual production concern for commercial pistachio growers in California. The disease affects foliage and fruits and under optimal conditions the fungus can defoliate a tree in late summer and autumn (Pryor and Michailides, 2002). Although cultural practices such as irrigation management and pruning, to increase air movement and decrease air humidity in the orchard, can help to manage ALB, the disease is best controlled

by the use of multiple fungicide applications. Fungicides registered for control of Alternaria late blight include sterol demethylation inhibitors (DMIs), respiration inhibiting fungicides including quinone outside inhibitors (QoIs), and succinate dehydrogenase inhibitors (SDHIs). Respiratory inhibiting fungicides have been a key component in Alternaria late blight fungicide spray programs. However, the rapid development of *Alternaria* populations resistant to these fungicides has caused serious challenges for pistachio growers. For instance, resistance to azoxystrobin (Abound®) has been detected in *Alternaria alternata, A. tenuissima,* and *A. arborescens* isolates from pistachio and almond orchards and cross resistance was determined to other registered QoIs (Ma et al., 2003; Luo et al., 2007; Avenot et al., 2008). The introduction of the first SDHI fungicide, boscalid in a pre-mixture along with pyraclostrobin (Trade name Pristine®) provided a high efficiency in controlling ALB even in orchards with widespread resistance to QoIs (Avenot et al., 2008). However, strains of *Alternaria* resistant to boscalid were detected within two seasons after the first registration and use of this fungicide on pistachio (Avenot and Michailides, 2007).

Fluopyram (*N*-[2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2 (trifluoromethyl) benzamide) is a new succinate dehydrogenase inhibitor (SDHI) recently launched by Bayer CropScience Co. The fungicide was recently registered on pistachio for ALB control as products premixed with demethylation inhibitor (DMI) tebuconazole (trade name: Luna experience) and QoI trifloxystrobin (trade name: Luna sensation). Resistance to boscalid in *Alternaria* was found to be associated with different mutations detected in the *SDH B, C,* and *D* genes of succinate dehydrogenase (Avenot et al., 2008, 2009). Pathogen populations that develop resistance to one fungicide simultaneously become resistant to other fungicides that are affected by the same gene mutation and the same resistance mechanism. Because widespread resistance to boscalid is prevalent in California pistachio orchards, it is crucial to assess the sensitivity of *Alternaria* to the new SDHI fluopyram and to generate data for the rational and sustainable use of this fungicide on pistachio. In a preliminary study, the sensitivity to fluopyram of selected *A. alternata* boscalid-resistant *SDHB, SDHC,* and *SDHD* mutants and wild-type isolates was determined by recording their relative mycelial growth in presence at 10 mg/ml fluopyram. All of the *A. alternata* isolates sensitive to boscalid were also sensitive to fluopyram while *A. alternata* boscalid-resistant isolates carrying the histidine to tyrosine mutation in the Ip gene were also found to be sensitive to fluopyram (Avenot and Michailides, 2009; 2010). *A. alternata* boscalid-resistant isolates carrying mutations in SDHC conferred a low resistance to fluopyram (Avenot and Michailides, 2010). These differences in cross-resistance patterns between SDHIs suggested differences in their intrinsic activity.

The objectives of this study were to (i) test the sensitivity of boscalid-resistant and -sensitive *Alternaria* isolates to fluopyram and select a discriminatory concentration to monitor the sensitivity to fluopyram, (ii) determine the level of sensitivity to boscalid and fluopyram for 125 isolates collected in 2010 from commercial pistachio orchard in different Counties of California, (iii) investigate if there is cross-resistance between boscalid and fluopyram, (iv) check whether any of the already known molecular mechanisms are determining this resistance.

MATERIAL and METHODS

Single-spored isolates of *A. alternata* were isolated from dormant buds collected from several pistachio orchards. Fungicides used were technical-grade fluopyram (a.i. 99.60% Bayer Crop Science Co.). Forty-one *A. alternata* isolates (28 boscalid-resistant mutants and 18 boscalid-sensitive isolates) from the collection of Kearney Agricultural Center were tested for their sensitivity to fluopyram. Fluopyram was dissolved in 100% acetone to obtain a stock solution of 10 g a.i. L^{-1} and aliquots were added to autoclaved PDA cooled to 45ºC to obtain PDA media amended with final concentrations 0 (control); 0.001; 0.005; 0.01; 0.05; 0.1; 0.5; 1.0; 5.0; and 10.0 µg fluopyram mL^{-1}. The concentration of acetone never exceeded 10 mL L^{-1}. A mycelial plug of 5 mm was taken from the edge of a culture and placed in the middle of a Petri dish containing media amended with fungicide or on the control. Each isolate was tested twice for the same concentration and the average value was used. After 6 days at 30ºC the radial growth of each isolate was measured (colony diameter). The original mycelial plug (5 mm) was subtracted from this measurement. Inhibition of growth was expressed as a percentage of the mean diameter of the untreated control. The EC_{50} value of an individual isolate was calculated by regressing the inhibition of mycelial growth against the log of the fungicide concentrations. To determine the discriminatory concentrate to discriminate between sensitive and resistant isolates to fluopyram, isolates were tested at 1, 2.5, 5, and 10 µg ml^{-1} fluopyram. Each isolate was tested twice for each fungicide and the mean growth (minus the original mycelial plug) was used to determine the inhibition of growth by the different fungicides. Based on the fluopyram sensitivity distribution and previous research by (Avenot and Michailides, 2010) the concentration of 5 µg ml^{-1} was selected as discriminatory concentration to monitor the sensitivity to fluopyram. The discriminatory concentration of 10 ppm boscalid was used to determine the sensitivity to boscalid. A mycelial

growth assay using discriminatory concentration for each fungicide was used to determine sensitivity to fluopyram and boscalid of 125 single-spore isolates of *A. alternata* isolated from different counties in California in 2010.

The mechanism of resistance to boscalid is caused by mutations resulting a substitution of a conserved histidine residue at position 277 in the AaSDHB subunit to tyrosine or arginine (Avenot et al., 2008b). Three different amino acid changes occurred in the AaSDHC (H134R) and AaSDHD (H133R and D123E) subunits (Avenot et al., 2009). Genomic DNA was extracted from mycelium of 10-days old colonies of 74 strains of *A. alternata* which were resistant or showed reduced sensitivity to one of the fungicides and 9 isolates that were sensitive to both fungicides. CAPS analyses were used to screen for the presence of the point mutation reportedly associated with boscalid resistance. Portions of the *AaSDHB*, *AaSDHC*, and *AaSDHD* genes potentially carrying mutations were PCR-amplified using relevant primers (Avenot et al., 2008, 2009) and directly digested with different restriction enzymes (Avenot et al., 2008, 2009) according to the manufacturer's instructions. Digestion products were then separated on agarose gels.

RESULTS and DISCUSSION

Use of the SDHI boscalid has been an important component in Alternaria late blight management programs. Fluopyram is a novel SDHI which was recently labeled in pre-mixed fungicides for use on pistachio. When resistance develops to a fungicide, all fungicides in the same chemical class are also affected with direct implications for disease management. Since widespread resistance to boscalid has been documented in pistachio orchards, we conducted this study to assess the sensitivity to of boscalid-resistant and -sensitive isolates to fluopyram. The EC_{50} values of fluopyram did not show a clear distinction between resistant and sensitive isolates and showed a wider range of EC_{50} values. They ranged from 0.0813 µg ml-^{1} to values higher than 100 µg ml-^{1}. Twenty-three of the 41 isolates (56%) that were tested showed an EC_{50} value below 1 µg ml-^{1}. Only 4 isolates had an EC_{50} value above 10 µg ml-1, of which two had a value above 100 µg ml^{-1}. The other 14 isolates showed EC_{50} values between 1 µg ml-^{1} and 10 µg ml-^{1} (Fig. 1). The discrepancy of cross-resistance pattern of fluopyram with a compound from the same cross-resistance group confirmed the higher intrinsic activity observed for this fungicide.

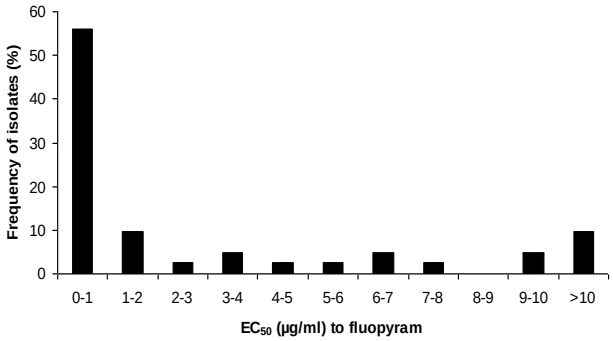

Fig. 1: Frequency distribution of effective concentration to inhibit 50% of mycelial growth (EC_{50} value) values to fluopyram for 41 *Alternaria alternata* isolates from the collection of Kearney Agricultural Research and Extension Center (University of California).

Routine monitoring of a large number of isolates is more easily accomplished by selecting a single discriminatory concentration. Mycelial growth tests performed at 1 µg ml-^{1}, 2.5 µg ml-^{1}, 5 µg ml-^{1}, and µg ml-^{1} fluopyram showed that the latter two concentrations could be used in monitoring studies as discriminatory concentrations for differentiating between sensitive and resistant isolates to fluopyram (data not shown). The sensitivities to boscalid and fluopyram were then tested for 125 isolates of *A. alternata* that were collected in 2010 from pistachio orchards in different counties in California. The entire experiment with 10 µg ml^{-1} boscalid and 5 µg ml^{-1} fluopyram was repeated. The mycelial growth inhibition values of the two experiments did not differ significantly (P = 0.595 and P = 0.185 for boscalid and fluopyram, respectively), therefore the average inhibition value of the two experiments was used in the data analysis. In total, 61 isolates were resistant to boscalid whereas 11 showed reduced sensitivity. There was only one isolate resistant to 5 µg ml^{-1} fluopyram and 16 other isolates had a reduced sensitivity for this concentration of fluopyram. 43 isolates were sensitive (>75% inhibition) to both fungicides (Table 1). Resistant isolates for fluopyram did not occur at the same spectrum as boscalid resistance. Forty-eight of the 83 tested isolate showed the presence of mutation H277Y. Two isolates showed the different mutation at the same codon, which resulted in another amino acid substitution (H277R). All these *SDHB* mutants were resistant to boscalid but these genotypes remained sensitive to fluopyram. Boscalid resistant isolates with H134-SDHC and H133R-SDHD alterations showed some reduced sensitivity to fluopyram and a cross resistance between the two SDHIs was found with lower resistance factors observed for fluopyram.

Table 1: Origin, phenotype, and mutation locations of the different genes from field isolates of *Alternaria alternata* tested at 10 µg ml^{-1} boscalid and 5 µg ml^{-1} fluopyram.

Isolate	Origin	Number[1]	Phenotype[2]			Mutation[3]
			Bosc.		Fluop.	
40-48-1	Madera county	73	R		S	N.F.
40-48-7	Madera county	47	RS		RS	N.F.
SVF-B3-9	Kern county	87	RS		RS	N.F.
KAC-1	Fresno county	32	S		RS	N.F.
40-42-4	Madera county	72	S		S	N.F.
SVF-B3-2	Kern county	77	S		S	N.F.
KAC-9	Fresno county	34	S		S	N.F.
Fag3	Kings county	71	S		S	N.F.
ORA2-2	Tulara county	88	S		S	N.F.
ORA-3-7	Tulara county	81	S		S	N.F.
GAT-3	Kern county	80	S		S	N.F.
COR-A-1	Kings county	66	S		S	N.F.
COR-A-2	Kings county	67	S		S	N.F.
DRU-F-6	Kings county	91	S		S	N.F.
Fag7	Kings county	28	R		S	H277Y (B)
23-01-06	Madera county	37	R		S	H277Y (B)
40-48-2	Madera county	41	R		S	H277Y (B)
40-48-4	Madera county	45	R		S	H277Y (B)
40-48-8	Madera county	48	R		S	H277Y (B)
40-48-9	Madera county	49	R		S	H277Y (B)
40-48-10	Madera county	74	R		S	H277Y (B)
40-42-5	Madera county	52	R		S	H277Y (B)
40-42-7	Madera county	51	R		S	H277Y (B)
40-42-8	Madera county	50	R		S	H277Y (B)
40-42-9	Madera county	79	R		S	H277Y (B)
39-18-3	Madera county	22	R		S	H277Y (B)
39-18-6	Madera county	23	R		S	H277Y (B)
DBGr7	Kern county	19	R		S	H277Y (B)
DBGr8	Kern county	20	R		S	H277Y (B)
Fag5	Kings county	27	R		S	H277Y (B)
Fag8	Kings county	29	R		S	H277Y (B)
ORA-3-6	Tulara county	59	R		S	H277Y (B)
SVF-B3-3	Kern county	62	R		S	H277Y (B)
SVF-E4-1	Kern county	75	R		S	H277Y (B)
GAT-1	Kern county	63	R		S	H277Y (B)
GAT-2	Kern county	70	R		S	H277Y (B)
GAT-5	Kern county	69	R		S	H277Y (B)
GAT-9	Kern county	78	R		S	H277Y (B)
GAT-10	Kern county	64	R		S	H277Y (B)
COR-A-10	Kings county	54	R		S	H277Y (B)
KAC-8	Fresno county	33	R		S	H277Y (B)
23-01-04	Madera county	55	R		S	H277Y (B)
23-01-05	Madera county	36	R		S	H277Y (B)
23-01-07	Madera county	38	R		S	H277Y (B)
23-01-09	Madera county	39	R		S	H277Y (B)
40-48-3	Madera county	44	R		S	H277Y (B)
40-42-1	Madera county	40	R		S	H277Y (B)
DBGr5	Kern county	17	R		S	H277Y (B)
DBGr6	Kern county	18	R		S	H277Y (B)
Fag9	Kings county	30	R		S	H277Y (B)
SVFA-5	Kern county	89	R		S	H277Y (B)
SVF-B3-1	Kern county	76	R		S	H277Y (B)
SVF-B3-6	Kern county	95	R		S	H277Y (B)
SVF-E4-6	Kern county	84	R		S	H277Y (B)
SVF-E4-9	Kern county	42	R		S	H277Y (B)
GAT-4	Kern county	92	R		S	H277Y (B)
GAT-6	Kern county	93	R		S	H277Y (B)
39-18-8	Madera county	24	RS		S	H277Y (B)
SVFA-6	Kern county	86	RS		S	H277Y (B)
SVF-E4-2	Kern county	83	RS		S	H277Y (B)
Fag2	Kings county	26	RS		S	H277Y (B)
GAT-7	Kern county	68	RS		S	H277Y (B)
SVFA-9	Kern county	90	RS		S	H277R (B)
SVF-E4-4	Kern county	61	RS		S	H277R (B)
DBGr4	Kern county	16	R		S	H277R (B) H134R (C)

DBGr9	Kern county	21	R		RS	H277Y (B) H134R (C)
23-01-01	Madera county	57	R		R	H134R (C)
23-01-02	Madera county	35	R		RS	H134R (C)
40-48-5	Madera county	46	R		RS	H134R (C)
40-42-10	Madera county	56	R		RS	H134R (C)
39-18-9	Madera county	25	R		RS	H134R (C)
DBGr1	Kern county	13	R		RS	H134R (C)
DBGr3	Kern county	15	R		RS	H134R (C)
Fag10	Kings county	31	R		RS	H134R (C)
ORA-3-8	Tulara county	58	R		RS	H134R (C)
SVF-B3-5	Kern county	94	R		RS	H134R (C)
SVF-E4-5	Kern county	43	R		RS	H134R (C)
40-42-3	Madera county	53	R		S	H134R (C)
DBGr2	Kern county	14	R		S	H134R (C)
SVF-E4-3	Kern county	60	R		S	H134R (C)
SVF-E4-8	Kern county	82	S		S	H134R (C)
COR-A-9	Kings county	65	S		S	H134R (C)
SVF-E4-7	Kern county	85	R		RS	H133R (D)
N[4] = 42						

[1] Number that is used in molecular analysis and electrophoresis agarose gels.
[2] Phenotype was based on relative mycelial growth: R=<50, RS = 50-75, S=>75
[3] Mutation found by running PCR with known primers with gene (B, C, or D). N.F. means that no previous known mutation caused this phenotype of the isolate.
[4] There were 42 isolates that did not show any resistance for the tested fungicides at the first experiment and therefore were not tested for mutations.

In this study, a small number of isolates showed a loss of sensitivity to fluopyram, even though boscalid was the only fungicide applied in pistachio orchards where these isolates were collected. This suggests that additional unique mechanism of resistance to fluopyram may exist. Characterization of the *AaSDH* genes for these particular isolates is in progress and could reveal unknown mutations associated with resistance to fluopyram. The emergence and spread of isolates expressing high level of resistance to fluopyram could have detrimental consequences for the sustainable use of fluopyram in ALB management strategy. Therefore, it is important to maintain the efficacy of fluopyram for as long as possible by following resistance management strategies.

SELECTED REFERENCES

Avenot HF and Michailides TJ, Progress in understanding molecular mechanisms and evolution of resistance to succinate dehydrogenase inhibiting (SDHI) fungicides in phytopathogenic fungi. *Crop Prot* 29:643–651 (2010).

Avenot HF, Sellam A, Karaoglanidis G and Michailides TJ, Characterization of mutations in the iron sulfur subunit of succinate dehydrogenase correlating with boscalid resistance in *Alternaria alternata* from California pistachio. *Phytopathology* 98:736–742 (2008).

Avenot H, Sellam A and Michailides TJ, Characterization of mutations in the membrane-anchored subunits AaSDHC and AaSDHD of succinate dehydrogenase from *Alternaria alternata* isolates conferring field resistance to the fungicide boscalid. *Plant Pathol* 58:1134–1143 (2009).

Ma Z, Felts D, Michailides TJ, Resistance to azoxystrobin in *Alternaria* isolates from pistachio in California. *Pestic. Biochem. and Physiol*. 77: 66-74 (2003).

Pryor B, Michailides TJ, Morphological, pathogenic, and molecular characterization of *Alternaria* isolates associated with Alternaria late of pistachio. *Phytopathology*. 92: 406-416 (2002).

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Comparative efficacy of microbial and chemical insecticides on four major lepidopterous pests of rice and their (insect) natural enemies

ABSTRACT
Two microbial (halt and dipel), three chemical insecticides (bifenthrin 10 EC , lambda-cyhalothrin 5 EC and clopyriphos 20 EC) and one neem formulation (multineem) were compared for efficacy on four major lepidopterans pest and their natural enemies in replicated field trials at Uttar Dinajpur district, West Bengal, India. Halt was evaluated at different combinations with lambda-cyhalothrin in a second trial. The results showed that the microbial formulations and plant extracts were lethal to stem borer and leaf roller larvae but non-lethal to their natural enemies. The synthetic chemicals on the other hand caused mortality to both pest and natural enemies. However all the types of insecticides enhanced paddy grain

yield considerably. Application of halt followed by lambda-cyhalothrin was found prudent than other formulations so evaluated.
Key words: paddy, pest, microbial insecticides, plant extract formulation, chemical insecticides

INTRODUCTION

Yellow stem borer (*Scirpophaga incertulas* Wlk.), pink stem borer (*Sesamia inferens* Wlk.) white stem borer (*Tryporyza innontata* Wlk.) and leaf folder (*Cnaphalocrosis medinalis* Guen.) are reported to be the major lepidopterous pests of rice fields in West Bengal, India (Chakraborty *et al*., 2010). Out of the three borer species, *S. incertulas*, predominates (Satpathi *et al*. 2005). These insect pests are currently being controlled by the application of broad spectrum insecticides three times at monthly intervals during the growing season. However, these broad spectrum materials are highly toxic to insect natural enemies (Hamilton and Attia, 1976). On the other hand, *Bacillus thuringiensis* Berl, is potentially active against many lepidopterous pest species imparting very little lethality to natural enemy complexes (Fadare *et al*. 1998). Guild of natural enemies of lepidopteron pests grossly includes both parasites (braconid wasp) and predators (spider, coccinellid beetle and odonate fly). Design of a suitable programme depending on the lethality of insecticides which would endure the beneficial species and cause the mortality of destructive ones is urgently desired. The efficacy of *B. thuringiensis* could be increased by the addition of sublethal doses of pesticides and could be used in such a programme. Here we compare the efficacy of two microbial insecticides (halt and dipel), one neem formulation (multineem) and three other chemical insecticides (bifenthrin, lambda-cyhalothrin and clopyriphos) on yellow stem borer , pink stem borer, white stem borer, leaf folder and their natural enemies.

MATERIALS AND METHODS

Study was conducted during four consecutive *kharif* crop years (2005-2008) in the field of paddy cultivar *Swarna mashuri* (MTU 7029). Transplantation to main field was done with 35-day old seedlings at 10 x15 cm spacing on 20-22 standard meteorological weeks (SMW). The soil of the experimental field was sandy loam with PH value 6.2 and EC value 0.29 mmhs/cm. N, P_2O_5 and K_2O was 315, 76 and 367 kg/ha respectively. During land preparation, each plot received 150:60:60 kg/ha NPK as basal dose. Field experiment was done following national protocol with befitting modifications (Khushawa, 1995). The treatments were arranged in a randomized complete block design with four replications. Each plot was 10m x 10m by size. Plots with heavy infestation of these concerned pests were chosen for this study.

There were two sets of experiments (set I and set II). In the first phase (set-I) six insecticide formulations were selected (Table 1). The formulations comprised of two microbials [halt (at 1500 gm/ha) and dipel (at 2000 gm/ha)], one neem formulation [multineem: 3750 ml/ha] and three chemical insecticides, [bifenthrin 10 EC (at 1150 ml/ha), lambda-cyhalothrin (at 375 ml/ha), clopyriphos 20 EC (at 1875 ml/ha)]. Relative efficacy of these formulations was evaluated with hand operated calibrated knapsak sprayer (hollow cone NMD 60450 nozzle, droplet diameter 1.6 mm, droplet size. 140 lm, discharge 450 ml/min at 40 psi pressure and distance between nozzle and target 30–45 cm) at 400 l/ha.

Table 1: List of insecticides used during experiment

Treatments	Insecticide formulation	Trade name	Company	Category	Applied dose/ha	
					a.i /L	Formulation /ha
T1	Bifenthrin 10 EC	Talstar 10 EC	FMC India	synthetic pyrethriod	25	1150ml
T2	Lambda-cyhalothrin 5 EC	Kerate 5 % EC	Syngenta	synthetic pyrethriod	0.5ml/l	375 ml
T3	Clopyriphos 20 EC	Darsban 20 EC	D-nosile	organophosphate	2.5ml/l	1875 ml
T4	*Bacillus thuringiensis* var. kurstaki 3.5 L	Dipel 8 L	Kemineva	microbial	0.5-2 kg/ha	2000g
T5	*Bacillus thuringiensis* var. kurstaki 5 WP	Halt	Wockhardt	microbial	2 gm a.i/L	1500g
T6	Azadirachtin formulation	multineem 0.03 % EC	Multiplex	plant extract	5ml/l	3750 ml
T7 (control)	Water spray only					

In the second phase (set-II) two insecticides out of the six formulations to suppress pest induced damage were selected. These were one microbial (halt) and one chemical insecticide (lambda-cyhalothrin). Different grade formulations of these two insecticides were prepared (table 5 [C1-C4]). In general, all the formulations were applied to the field at two times; at 30 and 70 days after seedling transplantation (DAT). But for multineem an additional dose was applied at 50 DAT. Plot without insecticide application was considered as control (T5).

Extent of pest infestation was assessed in terms of the crop damage symptoms. Damage due to all stem borer species was noted collectively. Incidence of dead heart+ white head due to stem borer and the occurrence of folded leaves due to leaf folder infestation were recorded accordingly. To count the adult incidence and the extent of damage 25 rice hills were randomly selected from each plot and the symptoms were recorded. Pre and post spray sampling of population of natural enemies (especially the predators) were carried out with an aerial net. Eggs, larvae and pupae forms of both the pests were taken to the laboratory and the extent of parasitization depending on the emergence of parasites was also recorded.

Data collected was subjected to statistical analysis. Efficacy of each formulation was assessed in

consideration of pre and post application count of both pest and natural enemy population, incidence of damage symptoms and the generation of yield in comparison to control plots.

RESULTS

Experiment I

All the insecticide formulations effectively suppress the pest population and the consequences of damage symptoms (Table 2). Incidence of adult borer population during post spray count ranged from 3.51 to 3.67 individuals/hill for chemical insecticides, from 2.72 to 3.12 individuals/hill for microbial insecticides and 2.89 individuals/hill for plant extract formulations respectively. The control plot has registered 5.72 adult individuals/hill. On the other hand incidence of leaf folder population during post spray count ranged from 4.35 to 4.81 individuals/hill for chemical insecticides, from 3.85 to 4.12 individuals/hill for microbial insecticides and 3.91 individuals/hill for plant extract formulations respectively. The control plot has registered 5.34 adult individuals/hill.

Table 2: Incidence of pest population and the extent of damage under different treatments

Treatments	Pest incidence*			
	Stem borer		Leaf folder	
	A	B	A	B
T1	6.49 (2.92)	3.51 (2.00)	7.20 (2.77)	4.35 (2.20)
T2	7.12 (3.13)	3.85 (2.09)	8.3 (2.97)	5.03 (2.35)
T3	6.79 (3.07)	3.67 (2.04)	7.9 (2.90)	4.81 (2.30)
T4	5.77 (2.85)	3.12 (1.90)	6.81 (2.70)	4.12 (2.15)
T5	5.03 (2.76)	2.72 (1.79)	6.41 (2.62)	3.85 (2.09)
T6	5.35 (2.78)	2.89 (1.84)	6.50 (2.64)	3.91 (2.10)
T7 (control)	8.73 (3.22)	5.72 (2.49)	8.82 (3.05)	5.34 (2.42)
Critical Difference (p=0.05)	0.31	0.56	0.42	0.39

* Individuals/hills, A: before application B: after application
Figure in the parenthesis are square root transformed value

Results from both the fields varied considerably. Infestation to paddy at early and late growth stage of paddy results in dead heart (DH) and white head (WH) respectively. Extent of DH and WH generation in plots treated with chemical insecticide ranged from 4.78-5.78%. While the corresponding value for microbial and plant extract application were 4.18-4.27% and 4.27% respectively. These values were significantly lower (P =0.05) than that of chemical insecticide treated plots.

In control plot 12.89% DH and WH was counted. Instances of rolled leaf formation due the gluing of the growing leaf margin by leaf folder larvae varied significantly (P = 0.05) in insecticide treated and untreated plots. The untreated control plot has registered 10.04% rolled leaf. Chemical insecticide and microbial treated plots has registered 3.95-4.03% and 3.12-3.43% rolled leaf (Table 3). Post harvest grain yield ranged from 28.85-32.16 q/ha for chemicals and 35.84-38.54 q/ha for the microbial treatment and the results were statistically different. In case of plant extract (neem formulation) treated plots yield was 36.51 q/ha. However, yield from the control plots was 24.59q/ha and this was significantly lower than those from the sprayed treatment (P = 0.05). Increase of yield from sprayed plots over the control plots ranged from 30.96 to 31.18 for the microbials and 32.04 to 32.68 for the chemical insecticides (Table 3). The mean numbers of parasites and predators recorded from plots sprayed with microbial differed insignificantly from those of the unsprayed control plots. In plots treated with chemical insecticides the mean numbers of parasites and predators were low than both the microbial and plant extract treated fields (Table 4). Grossly the numbers of coccinellid recorded from each plot were relatively higher than other natural enemies. An increase of both parasites and predator count was generally noted following the application of the microbial. But count was either same or reduced when chemical insecticide was applied. Maximum number of natural enemies was recorded in control plots. The numerical abundance of spider, predatory bug, beetle and odonate fly was 8.27, 5.98, 7.32, 4.68 and 3.45 individuals/10 hills respectively. The minimum incidence was recorded in bifenthin (T1) plots and the values were 5.67, 3.45, 4.56, 1.45 and 2.23 individuals/10 hills respectively.

Table 3: Extent of damage by pests under different treatments

Treatment	Extent of damage		Yield	
	Stem borer	Leaf folder	Post harvest (q/ha)	Yield increase over control (%)
T1	4.78 (2.30)	3.95 (2.11)	31.25	30.96
T2	5.78 (2.51)	4.49 (2.33)	28.85	30.40
T3	5.08 (2.36)	4.03 (2.13)	32.16	31.18
T4	4.32 (2.20)	3.43 (1.98)	35.84	32.04
T5	4.18 (2.16)	3.12 (1.90)	38.54	32.68
T6	4.27 (2.18)	3.34 (1.96)	36.51	32.20
T7 (control)	12.89 (3.66)	10.04 (3.25)	24.59	-
Critical Difference (p=0.05)	0.67	0.51	0.92	0.67

A: individuals/4m^2, B: Extent of damage/100 hills
Figure in the parenthesis are square root transformed value

Table 4: Incidence of natural enemy population under different treatments

Treatments	Natural enemy incidence*				
	predator				Parasite
	spider	bug	Coccinellid beetle	Odonate fly	
T1	5.67 (2.48)	3.45 (1.99)	4.56 (2.25)	1.45 (1.40)	2.23 (1.65)
T2	4.98 (2.34)	3.58 (2.02)	4.47 (2.23)	1.39 (1.37)	1.45 (1.40)
T3	5.12 (2.37)	3.41 (1.98)	4.51 (2.24)	2.41 (1.71)	2.09 (1.61)
T4	7.98 (2.91)	5.79 (2.51)	7.19 (2.77)	4.51 (2.24)	3.32 (1.95)
T5	8.12 (2.94)	5.95 (2.54)	7.26 (2.79)	4.63 (2.26)	3.41 (1.98)
T6	8.02 (2.92)	5.87 (2.52)	7.21 (2.78)	4.58 (2.25)	3.39 (1.97)
T7 (control)	8.27 (2.96)	5.98 (2.55)	7.32 (2.80)	4.68 (2.28)	3.45 (1.99)
Critical Difference (p=0.05)	0.45	0.41	0.63	0.13	0.11

Experiment II

One microbial (halt) and one chemical (lambda-cyhalothrin) insecticide is further selected among of the applied six insecticide formulations. In consideration of efficacy, halt is the best microbial insecticide in set-I experiment. While the impact of lambda-cyhalothrin as chemical insecticide was found least. Different grade combinations of such microbial and chemical insecticides were prepared (Table 5). In consideration of the incidence of pest population and the extent of damage symptoms, all the grades were found superior to the untreated control plots. Least damage (0.71% DH+WH and 0.67% rolled leaf) for both the pest was recorded in C4 treatment. An increase of 38.02% grain yield over the control was noted in C4. Among the treatments, extent of damage was maximum (2.09% DH+WH and 3.14% rolled leaf) in C2. Degree of damage in C3 (1.05% DH+WH and 1.42% rolled leaf) was immediately followed by C1 (1.54% DH+WH and 2.36% rolled leaf).

Table 5: Extent of damage and yield generation under different combinations of halt and lambda-cyhalothrin

Insecticide combinations	Insecticides and dose		Extent of Damage (%)		Increase of grain yield over control (%)
	Halt	Lambda-cyhalothrin	Stem borer	Leaf folder	
C1	1.0 g/L	0.75 ml/L	1.54 (1.43)	2.36 (1.69)	34.87
C2	1.0 g/L	0.25 ml/L	2.09 (1.61)	3.14 (1.91)	32.40
C3	2.5 g/L	0.25 ml/L	1.05 (1.24)	1.42 (1.39)	36.75
C4	2.0 g/L	0.50 ml/L	0.71 (1.10)	0.67 (1.08)	38.02
C5 (Control)	-	-	12.89 (3.66)	10.04 (3.25)	-
Critical Difference (p=0.05)			0.45	0.12	1.23

(-): Not applicable
Figure in the parenthesis are square root transformed value

DISCUSSION

During post application count, all the insecticide formulations were effective in reducing the level of DH+WH and the incidence of rolled leaves. However in consideration of the degree of damage suppression, all the formulations differed significantly. In suppressing stem borer induced damage, the microbials and plant extract performed significantly better (P = 0.05) than the chemicals. Grain yield from both microbial and chemical insecticide treated plots were relatively higher than that of the control plots. Application of microbials increases the yield in average more than 32% while that of chemicals it was 30.50%. Such high yield could be due to the effective protection of the growing foliage from larval ingestion which consequently enhanced the quantity and quality of the end products. In all the instances count of the natural enemies before insecticidal application was more than the count at post application. However, in case of microbials and plant extracts such decrease was marginal and such difference is statistically insignificant.

In case of chemicals, a drastic fall in count during post application was registered. Apparently, application of microbials and plant extracts allows the survival and multiplication of the natural enemies but disallows the destructive pest populations. Microbials are mostly targeted to the soft larval stage of insect life cycle. The larva due to their gregarious habit roams in the plant canopy and thus obviously made a ready contact to the spray formulation and ultimately periled. Plant extracts acts mostly as deterrent to the pest population. On the other hand, the probability of ready contact of grub or lymph present in their lifecycle of natural enemies with the formulation was less due to their comparatively small body size. Further some natural enemy completes lifecycle outside the fields and only the adult morph invades the rice crop. Contrary to this the chemicals caused the mortality of both the beneficial and destructive species. Chemicals are targeted to all the stages and morphs of life cycle of both pest and natural enemies. They penetrate to insect system either by gustatory or contact. Chakraborty *et al*. (2010) have enlisted a list of paddy pest natural enemies from this area. Natural enemies as he reported broadly include both parasite and predators. Coccinellid beetle, odonate fly, spider and bug are the important predatory groups. Results further demonstrate the existence of a mechanism in tropical irrigated rice systems that support high levels of natural biological control. This mechanism depends on season long successional processes and interactions among a wide array of species, many of which have hitherto been ignored as important elements in a rice ecosystem.

Combinations of different proportions of halt and lambda-cyhalothrin were effective in reducing the level of both yellow stem borer and leaf folder larval population significantly in the present study. Grain yield from all the treated plots were significantly higher than that from the control. From the experiment II, it is evicted that treatment C3 (application of halt-2g/l and lambda-cyhalothrin-0.5 ml/l) is the best combination. Incidence of both the pests were significantly low than the control. Yield from C3 was higher than all treatments in experiment II. Ali *et al*. (1973) have noted that spray formulations of halt and thuricide effectively suppressed grape leaf-folder *Desmia funeralis* (Hubn.) population with least residual toxicity. But from the present study a single formulation of either microbial or chemical insecticide less effective for pest suppression and hence not be advisable. Microbial insecticides, being highly selective conserve the populations of parasites and predators as well as other beneficial species while they suppress lepidopterous populations for which they are specific. An application of chemical insecticides at early growth stages of paddy disallows the settlement of natural enemies from the nearby field. This generates an agreeable condition for the accommodation and subsequent speedy multiplication of pest population especially the loppers.

Natarajan (1990) from Tamil Nadu have reported that botanical pesticides followed by microbial pesticides expressed less lethality to the coccinellid beetle population in descending order. He has further noted that high reduction of beetle population following the application of triazophos, monocrotophos or methamidophos in paddy field. Bull *et al*. (1979) had reported a deliberate suppression of beneficial species populations with methyl parathion and subsequent outbreak of *Heliothis* sp. on cotton fields. Saljogi *et al*.(2002) have reported that Padan 4 G (cartap-hydrochloride) was found to be the most effective in reducing rice stem borer infestation, followed by Regent 300 EC (fipronil) @ 197.6 ml/ha. Firake *et al*. (2010) have experimented with a batch of insecticides in rice field. They have concluded that the efficacy of Dipel (*B.thuringiensis* var. kurstaki) was the previous of the last within the batch. Campbell *et al*. (1991) from north California have reported that *B. thuringiensis* formulation did not cause reduction of *Trichogramma exiguum* and *Trichogramma pretiosum* parasitism on *Heliothis zea* egg in tomato fields. In India, 1.3 to 10.2 fold incidence of egg mass parasitism of leaf folder eggs was noted in bio-control plots compared to control as reported by Bentur *et al*. (1989). Morris *et al*. (1975) had commented that simultaneous application of both microbial and chemical insecticides though not significantly different result from thuricide or lambda-cyhalothrin alone. When lambda-cyhalothrin was applied in combination with *B.thuringiensis*, there are no risks as due to their compatibility (Morris 1975). Though the chemical insecticide may have adverse effects on parasites and predators in the agro-ecosystem, application of microbial insecticides with low supplemental doses of chemical insecticides has been thus suggested for integrated control of rice insect pests. The application of neem based formulation as an alternative to the chemical insecticides is also advocated by Samal *et al*. (2007). Bast and effective control of rice pest population and side by side the restoration of natural enemy complex was possible when sequential application of halt followed by lambda-cyhalothrin was adopted. The potential application of bio-formulations followed by chemical insecticide for the effective control of rice leaf folder is advocated by Patil *et al*. (2009). Rath (2006) have also documented that cypermethrin in combination with neem formulation effectively protects rice crop. Sabbour *et al*. (2005) from Egypt have noted that bio-pesticides can control lepidopteran insect pests of cabbage.

Approach of the present study is aimed to reduce the injudicious application of chemical insecticides, to subsume the insect pest populations and to enhance the activity of parasite, predator and other beneficial insects. Present study has elaborated that bio-formulations are as good as the commercially available chemical insecticides to check lepidopterous pests.

REFERENCES

Ali Niazee NJ, Jensen FL (1973). Microbial control of the grape leaffolder with different formulations of *Bacillus thuringiensis*. J. Econ. Entomol. 66: 151 – 158.

Bentur J S, Kalode M B, Rajendran B, and Patel V S (1989). Field evaluation of egg parasitoid Trichogramma japonicum Ash. (Hymenoptera, Trichogrammatidae) against the rice leaf folder: Chaphalocrocis medinalis (Guene) (Lepidoptera:Pyralidae) in India.J. Appl.Ent.117:257-261.

Bull DL, House VS, Ables JR and Morrison RK (1979). Selective methods for managing insect pests of cotton. J. Econ. Entomol. 72: 841–846.

Campbell C D, Walgerbach J F, and Kannedy G G (1991). Effect of parasitoids on lepidopterious pests in insecticide treated and untreated tomatoes in western Carolina.J.econ.Entomol.84:1882-1887.

Chakraborty K and Deb D C (2010). Predators of paddy insect pests in Uttar Dinajpur, West Bengal, Insect Environ.15(4): 191.

Fadare TA, Osisanya EO (1998). Field evaluation of microbialinsecticides on cotton bollworms and their natural enemies. Nig.Journ. Sci. 32: 72-75

Firake D M , Pande R and Karnatak A K (2010). Evaluation of microbial and some chemical insecticides against yellow stem borer and leaf folder of rice. J. Insect Sci. 23(2): 150-153.

Hamilton JR, Attia FI (1976). The susceptibility of the parasite *Apantelesglomeratus* (L.) Braconidae to Insecticides. J. Entomol. Soc. Aust. 9:24 – 25.

Kalode M B (2005).Insect pest of rice and their management in Rice in Indian Perspective edited by S.D .Sharma and B.C.Nayak. Today and Tomorrow Printers and Publishers (India).819-854.

Kushwaha K S (1995). Chemical control of Rice stem borer, *Scirpaphaga incertulas* (Walker) and leaf folder *Cnaphalocrocis medinalis* Guenee on Basmati. J Insect Sci.8(2): 225-226.

Matteson P C (2000). Insect pest management in Tropical Asian Irrigated rice. Ann. Rev. Entomol.45 : 549-574

Morris ON (1975). Effect of some chemical insecticides on the germination and replication of commercial *Bacillus thuringiensis*. J.Invertebr. Pathol. 26: 198 – 204.

Morris ON, Armstrong JA (1975). Preliminary field trials, with *Bacillus thuringiensis*. Chemical insecticide combinations in the integrated control of the spruce/budworm *Choristomeura fumiferana*. Can. Ent.107: 1281 – 1288.

Natarajan K (1990). Natural enemies of *Bemisia tabaci* Genn. And effect of insecticides on their activity J. Bio. Control. 4(2): 86-88.

Patil R S and Nandihalli B S (2009). Efficacy of promising botanicals against red spider mite on Brinjal. Karnata J. Agric. Sci. 14:480-481.

Rath P C (2006). Insect and non-insect pest of rice and their control.CRRI, Technology bulletin 44:4

Sabbour M M and Sahab Z A F (2005). Efficacy of some microbial control agent against cabbage pests in Egypt. Pak J.Biol. Sc.8(10):1351-1356.

Saljoqi AU R, Khan M , Abdullah K and Latif A (2002). Evaluation of Fipronil for the management of rice stem borer. Sar. J.Agric. **18**(1): 59-61.

Samal A Bhattacharya D K and Nanda L R (2007). Eco-friendly management of rice leaf folder and case worm in Odisha. National Symphosium on sustainable pest management for safe environment organized by OUAT, Bhubaneswar 6-7, December 33-34.

Satpathi, C. R., Mukhopadhyay, A. K., katti,G., Pasalu, I.C. and Venkateswarlu, B.,2005, Quantification of the role of natural biological control in farmers' rice field in West Bengal. Indian J. Entomol., 67 (3): 211-213.

Wakil W, Hussain M, Akbar R. and Gulzar A (2001). Evaluation of different insecticides against rice stem borer and rice leaf folder. Pak.J. Agric. Sci., 38: 49–50.

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Research in Resistance Management

LETHAL AND SUBLETHAL EFFECT OF INSECTICIDE OVER DIFFERENT Chrysoperla carnea (STEPHENS) (NEUROPTERA: CHRYSOPIDAE) INSTARS

ABSTRACT
The chemical and biological control in sustainable management requires knowing the risks, selectivity and conditions of use of insecticides, in that matter, tolerance of Chrysoperla carnea (Stephens) to field concentration abamectin, endosulfan and profenofos was compared. Profenofos was the insecticide that showed the higher toxicity to larvae and adults. Abamectin showed toxicity over 1st instar larvae and a negative effect on oviposition and endosulfan had adverse effects on eggs, 1st instar, 2nd instar and had no negative effect over 3rd instar pupae.
Key words: integrated control, selectivity, tolerance

INTRODUCTION

Chrysoperla carnae (Stephens) is a voracious generalist predator in agricultural systems (Tauber et al., 2000), having a wide host range (McEwen et al., 2001), and effective as a biological control agent (Hagley and Miles, 1987). However, in some cases, using only biological control agents is not enough to provide adequate control, but through its integration with other management tools, can be a source of sustainable control (Cock, 1994). The indiscriminate use of insecticides can jeopardize biological control success due to its toxic effects on natural enemies; therefore it is important to know the risks, selectivity and conditions of use of these products to maximize compatibility between chemical and biological control (Stevenson and Walters, 1983). The effect of insecticides on beneficial arthropods were measured first by bioassays, usually designed to assess the acute toxicity of individuals of specific age and size, in order to estimate their mortality through products with lower toxicity on non-target organisms (Purcell et al., 1994). The estimated lethal dose for acute toxicity test and selectivity is only a partial measure of the total effect of insecticides on these beneficial organisms; in addition to direct mortality induced by pesticides, sub-lethal effects on arthropod physiology and behavior should be considered for a complete analysis of its impact (Desneux et al., 2007). Sub-lethal effects are defined as effects on individuals which survive exposure to a pesticide and can show a reduction in cycle life, rate of development, fertility, fecundity, change in sex ration and changes in feed and search capacity and oviposition (Desneux et al., 2004). Therefore, the objective of this research was to evaluate the lethal and sub-lethal effects of endosulfan, abamectin and profenofos over C. carnea under laboratory conditions.

MATERIALS AND METHODS

Insect colony: Lacewing colony was obtained from eggs provided by the Centro de Reproduccion de Organismos Beneficos (CROB) of the state of Coahuila, placed individually in plastic cups (3 cm x 3 cm) and after emergence, larvae were fed with Sitotroga cereallela eggs and maintained under controlled conditions at a temperature of 25 ± 2° C and 70 ± 10% relative humidity.

Insecticides: Insecticides evaluated were endosulfan (EC Thiodan 33%), abamectin (EC Agrimec 1.8%) and profenofos (73% Curacrón EC) whose field concentration were 180, 18, 1400 ppm L-1, respectively, and the control in which only water was applied.

Treatments: Treatments consisted of a petri dish (6 cm diameter) with 50 individuals per developmental stage (eggs, larvae 1-3, pupae and adult), individually placed in each petri dish. Approximately 2 mg per cm^2 of the insecticide solution was sprayed in each case and subsequently transferred into individual containers for food as appropriate. Mortality for larval stages, eggs, and pupae was assessed daily until the adult stage, while in treated adults only ovipostion rate was measured. Larval stages were fed every other day with S. cereallela eggs. Death was record when insect displacement was less than the length of its body, after stimulating them with a brush. All bioassays were performed at a temperature of 25 ± 2°C and relative humidity of 70 ± 10%.

Statistical analysis: Mortality data, life cycle in days and oviposition were analyzed with a one-way ANOVA using the GLM SAF/ STAT (SAS, 2001 procedure), and means of the least squares were compared using Tukey (p≤0.05_ (SAS, 2001).

RESULTS AND DISCUSSION

Evalutaion of commercial doses of endosulfan, profenofos and abamectin over eggs of C. carnea are shown in Table 1. Endosulfan and abamectin

showed higher cumulative mortality with 20 and 30% respectively. For life cycle duration, all treatments showed similar results; for ovipostion, profenofos was the insecticide that had the higher average (35.2 eggs per female), while for sex ration, abamectin presented the greater difference (21.12, female: male). Regarding endosulfan, Carvalho et al., (2002), evaluating the toxic effect of this insecticide on eggs of C. externa (Hagen), found low mortality and reported no mortality on 1st instar larvae. Rimoldi et al., (2008), reported less than 20% mortality of eggs of the same species treatd with endosulfan; however, these authors consider it highly toxic because in subsequent stages of development from eggs treated, mortality was 100%. All these data sugges that this insecticide is not recommended for inclusion in Integrated Pest Management (IPM) systems. In relation to abamectin, there are reports which indicate tolearance of C. carnea to this product, for example, Giolo et al., (2009), reported low toxicity on the complete cycle (egg, larva, pupa and adult) of C. carnea, which differs to results found in this investigation. In relation to the other biological parameters, Giolo et al., (2009) mentioned that there was no effect on fertility of adults of C. carnea from eggs treated with abamectin.

Table 1: Cumulative mortality (%) (± SE), days of development and oviposition of *Chrysoperla carnea* treated on the egg stage.

Treatment	% mortality						Development days					Oviposition			
	E	L1	L2	L3	P	A	L1	L2	L3	P	A	E	α	β	%T
Control	50	12	0	0	0	0	4.5^a	3^a	3^a	6.5^a	4.5^a	29 ± 1.09^a	21	21	16
Endosulfan	50	16	0	0	0	0	4.5^a	3^a	3^a	6.5^a	4.5^a	32.2 ± 2.11^b	23	19	20
Abamectin	50	30	0	0	0	0	4.5^a	3^a	3^a	6.5^a	4.5^a	32.1 ± 2.17^b	21	14	30
Profenofos	50	14	0	0	0	0	4.5^a	3^a	3^a	6.5^a	4.5^a	35.2 ± 3.16^b	23	20	14

α: Females, β: Male,% T: total mortality.

In the case of C. carnea treated in 1st instar larvae (Table 2), profenofos was very toxic to this developmental stage, with 100% mortality, followed by abamectin with 50% mortality. Life cycle based on development days, the control without treatment showed differences for 3rd instar larvae and pupae stages as compared with abamectin and endosulfan; for ovipostion, endosulfan had the higher average 34.6 eggs per female, and no difference was found in relation of sex ratio.

Table 2: Cumulative mortality (%) (± SE), days of development and oviposition of *Chrysoperla carnea* treated in first instar stage.

Treatment	% mortality					Development days				Oviposition			
	L1	L2	L3	P	A	L2	L3	P	A	H	α	β	%T
Control	12	0	2	4	0	3^b	3^b	6.5^b	4.5^b	28.9 ± 1.19^b	26	15	18
Endosulfan	16	2	2	0	0	3.5^b	4^c	8.5^c	4.5^b	34.6 ± 2.33^c	24	16	20
Abamectin	14	30	2	2	2	3.5^b	4^c	8.5^c	4.5^b	27.3 ± 1.46^b	15	10	50
Profenofos	100	0	0	0	0	0^a	0^a	0^a	0^a	0^a	0	0	100

α: Females, β: Male,% T: total mortality.

In larvae treated on 2nd instar, profenofos was very aggressive (Table 3), having 100% mortality as compared with other treatments that had 28% and 20% mortality for endosulfan and abamectin respectively. For the duration of the life cycle, endosulfan and abamectin caused a decrease in 3rd instar larvae as well as in the pupal and adult stages in the effet on oviposition, endosulfan had the higher oviposition average with 32.1 eggs per female and a sex ratio of 23.13, female: male, whereas abamectin had a negative effect on the predator with 19.1 eggs per female.

Table 3: Cumulative mortality (%) (± SE), days of development and oviposition of *Chrysoperla carnea* treated in second instar stage.

Treatment	%of mortality				Development days			Oviposition			
	L2	L3	P	A	L3	P	A	E	α	β	%T
Control	4	2	0	0	3^b	6.5^c	4.5^b	29.9 ± 1.89^c	26	21	6
Endosulfan	24	2	0	2	1.5^a	5^b	5.5^c	32.1 ± 2.23^d	23	13	28
Abamectin	18	2	0	0	1.5^a	5^b	5.5^c	25.8 ± 1.05^b	22	15	20
Profenofos	96	4	0	0	5.5^c	0^a	0^a	0^a	0	0	100

Profenofos 96 4 0 0 5.5c 0a 0a0a 0 0 100
α: Females, β: Male,% T: total mortality.

Profenofos was the most damaging product causing 100% mortality over 3rd instar C. carnae larvae (Table 4). For the duration of the cycle based on development days, the control without treatment had the least time to develop (6.5), whereas in abamectin and endosulfan, the pupal stage lasted 8.5 days. For ovipostion, the insecticide endosulfan and the control had the higher average with 31.7 eggs per female. Abamectin showed an adverse effect on ovipostion with 19.1 eggs per female and no effect effect was obtained in regard to sex ratio where all treatments presented a similar sex proportion.

Table 4: Cumulative mortality (%) (± SE), days of development and oviposition of *Chrysoperla carnea* treated on third instar stage.

Treatment	% mortality			Development days		Oviposition			
	L3	P	A	P	A	E	α	β	T
Control	6	0	0	6.5^{a}	4.5^{b}	29.2±2.16	23	24	6
Endosulfan	0	0	2	8.5^{b}	4.5^{b}	31.7±2.44	27	22	2
Abamectina	8	0	0	8.5^{b}	4.5^{b}	19.1±1.11	22	24	8
Profenofos	98	2	0	6.5^{a}	0^{a}	0	0	0	100

α: Females, β: Male,% T: total mortality.

In this regards, we can mention that the insecticide profenofos, is reported as an insecticide extremely toxic to different larval stages of C. carnea (Nasreen et al., 2003), results similar to those reported in this study. For the insecticide abamectin, Good and Freitas (2004) reported low mortality in the three larval instars of C. externa exposed to this insecticide, however, in this study, a high mortality (50%) was found for 1st instar larvae. In relation to the fecundity, Giolo et al., (2009), mentioned that there was no effect on fecundity of C. carnea, from larvae treated with abamectin, but in this research it was a marked decrease in the average number of eggs per female when all larval stages were treated with this insecticide. Endosulfan is classified as harmless in most evaluations conducted on larvae of this predator (Silva et al., 2005), results similar to these results found in this investigation for 3rd instar larvae (2% mortality). However, there are reports stating that the product is highly toxic to larvae of C. carnea, (Ulhôa et al., 2002), similar to those reported in this study with mortalities of 20 to 28% for 1st and 2nd instars respectively. Furthermore, Silva et al., (2005), found that the three larval instars exposed to endosulfan, the duration for each instar larva was not affected, nor the subsequent phases of development and also did not have an adverse effect on fertility and fecundity.

In the case of C. carnea treated pupa, Table 5, which all treatments caused low mortality to the predator pupal stages; however, abamectin and profenofos had an effect on development time in the adult stage. For oviposition, the insecticide abaemectin presented the lower average (40.8) eggs per female compared with the other treatments while no effect was found in sex ratio where all showed a similar proportion.

Table 5: Cumulative mortality (%) (± SE), days of development and oviposition of *Chrysoperla carnea* treated in pupal stage.

Treatment	% mortality		Development days	Oviposition			
	P	A	A	E	α	β	%T
Control	0	0	4.5^{b}	$42.7±2.24^{b}$	29	21	0
Endosulfan	4	0	4.5^{b}	$43.9±2.45^{b}$	29	19	4
Abamectin	0	0	3.5^{a}	$40.8±2.16^{a}$	31	19	0
Profenofos	0	2	3.5^{a}	$43.3±2.34^{b}$	31	18	2

α: Females, β: Male,% T: total mortality.

In relation to endosulfan, there are conflicting reports, Silva et al., (2006), reported this insecticide safe on pupae of C. externa, they also mentioned that endosulfan had no influence on the duration of pupal stage, ovipostion period, fecundity and fertility of adults from pupae treated, similar to the results in this study. On the other hand, Godoy et al., (2004), for the insecticide abamectin, reported that when pupae of C. externa were treated with this insecticide, did not show an adverse effect on mortality, fecundity and fertility of adults from pupae treated; however, in this study, no effect was observed on mortality, but had a reduction of fecundity.

Finally, for treated adults, Table 6 shows that profenofos caused a 100% mortality, whereas endosulfan and abamectin only caused a 20 and 12% mortality, respectively; abamectin and endosulfan had an effect on oviposition with 24.5 and 26.4 eggs per female respectively as compared with the untreated control that had a higher average (41.4) eggs per female, while no effect was found on sex ratio, although abamectin presented a greater difference (20:12 female: male).

Table 6: Cumulative mortality (%) (± SE), days of development and oviposition of *Chrysoperla carnea* treated in adult stage.

Treatment	% mortality	Oviposition			
	A	E	α	β	%T
Control	6	$41.4±2.25^{d}$	24	23	6
Endosulfan	20	$26.4±1.13^{c}$	23	17	20
Abamectin	12	$24.5±2.11^{b}$	24	20	12
Profenofos	100	0^{a}	0	0	100

In this regard we can mention that the insecticide profenofos, is reported as an insecticide extremely toxic to different larval stages and adults of C. carnea (Nasreen et al., 2003), results similar to those reported in this study; however, Godoy et al., (2004), reported that abamectin does not affet mortality of C. carnea but does affect fertility. Finally, in regard to endosulfan, adults of C. externa exposed to this insecticide, Ulhôa et al., (2002), found high mortality and Silva et al., (2006) mentioned it safe to use and also mention that endosulfan did not affect fertility on treated adults.

CONCLUSION

The insecticide profenofos showed a high toxicity to all larval stages and adults of Chrysoperal carnea. Abamectin had detrimental effects in 1st instar larvae, also showed a negative effect on adult ovipostition from treated larvae. Endosulfan caused adverse effects on eggs, 1st and 2nd instar larvae, and not to 3rd instar larvae and pupa. Therefore, abamectin and endosulfan are better products to use in Pest Management scheme as long as the doses used are the recommended low and intermediate dosages.

LITERATURE CITED

Bueno A. F., and S. Freitas. 2004. Effect of the insecticidas abamectin and lufenuron on eggs and larvae of Chrysoperla externa under laboratory conditions. Biocontrol. 49: 277-283.

Carvalho G. A., C. F. Carvalho, B. Souza e J. L. R. Ulhoa. 2002. Seletividade de insecticidas a Chrysoperla externa (Hagen) (Neuroptera:Chrysopidae). Neotropical Entomology. 31(4): 615-621.

Cock, M. J. W. 1994.Integrated management of whitefly pest problems in the Middle and Near East with special emphasis on biological control.The Arab Journal of Plant Protection. 12: 127-136.

Godoy M. S., G. A. Carvalho, J. C. Moraes, L. V. Cosme, M. M. Goussain, C. F. Cavalho e A. Morais. 2004. Seletividade de seis insecticidas emcitros a pupas e adultos de Chrysoperla externa (Hagen) (Neuroptera: Chrysopidae). Neotropical Entomology. 33(3): 359-364.

Desneux N, E Wajnberg, X Fauvergue, S Privet, L. Kaiser. 2004. Sublethal effects of a neurotoxic insecticide on the oviposition behavior and the patch-time allocation in two aphid parasitoids, Diaeretiella rapae and Aphidius matricariae. Entomol. Exp. Appl. 112:227–35

Desneux N., A. Decourtye, and J.-M. Delpuech. 2007.The Sublethal Effects of Pesticides on Beneficial Arthropods. Annu. Rev. Entomol. 52:81-106

Giolo F. B. P. Medina, A, D, Grutzmacher, and E. Viñuela. 2009. Effects of pesticides commonly used in peach orchards in Brasil on predatory lacewing Chrysoperla carnea under laboratory conditions. Biocontrol. 54: 625-635.

Hagley, E. A. C., and N. Miles. 1987. Release of Chrysoperla carnea Stephen (Neuroptera: Chrysopidae) for control of Tetranychus urticae Koch (Acarina:Tetranychidae) on peach grown in a protected environment structure. Canadian Entomologist. 119: 119-205.

McEwen, P. K., T. R. R. New, A. Whittington. 2001. Lacewing in the crop management. Cambridge University Press. 546 p

Nasreen A., G. Mustafa, and M. Ashfaq. 2003. Selectivity of some insecticides to Chrysoperla carnea Stephen. (Neuroptera: Chrysopidae) in laboratory. Pak. J. Biol. Sci. 6: 536-538.

Purcell, M. F., J. D. Stark, and R. H. Messing. 1994. Effects of insecticides on three tephritid fruit flies and associated braconid parasitoids in Hawaii. Journal of Economic Entomology. 87: 1455-1462.

Rimoldi F., M. I. Schneider, and A. E. Ronco.2008. Susceptibility of Chrysoperla externa eggs (Nueroptera: Chrysopidae) to conventional and biorational insecticides. Environ. Entomol. 37(5): 1252-1257.

SAS Institute 2001.SAS/STAT User's Guide.SAS Institute Inc., Cary, NC.

Silva, R.A.S.; Carvalho, G.A.; Carvalho, C.F.; Reis, P.R.; Pereira, A.M.A.R.; Cosme, L.V. 2005. Toxicidade de productos fitossanitários utilizados na cultura do cafeeiro a larvas de Chrysoperla externa (Hagen) (Neuroptera: Chrysopidae) e efeitos sobre as fases subseqüentes do desenvolvimento do predador. Neotropical Entomology. 34(6): 951-959.

Silva, R.A.S.; Carvalho, G.A.; Carvalho, C.F.; Reis, P.R.; Souza B., Pereira A. M. A. R. 2006. Acao de productos fitossanitarios utilizados em cafeeiros sobre pupas e adultos de Chrysoperla externa (Hagen, 1861) (Neuroptera: Chrysopidae). Ciencia rural. 36(1): 8-14.

Stevenson, J. H., and J. H. H. Walters. 1983. Evaluation of pesticides for use with

biological control. Agriculture, Ecosystems and Environment. 10: 201-215.

Tauber, M. J., C. A. Tauber, K. M. Daane, and K. S. Hagen. 2000. Commercialization of predators: recent lessons from green lacewings (Neuroptera: Chrysopidae: Chrysoperla). American Entomologist. 46: 26-38.

Ulhôa, J.L.R., A.C. Geraldo, F.C. César and S. Brígida. 2002.Ação de inseticidas recomendados para o controle do curuquerê-do-algodoeiro para pupas e adultos de Chrysoperla externa(Hagen, 1861) (Neuroptera: Chrysopidae). Ciência e Agrotecnologia, Lavras, v.26, p.1365-1372, 2002. (Edição Especial).

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Investigation on the Efficacy of *Verticillium lecanii* for Biological Control of Whitefly (*Bemicia tabaci*)

ABSTRACT
Efficacy of entomopathogenic fungus, *Verticilium lecanii* was evaluated against assorted populations of *Bemicia tabaci* nymphs in *vitro*. The nymphal population was subjected to various concentration of bioagent *viz.*, 10^9 – 10^6 spores /ml alone and in combination with various oils *viz*., Neem oil, D-C-Tron plus oil and liquid paraffin oil at 1% concentration, respectively, replicated thrice. Dose mortality (LC_{50}) and time mortality (LT_{50}) response of Whitefly nymphs against various treatments formed the basis of comparison. Dimethoate @ 0.05 per cent, the application of standard check was the most effective treatment amongst the all. In case of bioagent application, *V. lecanii* @ 10^9 spores /ml in combination with Neem oil and D-C-Tron plus oil at 1 % concentration, respectively were the promising treatments in terms of lower time to registered 50 per cent mortality in test population and higher mortality inflicted. Higher spore load of fungus was more lethal in terms of mortality inflicted, statistically significant over untreated control, though less effective as compared to application of standard check Dimethoate. The probit analysis reveals the possibility of utilization of *V. lecanii* as a potent bio agent against *Bemicia tabaci*.
Keywords: Biocontrol, *Verticillium lecanii*, *Bemicia tabaci*, Oil base formulations

INTRODUCTION

Whitefly (*Bemicia tabaci*) is a major polyphagous pest of several economically important crops and an efficient vector of Yellow vein mosaic virus, which is the root cause of damage. There has been increased concern and awareness regarding adverse effect of chemicals on ecosystem *viz*., objectionable residues, and fast development of resistance in insect pests demanding higher pesticides application along with ill effect on natural enemies. Several workers have concentrated on bio-control as cheap, eco-friendly and a potential source of pest management. Studies on the effect of various entomopathogenic microorganisms for control of Whitefly (*Bemicia tabaci*) are prominent amongst them. The efforts were made to search not only economical but also effective, eco-friendly alternative measure to insecticides for the management of Whitefly.

Earlier studies show that *Verticillium lecanii* is a useful biological agent against Whitefly (Ekbom, 1979). These fungi are attacking on nymphs and adults stuck to the leaf underside by means of a filamentous and somewhat crystalline mycelium. *V. lecanii* is a widely distributed fungus, which can cause large epizootics in tropical and subtropical regions, as well as in warm and humid environments (García and López, 1997 in Nunez *et al*, 2008). Considering the potential of *Verticillium lecanii* in Whitefly management, their "eco friendly" nature and mammalian safety the present investigation was planned to evaluate the bioefficacy of these entomopathogenic fungus against Whiteflies with a view to include these components in the pest management strategies of Whiteflies.

MATERIALS AND METHODS

Bioassay experiment was planned with six treatments and replicated thrice in the laboratory of Department of plant pathology, College of Agriculture, Nagpur. Different concentrations of entomopathogenic microorganism *V. lecanii* alone and its oil base formulations were evaluated against assorted populations of nymphs of *Bemicia tabaci*. For preparation of formulations of spore suspension in different oils, spores from young culture grown on PDA medium were suspended in one percent oil (Neem oil, D-C-Tron plus oil and liquid paraffin oil, separately) with 0.05 percent aqueous tween 80. The concentration was estimated using haemocytometer. It was adjusted to $1x10^6$, 10^7, 10^8 and 10^9 spores/ml. The adult whiteflies were collected with the help of aspirators from the cotton fields of College of Agriculture, Nagpur for infection.

For bioassay procedure for nymphs of *B. tabaci*, third instar nymphs were used for all screening bioassay procedures. Individual cotton leaves with more or less uniformly distributed insects (5 to l0 insects/cm^2) were selected prior to fungal inoculation. Leaf sectors with approximately 50 to 100 insects were used. These leaf pieces bearing nymphs were immersed in a spore suspension for l0 sec; control leaves were immersed in 0.005% Triton x 100 for the same length of time. To prevent development of saprophytic fungi, treated leaves were placed for 20-30 min. on filter paper to remove excess moisture. The leaves were then placed in petri dishes, undergoing incubation in a growth chamber at alternating temperatures of 25 ^{o} C (14 h in light) and 20 ^{o} C (10 h in the dark). Relative humidity close to 100% was reached by placing a moist filter paper in each petri dish. For aeration purposes, each petri dish was opened daily for 25-30 min. This procedure was necessary to avoid development of saprophytic fungi on Whitefly honeydew. The number of insects per leaf sector was counted prior to inoculation. The percentage of dead insects following inoculation and infection was recorded daily. The natural mortality in control leaves was usually lower than 10% and was not subtracted from the obtained mortality percentage. The observation on mortality of assorted population of nymphs of *Bemicia tabaci* was based on mycotic effects of the entomopathogenic microorganism *V. lecanii and* mycelial growth on the insect body. These observations were recorded for 7 days at 24 hours interval, commencing the first observation 24 hrs after application of the treatment during all the sets of experiment (Gindin *et. al* 2000). The mortality data was recorded to find the effect of various treatments on assorted population of nymphs of *Bemicia tabaci*, subjected to probit analysis for estimation of dose mortality (LC_{50}) and time mortality (LT_{50}) response of treatments (Finney, 1972).

RESULT AND DISCUSSION

The most effective treatment, *Verticillium lecanii* with 1% neem oil formulation, registered a lowest median lethal concentration (LC_{50}) value of 5.8 to $7.5x10^6$ spores /ml i.e. lowest concentration required to kill the 50% of nymphal population of Whitefly followed by *V. lecanii* with 1% D-C-Tron plus oil registered median lethal concentration (LC_{50}) of 1.8 to $8.5x10^7$ spores/ml, (Table 1). Whereas *V. lecanii* alone registered median lethal concentration of 5.2 to 8.8 x 10^7 spores /ml for all three sets of experiment (Table 2). *V. lecanii* with 1% liquid paraffin oil registered the same lethal concentrations as obtained in the alone trial. This means that the formulations of *V. lecanii* with liquid paraffin oil did not inflict any significant effect on mortality of Whitefly nymphs as found in formulations of neem oil and D-C-Tron plus oil.

Table 1: Concentration –mortality (LC_{50}) response of *Bemicia tabaci* nymphs to different oil base formulations

Treat.	Repl	n	LC_{50} (Days)	95%FL	Slope	Hete.	Chisqure
1%N + *V .l*	RI	250	6.3 x 10^{6}	1.0 x10^{3}- 3.3 x10^{7}	0.49 ± 0.12	0.90	1.79
	RII	250	5.8 x 10^{6}	1.0 x10^{3}- 3.2 x10^{7}	0.48 ± 0.12	0.18	0.36
	RIII	250	7.5 x 10^{6}	6.2 x10^{2}- 4.2 x10^{7}	0.45 ± 0.11	0.20	0.40
1%D + *V. l*	RI	250	1.8 x 10^{7}	0.6 x10^{6}- 7.8 x10^{7}	0.46 ± 0.11	0.13	0.27
	RII	250	8.5 x 10^{7}	5.9 x10^{6}- 3.1 x10^{8}	0.42 ± 0.10	0.17	0.35
	RIII	250	5.1 x 10^{7}	9.1 x10^{6}- 1.4 x10^{8}	0.61 ± 0.12	0.09	0.18
1%P + *V. l*	RI	250	8.8 x 10^{7}	1.2 x10^{7}- 2.4 x10^{8}	0.52 ± 0.11	0.31	0.63
	RII	250	1.0 x 10^{8}	2.4 x10^{7}- 4.8 x10^{8}	0.48 ± 0.10	0.48	0.97
	RIII	250	7.4 x 10^{7}	1.1 x10^{7}- 2.1 x10^{8}	0.55 ± 0.11	0.28	0.55

*(P = 0.05@ d. f. = 5.991 and at @5 d. f. =11.070 and P = 0.1 @ 2 d. f. =4.605 and at @5 d. f. = 9.236)

**: (The Chi Squre values compared with table values at respective degree of freedom for any real deviation.)

n = no. of nymphs treated.

LC_{50} = Median lethal concentration

F.L. = Fiducial Limit

Note : 1%N+*V.l* : *V lecanii* was used at different concentration viz., 10^9,10^8,10^7 and 10^6 spores/ml along with 1 % Neem oil, respectively.

1%D+*V.l*: *V lecanii* was used at different concentration viz., 10^9,10^8,10^7 and 10^6 spores/ml along with 1 % Dctron plus oil, respectively.

1%P+*V.l*: *Verticillium lecanii* was used at different concentration viz., 10^9,10^8,10^7 and 10^6 spores/ml along with 1 % Liquid paraffin oil, respectively.

Table 2: Concentration –mortality (LC_{50}) response of *Bemicia tabaci* nymphs for Set I, Set II, and Set III

Set No.	Repl.	n	LC_{50}	95%FL	LC_{90}	95%FL	Slope	Hete.	Chi Squre
I	I	250	5.2 x 10^{7}	7.4 x 10^{5} - 1.5 x 10^{8}	9.6 x 10^{9}	3.0 x10^{9} – 8.6 x10^{11}	0.57 ± 0.15	0.78	1.56
	II	250	7.1 x 10^{7}	1.1 x 10^{7} - 2.0 x 10^{8}	1.9 x 10^{10}	5.3 x10^{9} – 2.4 x10^{11}	0.53 ± 0.10	0.17	0.34
	III	250	7.5 x 10^{7}	1.4 x 10^{7} - 2.0 x 10^{8}	1.1 x 10^{10}	3.7 x10^{9} – 9.7 x10^{11}	0.59 ± 0.11	0.20	0.40
II	I	250	6.3 x 10^{7}	1.0 x 10^{7} - 1.7 x 10^{8}	1.2 x 10^{10}	3.9 x10^{9} – 1.1 x10^{11}	0.56 ± 0.11	0.03	0.07
	II	250	6.3 x 10^{7}	5.7 x 10^{5} - 2.1 x 10^{8}	5.0 x 10^{10}	9.8 x10^{9} – 2.2 x10^{12}	0.44 ± 0.10	0.51	1.02
	III	250	7.1 x 10^{7}	9.5 x 10^{5} - 2.1 x 10^{8}	2.5 x 10^{10}	6.3 x10^{9} – 4.3 x10^{11}	0.50 ± 0.11	0.32	0.65
III	I	250	8.8 x 10^{7}	1.2 x 10^{7} - 2.6 x 10^{8}	3.3 x 10^{10}	7.9 x10^{9} – 6.6 x10^{11}	0.50 ± 0.11	0.10	0.21
	II	250	8.1 x 10^{7}	1.0 x 10^{7} - 2.4 x 10^{8}	2.6 x 10^{10}	6.7 x10^{9} – 4.7 x10^{11}	0.51 ± 0.11	0.20	0.46
	III	250	8.2 x 10^{7}	5.4 x 10^{5} - 1.6 x 10^{8}	1.8 x 10^{10}	4.8 x10^{9} – 3.1 x10^{11}	0.50 ± 0.11	0.48	0.95

* (P = 0.05@ d. f. = 5.991 and at @5 d. f. =11.070 and P = 0.1 @ 2 d. f. =4.605 and at @5 d. f. = 9.236)
**: (The Chi Squre values compared with table values at respective degree of freedom for any real deviation.)
n = no. of nymphs treated.
LC $_{50}$ = Median lethal concentration
F.L. = Fiducial Limit
Note: *Verticillium lecanii* was used at different concentration viz., 10^{9},10^{8},10^{7} and 10^{6} spore/ml, respectively.

The median lethal time (LT_{50}) values, i.e. time or days required to kill 50% of nymphal population, were lowest due to *V. lecanii* with 1% neem oil with (LT_{50}) value in the range of 2.3 to 4.6 days when spore load was in order of 10^{9} to 10^{6} spores/ ml (Table 3). Where as *V. lecanii* alone brought about 50 per cent mortality in range of 4.1 to 6.4 days for all the three sets of experiment with suspension strength of 10^{9} to 10^{6} spores/ml respectively. It was observed that all the treatments were superior over control and managed to bring about significant mortality in test population. Application of Dimethoate (0.05%) proved to be the most effective treatment in terms of lowest median lethal time (LT_{50}) (Table 4).

Table 3: Time –mortality (LT_{50}) response of *Bemicia tabaci* nymphs due to 1 % Neem oil base formulations

Treat.	Conc.	Repl	n	LT_{50} (Days)	95%FL	Slope	Hete.	Chisqure
T_1	1.9 X 10^{9}	I	350	2.3	2.03 - 2.67	2.92 ± 0.30	0.43	2.13
		II	350	2.5	2.20 - 2.84	3.03 ± 0.04	0.07	0.34
		III	350	2.4	2.10 - 2.75	2.96 ± 0.30	0.25	1.26
T_2	3.1 X 10^{8}	I	350	3.1	2.81 - 3.48	3.38 ± 0.34	0.48	2.38
		II	350	2.9	2.58 - 3.27	3.15 ± 0.32	0.79	3.94
		III	350	3.2	2.74 - 3.59	3.26 ± 0.33	0.67	3.35
T_3	2.7 X 10^{7}	I	350	4.0	3.41 - 4.56	3.96 ± 0.42	0.61	3.07
		II	350	3.9	3.69 - 4.40	4.18 ± 0.44	0.35	1.77
		III	350	3.8	3.26 - 4.12	4.20 ± 0.49	0.84	3.37
T_4	3.6 X 10^{6}	I	350	4.3	4.02 - 4.79	4.24 ± 0.47	0.35	1.74
		II	350	4.3	3.96 - 4.74	4.04 ± 0.45	0.27	1.34
		III	350	4.6	4.21-5.10	4.23 ± 0.49	0.89	4.44
T_5	0.05 %	I	350	1.1	0.94 - 1.66	5.62 ± 1.18	0.63	3.13
		II	350	1.5	1.45 - 2.29	7.83 ± 2.14	0.89	4.44
		III	350	1.3	1.03 - 1.67	4.77 ± 0.79	0.55	2.77
T_6	Control	I	350	22.8	13.70 - 92.46	2.20 ± 0.55	0.40	2.01
		II	350	16.8	11.37 - 51.05	3.11 ± 0.78	0.61	3.04
		III	350	17.7	11.72 - 36.92	2.93 ± 0.73	0.78	3.92

*(P = 0.05@ d. f. = 5.991 and at @5 d. f. =11.070 and P = 0.1 @ 2 d. f. =4.605 and at @5 d. f. = 9.236)
**: (The Chi Squre values compared with table values at respective degree of freedom for any real deviation.)
n = no. of nymphs treated**.**
LT $_{50}$ = Median lethal Time
F.L. = Fiducial Limit

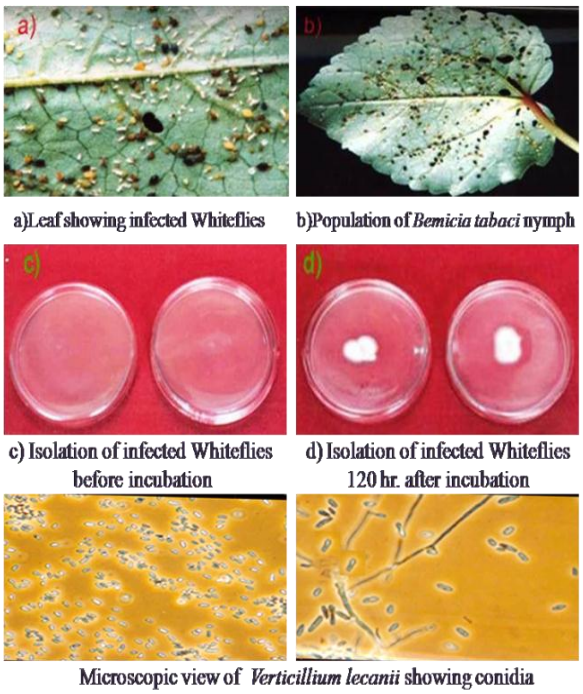
Table 4: Time –mortality (LT_{50}) response of *Bemicia tabaci* nymphs to different concentrations of *V. lecanii*

Treat	Conc.	Repl	n	LT_{50} (Days)	95%FL (Days)	LT_{90} (Days)	95%FL (Days)	Slope	Hete.	Chisqure
T_1	1.9 X 10^{9}	I	350	4.1	3.76 – 4.53	8.8	7.55 – 11.08	3.85 ± 0.42	0.27	1.37
		II	350	4.4	3.96 – 4.75	9.0	7.74 – 11.39	4.00 ± 0.44	0.20	1.01
		III	350	4.3	3.68 – 4.61	8.8	7.61 – 11.05	4.10 ± 0.45	0.34	1.69
T_2	3.1 X 10^{8}	I	350	4.7	4.61 – 5.11	9.2	7.89 – 11.48	4.39 ± 0.50	0.84	4.19
		II	350	4.9	4.54 – 5.51	10.4	8.72 – 13.79	3.98 ± 0.48	0.31	1.57
		III	350	4.9	4.59 – 5.50	10.5	8.64 – 12.98	3.99 ± 0.47	0.72	0.33
T_3	2.7 X 10^{7}	I	350	5.3	4.68 – 5.71	11.4	9.35 – 15.92	3.62 ± 0.45	0.67	3.33
		II	350	5.5	5.10 – 6.27	11.5	9.41 – 14.52	4.10 ± 0.47	0.47	0.32
		III	350	5.1	4.28 – 5.36	10.8	9.10 – 15.78	3.94 ± 0.53	0.06	2.33
T_4	3.6 X 10^{6}	I	350	5.7	5.12 – 6.44	12.4	10.00 – 14.55	3.73 ± 0.49	0.28	1.40
		II	350	5.8	5.21 – 6.57	12.5	10.05 – 18.09	3.71± 0.50	0.09	0.47
		III	350	5.9	5.36 – 6.78	12.6	10.13 – 18.31	3.89± 0.52	0.24	1.21
T_5	0.05 %	I	350	1.5	0.79 – 1.45	2.8	2.29 – 3.79	3.30 ± 0.40	1.19	3.40
		II	350	1.0	0.84 – 1.29	2.6	2.25 – 3.16	3.35 ± 0.43	0.62	2.57
		III	350	1.2	0.73 – 1.36	2.7	2.20 – 3.55	3.22 ± 0.41	1.04	2.47
T_6	Control	I	350	21.2	12.96 – 40.20	78.0	33.38 – 902.20	2.20 ± 0.55	0.40	2.01
		II	350	19.2	11.72 – 36.92	54.2	43.11 – 406.93	2.93 ± 0.73	0.78	3.92
		III	350	21.0	11.37 – 51.05	61.0	21.62 – 328.02	3.11 ± 0.78	0.61	3.04

*(P = 0.05@ d. f. = 5.991 and at @5 d. f. =11.070 and P = 0.1 @ 2 d. f. =4.605 and at @5 d. f. = 9.236)
**: (The Chi Squre values compared with table values at respective degree of freedom for any real deviation.)
n = no. of nymphs treated.
LT $_{50}$ = Median lethal Time
F.L. = Fiducial Limit

Higher conidial concentration was more lethal and the mortality due to treatments differed significantly from untreated control. It is inferred that the mortality of nymphs was highest in *V. lecanii* with 1% neem oil and proved to be very effective formulation against the nymphal population of *Bemicia tabaci.* Burges (1998**)** reported that oil formulations increase the efficacy of infection by entomopathogenic fungi based on bioassay experiment. The present findings are in

agreement with their findings in respect to the efficacy of *V. lecanii* in combination with various oils. Hall (1982) estimated the LC_{50} value at 2.3 x 10^6 conidia/ml. Spraying a conidial suspension at a concentration of 10^8 conidia / ml seems to be an economical and effective way of controlling Whitefly. Kim (2002) reported that *V. lecanii* was an effective biological control agent against *Trialeurodes vaporariorum* in South Korean greenhouses by spraying with a suitable concentration of conidia in the suspension applied as inoculum at 10^8/mL with a temperature range of 20- 25°C. Bouhous and Larous (2012) observed based on the realised tests that the entomopathogenic fungus *V. lecanii* is particularly infectious for larval and adult stages, and slightly infectious for eggs. In larvae, LD_{50} is relatively low ($0.5x10^3$ spores/ml) in the experimentation conditions. The action time of the fungus reached its maximum in day 7. Ahmedi *et al*. (2004) found that suspension of *V. lecanii*, at 10^3 to 10^7 conidia/ml grown in liquid culture medium, was effective against 2nd stage of *T. tabaci* under controlled conditions. Thus, the infectivity, resultant mortality and LC_{50} value of *V. lecanii* against *Bemicia tabaci* in corroboration with present findings. As a result, *V lecanii* can be proposed for its incorporation into pest management programs that utilize biological control agents against Whitefly, *Bemicia tabaci.*



Figures

REFERENCES

Ahmadi LB, Askary H, Ashouri A.. 2004. Preliminary evaluation of the effectiveness of a *Verticillium lecanii* isolate in the control .*Commun Agril Applied Biological Sci.*, 69 (3):201-204.

Anitha Cherian, K. 1998 Management of tomato leaf curl virus through the control of Whitefly vector, *Bemicia tabaci* Genn, by entomopathogenic fungi. Ph. D. thesis, Univ. Agric. Sci., Banglore, 226pp.

Asiatic, J.M. and Zoebisch, T.G. 1992 Control of whitefly *Bemicia tabaci* (Gennadius) on tomato with biological and chemical insecticides. Manejo lntegr. Plagas 24-25:1-7.

Barson, G. 1976 Laboratory studies on the fungus *Verticillium lecanii*, a floral pathogen of the large elm bark beetle. *Ann. Appl. Biol*. 83: 207-214.

Burges, H.D. 1998 Formulation of mycoinsecticides In : Burges HD, ed. Formulation of microbial Biopesticides. Dordrecht : Kluwer, Academic Pulishers .131-185.

Byme, D.N. and Bellows, T.S.Jr. 1991 Whitefly biology. *Annu. Rev. Entomo*. 36:.431-457.

Chandler, D. Heale, J.B. and Gillespie, A.T. 1993 Germination of the entomopathogenic fungus *V. lecanii* on scales of the glass house Whitefly *Trialeurodes vaporarium Biocontrol Sci. Technol*. 3:161-164.

Easwaramoorthy, S and Jayraj, S. 1978 Effectiveness of the white halo fungus, *Cephalosporium lecanii* against field population of coffee green bug, *Coccus viridis J. Invert. Path* 32: 88-96.

Ekbom, B. S. 1979 Investigations on the potential of a parasitic fungus (*V. lecanii*) for biological control of the green house Whitefly (*Trialeurodes vaporariorum*) *Swedish J. agric. Res.* 9 :129-138.

Finney, D.J., 1972. Probit analysis, Cambridge university press, London, 318 P.P.

Gindin, G., Barash, I., Raccah, B., Singer, S., Ben-Ze'ev, I. and Klein, M. 1996. The potential of some entomopathogenic fungi as biocontrol agents against the onion thrips, *Thrips tabaci* and the western flower thrips, *Frankliniella occcidentalis*. *Folia Enmmol. Hung.* LVII: 37-42.

Gindin G., Geschtovt N.U., Raccah B. and Barash I. 2000. Pathogenicity of *V. lecanii* to different developmental stages of the silver leaf Whitefly *Bemicia argentifolii* . *Phytoparasitica* 28(3): c xxx-xxx

Hall, R.A. 1982 control of Whitefly, *Trialeurodes vaporariorum* and cotton aphid *Aphis gossypii* in glass house by two isolates of the fungus *V. lecanii Anuu Application Bio* 10: 1 - 11.

Helyer, N. 1993. *Verticillium lecanii* for control of aphids and thrips on cucumber. IOBC2WPRS Bull. 16:63- 66.

Hsiao, W.F., Bidochka, M.J. and Khachatourians, G.G. 1992 Effect of temperature and relative humidity on the virulence of the entomopathogenic fungus, *Verticillium lecanii*, towards the oat-bird berry aphid, *Rhophalosipum padi* (Horn Aphididae). *J. Appl. Enmmol*. 114: 484 - 490.

Hussey, N. W. 1958 Notes on a fungus parasitic on greenhouse whitefly. *Plant Pathol.* :71 - 72.

Kim, J. J.; Lee, M. H.; Yoon, C. S.; Kim, H. S.; Yoo, J. K.; and Kim, K. C. 2002. Control of Cotton Aphid and Greenhouse Whitefly With a Fungal Pathogen. *Journal of National Institute of Agricultural Science and Technology*, (NIAST): Korea.

Landa, A., Osborne, L., Lopoz, F. and Eyal, J. 1994 A bioassay for determining pathogencity of entomogenous fungi on Whitefly. *Bio-control*, 4: 341-350.

Masuda, T. and Kikuchi, O. 1992. Pathogenicity of *Verticillium lecanii* isolates to whitefly and aphids., *Appl. Entomol. Zool.* 36:239

Mangula, C., Nagraju and Muniappa V. 2003 Evaluation of different oil formulation of bioagents, *Bemicia tabaci* against different life stages of *Bemicia tabaci* the vector of tomato leaf curl virus: *Plant Dis. Reporter* 18(1): 25-28.

Meade, D. L. and Byme, D.N. 1991. The use of *V. lecanii* against submiginal instars of *Bemicia tabaci*. *J. Invertebrate. Pathol.* 57: 296 - 298.

Mostefa Bouhous and Larbi Larous (2012) Efficiency of the entomopathogenic fungus *Verticillium lecanii* in the biological control of *Trialeurodes vaporariorum*, (*Homoptera*: *Aleyrodidae*), a greenhouse culture pest. African Journal of Microbiology Research Vol. 6(10), pp. 2435-2442

Mot, H., Gindin, G., Ben-Ze'ev, I.S., Raccah, B., Geschtovt, N.U., Ajtkhozina, N. and Barash, I. 1996. Diversity among isolates of *Verticillium lecanii* as expressed by DNA polymorphism and virulence towards *Bemisia tabaci*. *Phytoprasitica* 24: 111-118.

Nier, T., Rivera, F., Bermudez, J.C., Dominguez, Y., Benavides, C..and Ulloa, M. 1991. First report in Mexico on the isolation of *Verticillium lecanii* from whitefly and *in vitro* pathogenicity tests on this insect. *Rev. Mex. Micol.* 7: 149-156.

Nunez, E., Iannacone1, J.; and Gomez, H. 2008. Effect of two Entomopathogenic fungi in controlling *Aleurodicus cocois* (curtis, 1846) (Hemiptera: Aleyrodidae). *Chilean J. Agric. Res.*Vol 68 (1).

Osborne, L.S. and Landa, Z. 1992.Biological control of whiteflies with entomopathogenic fungi. Fla. Eiztomol. 75: 456 - 471.

Perring, T.M., Cooper, A.D., Rodrigues, R.J., Farrar, C.A. and Bellows, T.S. Jr. 1993. Identification of a whitefly species by genomic and behavioral studies.*Science* 259: 74 - 77.

Ravensberg, W.J., Malais, M. and van der Schaaf, D.A. 1990. *Verticillium lecanii* as a microbial insecticide against glasshouse whitefly. Plvc. Brighton Crop Protection Conf on Pests and Diseases Vol. 1, pp. 265-268.

Saito, T. 1992 Control of *Thrips palmi* and *Bemisia tabaci* by a mycoinsecticidal preparation of *Verticillium lecanii*. *Proc. Kanto Tosan Plant Protection Soc.* (Shizuoka, Japan), pp. 209-210.

Saito, T. 1993. Control of two Whitefly species, *Bemicia tabaci* and *Traleurodes vaporariorum* by a preparation of *V. lecanii Proc. Kanto. Tosan Plant Protection Soc.* (Shizuoka, Japan) PP.221-222.

Steenberg, T. and Humber, R. 1999.Entomopathogenic potential of *Verticillium lecanii* and *Acremonium* species (Deuteromycotina: Hyphomycetes). *J. lnvertebrate Pathol*. 73: 309 - 314.

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Genetic Modeling of the Fipronil Resistance's Increase in the Population of Colorado potato beetle.

ABSTRACT
The effectiveness of insecticides has been reduced due to resistance formation in many populations of pests. We have constructed a discrete predictive genetic model of resistance in populations of Colorado potato beetle, based on the interaction of various factors. We test various hypotheses in the model for the development of strategies for overcoming resistance. This allows us to calculate a model with intergenic mediated interactions using phenetic markers of nonspecific resistance to environmental factors. Calculations show that use of minimal effective doses of insecticides leads to impaired development of the share of resistant individuals in populations of Colorado potato beetle. Calculations show a slow increase in the proportion of stable individuals in populations of Colorado potato beetle using the minimal effective dose of insecticide (low dose).
Key word: Colorado potato beetle, Leptinotarsa decemlineata, protection of resistance to insecticides, fipronil, population, genetic modeling, low dose of insecticide.

INTRODUCTION

Insecticides are currently the most effective means of control of insects. But the effectiveness of their use is reduced due to an increase of resistance (Roslavtseva, 2005, 2009; Sukhoruchenko, 2005; Benkovskaya et al, 2008; Baker et al., 2007; Alyokhin et al., 2008, 2009; Benkovskaya et al., 2009). Also by means of protection, the Colorado potato beetle currently available in the arsenal of agronomists have the potential to become a factor in forming new resistant populations and lose their original precious economic property (Sukhoruchenko et al, 2006). The development of strategies for the use of pesticides and other technological barriers to the formation of resistant populations of pests is not only a relevant scientific problem, but also is an important task for businesses, aimed both at protecting their capital investment in the creation of drugs or production of agricultural.

The effectiveness of insecticides is reduced due to the resistance formation in the populations of pests. We have constructed a discrete predictive genetic model of resistance in populations of Colorado potato beetle, based on the interaction of various factors. The emergence of insecticide resistance of insect populations is the result of instability of the gene pool and the selection that occurs in agricultural lands. Insecticides used in agrotechnology act as selective factors of selection of resistant phenotypes to them. Some phenotypes can overcome the effect of insecticides, stored selection, while non-adaptive phenotypes derived from reproduction cannot and eventually are eliminated. The effect of other factors as may reduce survival of stable phenotypes and improve it.

Our objective is to test hypotheses about the impact of various agricultural practices on the acceleration or deceleration of increase of resistance in populations of Colorado potato beetle. Since the population of insects always act as factors, it is necessary to have methods for evaluating the impact of each factor, as well as their set. To solve this problem requires a fairly simple and at the same time universal mathematical tool that lets you change the prediction set of so that the results obtained experimentally validate and refine.

Materials and Methods

We examine genetic model of population resistance to insecticides in the case *Leptinotarsa decemlineata* Say and fipronil. Fipronil acts on the nervous system of insects by binding to an allosteric site receptor gamma-aminobutyric acid (GABA), and blocks the chloride ion channel, which causes the death of the pest (Narahashi et al., 2007). Insect resistance to fipronil caused by the presence of mutation in the gene Rdl, encoding subunit of the GABA receptor (Li et al., 2006). The researchers point out that there is a mutation in one gene. Resistance to fipronil is inherited recessively, but at low doses of the drug and are partially in heterozygotes (Sayyed, Wright, 2004), suggesting an incomplete recessive allele.

We use data about resistance in the population of Colorado potato beetle on the South Ural (fig. 1) and data from the experiment of testing diagnostic dose of fipronil (0.0001% of the active substance) (table 1).

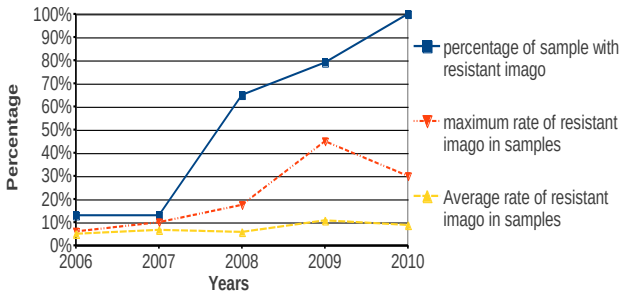

Figure 1: Increase in resistance to fipronil in the population of Colorado potato beetle on the South Ural.

Table 1: Changing the proportion of fipronil-resistant Colorado potato beetle with use of the Regent (fipronil). (Mardanshin et al., 2012, with addition of the authors).

Year	Sample	Rate of resistant imago
2007	Overwintering	0
2008	Overwintering	0
2009	Summery	0.1±0.019
2010	Overwintering	0.05±0.003
	Samery	0.25±0.05
2011	Overwintering	0.20±0.06

Formulas 1-3 are proposed on the basis of Hardy-Weinberg Law and population growth formula Fisher's describing the processes of increasing or decreasing the frequency of alleles in the selection of a function of time (Altukhov, 2004).

$$p_{n+1} = \frac{w_1 * p_n^2 + w_2 * p_n * q_n}{w} \quad (1)$$

$$q_{n+1} = \frac{w_3 * q_n^2 + w_2 * p_n * q_n}{w} \quad (2)$$

$$w = w_1 * p_n^2 + w_2 * p_n * q_n + w_3 * q^2 \quad (3)$$

Where p and q are the allele frequencies of genotypes in the amount of components 1, w - the selection coefficient, and w_1, w_2, w_3 – the selection coefficients of which genotype. The coefficient of selection is a complex function w (R, S or M). R – resistance (survival in the field), S – winter survival, M – morphotype survival.

We made models with one gene, determining resistance or sensitivity for fipronil (2 alleles: ss – susceptible, sr – heterozygote, rr – resistance). There is resistance (survival in the field) in the case of the **recommended dose** of the insecticide Regent R_1 = 0.06; R_2 = 0.20; R_3 = 1. There is resistance (survival in the field) in the case of **low dose (1/2 – 1/3 recommended dose)** R_1 = 0.125; R_2 = 0.35; R_3 = 1. We calculate these parameters according to the schedule of dose-effect and the lognormal distribution of the probability of a given dose each individuals (Bezel et al., 1994). Winter survival is S_1 = 1; S_2 = 0.7; S_3 = 0.4.

In addition, we made models with two genes: first gene is fipronil resistance (ss – susceptible, sr – heterozygote, rr - resistance), second gene – morphotype (AA – achromist, AM – intermediate, MM – melanist) (Benkovskaya et al., 2004; Benkovskaya et al., 2009; Benkovskaya, Udalov, 2011). There is morphotype survival for AA M1=M2=M3=1, for AMss M4=1, for (AMsr) M5=0.8, for AMrr M6=0.5, for MMss M7=1, for MMsr M8=0.5 and for MMrr M9=0.4 (table 2).

Table 2: Genotypes and the calculation model with two non-entangled genes (percentage calculated on the proportion of over-wintering the summer of last year, the shares are calculated from a summer share alleles and survival after treatment with insecticide).

genotypes and propotion		summering (s)	overwintering (ow)
AAss	$k^2 \times p^2$	$k^2 \times p^2 \times R_1$	$k^2 \times p^2 \times M_1$
AAsr	$k^2 \times 2 \times p \times q$	$k^2 \times 2 \times p \times q \times R_2$	$k^2 \times 2 \times p \times q \times M_2$
AArr	$k^2 \times q^2$	$k^2 \times q^2 \times R_3$	$k^2 \times q^2 \times M_3$
AMss	$2 \times k \times l \times p^2$	$2 \times k \times l \times p^2 \times R_1$	$2 \times k \times l \times p^2 \times M_4$
AMsr	$2 \times k \times l \times 2 \times p \times q$	$2 \times k \times l \times 2 \times p \times q \times R_2$	$2 \times k \times l \times 2 \times p \times q \times M_5$
AMrr	$2 \times k \times l \times q^2$	$2 \times k \times l \times q^2 \times R_3$	$2 \times k \times l \times q^2 \times M_6$
MMss	$l^2 \times p^2$	$l^2 \times p^2 \times R_1$	$l^2 \times p^2 \times M_7$
MMsr	$l^2 \times 2 \times p \times q$	$l^2 \times 2 \times p \times q \times R_2$	$l^2 \times 2 \times p \times q \times M_8$
MMrr	$l^2 \times q^2$	$l^2 \times q^2 \times R_3$	$l^2 \times q^2 \times M_9$
w mean		sum	sum
allels and propotion		calculate of allel's propotion	
A	k	$(k^2 \times p^2 + k^2 \times 2 \times p \times q + k^2 \times q^2 + k \times l \times p^2 + k \times l \times 2 \times p \times q + k \times l \times q^2)/w$	
M	l	$(l^2 \times p^2 + l^2 \times 2 \times p \times q + l^2 \times q^2 + k \times l \times p^2 + k \times l \times 2 \times p \times q + k \times l \times q^2)/w$	
S	p	$(k^2 \times p^2 + k^2 \times p \times q + + k \times l \times p^2 + k \times l \times p \times q + l^2 \times p^2 + l^2 \times p \times q)/w$	
R	q	$(k^2 \times q^2 + k^2 \times p \times q + k \times l \times q^2 + k \times l \times p \times q + l^2 \times q^2 + l^2 \times 2 \times p \times q)/w$	

RESULTS

We calculated the increase of resistance and reduction of biological effectiveness of different doses of the drug Regent (fipronil) in the model with winter survival (fig. 2). The difference between the increase of resistance in the case of recommended and low dose is 1-2 seasons.

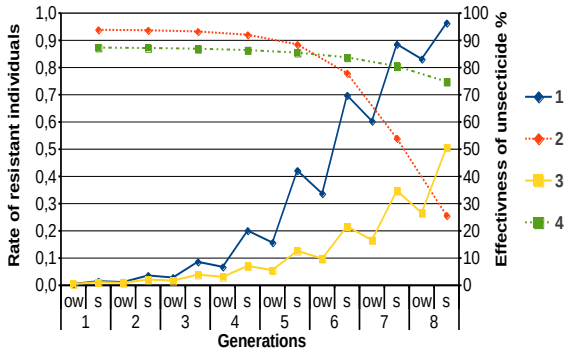

Figure 2: The results of mathematical calculations of increase resistance and reduce the biological effectiveness of different doses of the drug Regent

(fipronil) in the model with winter survival. Proportion of resistant individuals, and changes in the biological effectiveness of using the recommended dosage indicated (1) and (2), and the use of low-dose (3) and (4), respectively (ow - overwintered, s - summer generation).

We calculated the increase of resistance and reduction of biological effectiveness of different doses of the drug Regent (fipronil) in the model with morphotype survival (fig. 3). The difference between the increase of resistance in the case of recommended and low dose is 2-3 seasons.

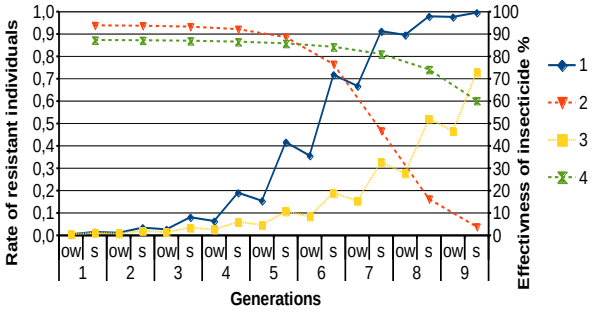

Figure 3: The results of mathematical calculations of resistance and reduce the biological effectiveness of different doses of the drug Regent (fipronil) in the model of two genes. Designations are given above (fig. 2).

Also we examined several hypotheses. We calculated the increase of resistance in the model at different doses of insecticide and, correspondingly, different survival in the field (fig. 4). The most appropriate dose of the relevant biological insecticide efficacy of 85% (R_1 = 0.15).

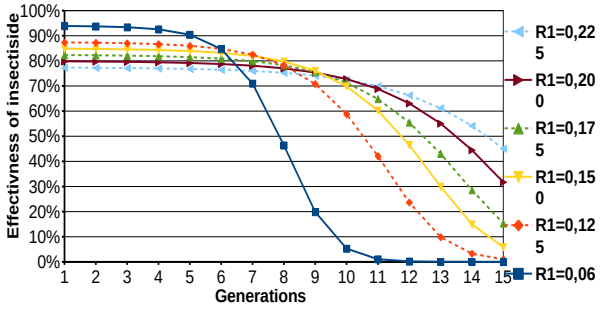

Figure 4: The results of mathematical calculations reduce the biological effectiveness of different doses of the Regent (fipronil) in the model of two genes (R1 — survival of sensitive in field).

And we examine in the model variants with minimal dose of two insecticides alternating with each other. In this variation slowing resistance is equal to 9-14 seasons.

CONCLUSION

A possible way to preserve the biological effectiveness can be the alternation of insecticides of different classes over a number of years. When new classes of insecticides will be entered into circulation, lower doses of it should be used to maintain of its biological effects and inhibition of increase of resistance in populations of Colorado potato beetle. We must focus on determining the minimum effective doses of drugs.

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REFERENCES

Alyokhin A., Baker M., Mota-Sanchez D., Dively G., and Grafius E. 2008. Colorado potato beetle resistance to insecticides // American Journal of Potato Research. 85: 395–413.

Alyokhin A. 2009. Colorado potato beetle management on potatoes: current challenges and future prospects // Fruit, Vegetable and Cereal Science and Biotechnology. Vol. 3. 1: 10–19.

Baker, M. B., Alyokhin A., A. H. Porter, D. N. *et al.* 2007. Persistence and inheritance of costs of resistance to imidacloprid in Colorado potato beetle // J. of Econ. Entomology. 100: 1871–1879.

Benkovskaya G.V., Leontieva T.L., Udalov M.B. 2008. Colorado potato beetle resistance to insecticides in the Southern Urals // Agrochemistry. 8: 55-59.

Benkovskaya G.V., Leontieva T.L., Udalov M.B. 2009. Colorado beetle resistance to insecticides in South Urals // Resistant Pest Management Newsletter. Vol. 19. 1: 3–4.

Benkovskaya G.V., Udalov M.B. 2011. Colorado Potato Beetles Investigations in the South Urals. New York: Nova Science Publishers. pp. 60.

Benkovskaya G.V., Udalov M.B., Poskryakov A.V., et al. 2004. Phenogenetic polymorphism of the Colorado potato beetle *Leptinotarsa decemlineata* Say is an indicator of resistance to insecticides // Agrochemistry. 12: 1–6.

Benkovskaya G.V., Udalov M.B., Khusnutdinova E.K. 2008. The genetic basis and phenotypic resistance of Colorado potato beetle to organophosphorus insecticides // Russian J. of Genetics. Vol. 44. 5: 638-644.

Bezel V.S., Bolshakov V.N., Vorobeichik E.L. 1994. Population ecotoxicology. Moscow: Nauka. pp. 80.

Narahashi T., Zhao X., Ikeda T., et al. 2007. Differential actions of insecticides on target sites: basis for selective toxicity // Human Experimental Toxicology. 26(4): 361–366.

Li A., Yang Y., Wu S., et al. 2006. Investigation of Resistance Mechanisms to Fipronil in Diamondback Moth (Lepidoptera: Plutellidae) // J. of Econ. Entomology. 99(3): 914–919.

Roslavtseva S.A. 2005. Colorado potato beetle resistance monitoring of insecticide // Agrochemistry. 2: 61-66.

Roslavtseva S.A. 2009. Insecticide resistance in populations of Colorado potato beetle // Agrochemistry. 1: 87-92.

Sayyed A.H., Wright D.J. 2004. Fipronil Resistance in the Diamondback Moth (Lepidoptera: Plutellidae): Inheritance and Number of Genes Involved // J. of Econ. Entomology. 97(6): 2043–2050.

Sukhoruchenko G.I. 2005. Status of resistance in crop pest species Russia in the early XXI century // Proceedings of the II All-Russia Congress of Plant Protection. Saint-Petersburg. 61-66.

Sukhoruchenko G.I., Dolzhenko V.I., Goncharov N.R. et al. 2006. The technology and methods of evaluation of side effects of pesticides. Saint-Petersburg.: All-Russia Institute of Plant Protection. pp. 52.

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Effect of photoperiod on organophosphate pesticide detoxification ability in midgut tissue of *Spodoptera litura* (Fab.)

ABSTRACT
Photoperiod is an important environmental factor which governs the physiological activity in insects. The proportion of photophase and scotphase also influence the enzyme activity. Present study is aimed at assessing the influence of light and dark phase on the organophosphate detoxification ability in *Spodoptera litura*. The study shows that Cyt P450 activity is higher, when scotphase is increased. While the activity of esterase increases with increased photophase. Result suggests that *S.litura* employs a differential array of detoxification enzymes with respect to the change in photoperiod for organophosphate detoxification.
Key words: Photoperiod; common cutworm; Esterase; Cyt P450; superoxide dismutase.

INTRODUCTION

The seasonal activity of many insects is governed by environmental factors like photoperiod, temperature and humidity. Among environmental factors photoperiod affects the growth and development of several insects (Musolin & Saulich, 1997). The roles of circadian clock-controlled molecular rhythms in adapting organisms to the environment are only beginning to be explored (Dubruille & Emery, 2008). The basic molecular mechanisms are shared in insects and mammals, a fundamental understanding of the functional significance of circadian rhythms in chemical exposures may facilitate strategies to reduce adverse events in humans, promote control of pest species, and reduce pesticide use (Hooven, et al., 2009). The detoxification enzyme kinetics in *S.litura* beet armyworm have been well documented when feeding on different host plants (Wang *et al*., 2003; Karthi et al., 2012) and different photoperiod, this relationship was investigated with focus on detoxification enzyme activity; therefore Cytochrome P450, SOD, Esterase enzyme activity were selected. Insecticide resistance is mainly attributed to decreased penetration, and enhanced detoxification by mixed function oxidases (MFO), esterases and glutathione-S-transferases. Cytochrome P450 enzymes are involved in the oxidation of a wide variety of drugs, carcinogens, steroids, pesticides, and other chemicals (Coon *et al*., 1992). In insects, it has been shown that the enzyme can metabolize insect hormones (Feyereisen et al., 1981), secondary plant chemicals (Dowd *et al*., 1983), and synthetic insecticides (Agosin *et al.*, 1985). In the present study, this relationship was investigated with focus on carboxylesterase (CarE), Cytochrome P450 and Superoxide Dismutase (SOD). CarE is an important detoxifying enzyme which distributes broadly in insects, playing an important role in the metabolism with various functions, especially on insecticide resistance (Wang *et al*., 2003). The present study is aimed at assessing the influence of photoperiod on organophosphate detoxifying ability of detoxification enzymes in *S. litura*.

MATERIALS AND METHODS

Insect culture and insecticide treatment

Larvae of *Spodoptera litura* were obtained from the laboratory stock maintained at insectary (Molecular Entomology Lab, Department of Biotechnology, Periyar University). The larvae were divided into two photoperiodic regimen of 12L: 12D; 0 L: 24D. Larvae were maintained castor leaves till 5^{th} instars. At 5^{th} instar the larvae were treated at field recommended dosage of monocrotophos (175 g/a.i) insecticide using

leaf dip method recommended by (IRAC; www.irac-online.org/resources/methods.asp).

Enzyme preparation

20 fifth instar larvae were separated and dissect remove the midgut tissues. The dissected larval tissue was homogenized on ice in homogenization buffer (0.1 M phosphate buffer, pH 7.2). The homogenate was centrifuged at 4°C, 10, 000 rpm for 15 min, and the solid debris and cellular material were discarded. The supernatant was decanted into a clean eppendorf tube, placed on ice and used immediately for enzymatic estimation of Mixed Function Oxidase (MFO), Esterase (EST), and Superoxide dismutase (SOD).

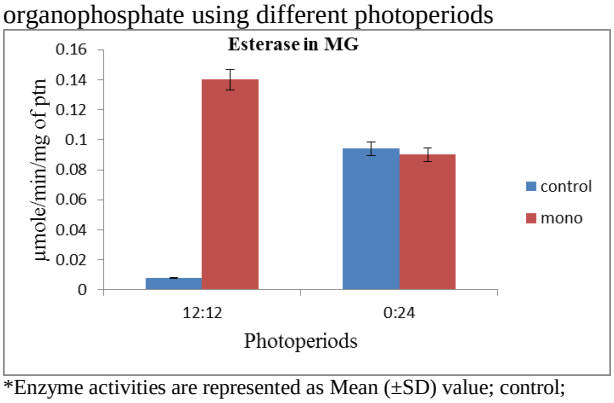
Enzyme assays

Enzyme assays are following methods, esterase assay (Kranthi et al., 2005); cytochrome P450 assay (Brogdon et al., 1998) and superoxide dismutase (Marklund & Marklund, 1974).

RESULTS AND DISCUSSION

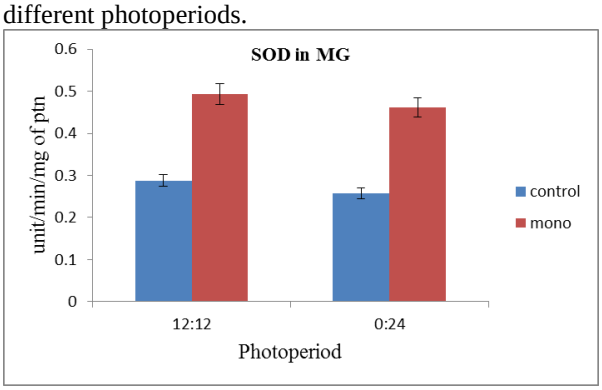
Esterase activity in *Spodoptera litura* larvae shows high in 12:12 photoperiod. SOD activity was high in both photoperiods (12:12; 0:24). Cytochrome P450 activity was high in 0:24 photoperiod as compared to control (Fig: 3). Several studies on insects circadian rhythms in ECOD activity reflecting mixed function oxidase activity, which has been associated with insecticide resistance and over expression of Cyt P450 in *Drosophila* (Sousa et al., 1995). Constant light which abrogates clock function, depressed rhythm in both enzymes activity (Esterase, Cyt P450) and rhythm is susceptibility to monocrotophos. Daily rhythms in expression of xenobiotic metabolizing enzymes may have evolved to anticipate the intake of plant allelochemicals, mycotoxins and other compounds ingested during daily feeding rhythms (Saunders, 2002; Shea et al., 2005). The above study suggests that there are differential detoxification enzyme profiles in response to changes in photoperiod. Suggesting that photo phase positively affects esterase activity while inhibits/reduces the activity of Cyt P450. The further studies on the relative contribution of detoxification enzymes to OP detoxification can further sustainable our findings and can help in dividing the timing of the insecticide sprays to achieve maximum efficiency in Insect control.

Figure 1: Detoxification enzyme profiles of esterase on fifth instar *Spodoptera litura* larvae exposed to organophosphate using different photoperiods



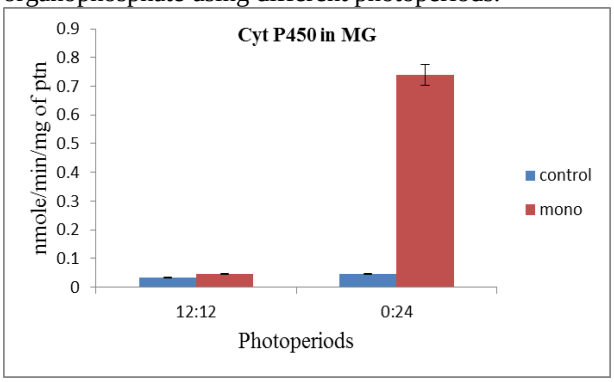
*Enzyme activities are represented as Mean (±SD) value; control; mono- Monocrotophos; MG- Midgut.

Figure 2: Detoxification enzyme profiles of Superoxide dismutase (SOD) on fifth instar *Spodoptera litura* larvae exposed to organophosphate using different photoperiods.



*Enzyme activities are represented as Mean (±SD) value; control; mono- Monocrotophos; MG- Midgut.

Figure 3: Detoxification enzyme profiles of MFO on fifth instar *Spodoptera litura* larvae exposed to organophosphate using different photoperiods.



*Enzyme activities are represented as Mean (±SD) value; control; mono- Monocrotophos; MG- Midgut.

REFERENCES

Musolin, D. L., and Saulich, A. K., 1997. Photoperiodic control of nymphal growth in true bugs (Heteroptera). Entomological Review. 77: 768-780.

Wang, M., Wu, C.C., Zhu, F.X., 2003. Studies on insecticide susceptibility of the beet armyworm fed on different host plants. Journal of Plant Protection. 30: 193-197. (In Chinese).

IARC Susceptibility test methods series. 2009: 3, Insecticide resistance action committee (www.irac-online.org/resoursces/methods.asp).

Kranthi, K.R., 2005. Insecticide resistance-Monitoring, Mechanisms and Management Manual. Central Institute for Cotton Research. Shankar Nagar PO, Nagpur. 155.

Brogdon, W.G., McAllister, J.C., and Vulule, J., 1998. Heme peroxidase activity measured in single mosquitoes identifies individuals expressing the elevated oxidase mechanism for insecticide resistance. Journal of American Mosquito Control Association. 13: 233-237.

Marklund, S.L., and Marklund, G., 1974. Involvement of the superoxide dismutase. European Journal of Biochemistry. 47: 469-474.

De Sousa, G., Cuany, A., Burn, A., Amichot, M., Rahmani, R. et al. 1995. A microflurometric method for measuring ethoxycumarin-O-deethylase activity on individual *Drosophila melanogaster* abdomens: insect for screening resistance in insect populations. Anal Biochem. 229: 86-91.

Saunders, D.S., 2002. Insect clocks, Amsterdam: Elsevier. Pp: 560.

Shea, S.A., Hilton, M.F., Orlova, C., Ayers, R.T., Mantzoros, C.S., 2005. Independent circadian and sleep/ wake regulation of adipokines and glucose in humans. Journal of Clinical Endocrinology Metabolism. 90: 2537-2544.

Karthi, S., Muthusamy, R., and Shivakumar, M.S., 2012. Influence of Diet on Pesticide Detoxification Ability of *Spodoptera litura* Exposed to Organophosphate and Synthetic Pyrethroid Insecticides. Resistant Pest Management Newsletter. 21 (2): 26-28.

Dubruille, R., & Emery, P., 2008. A Plastic Clock: How Circadian Rhythms Respond to Environmental Cues in *Drosophila*. Molecular Neurobiology. 38(2): 129-145.

Hooven, L.A., Sherman, K.A., Butcher, S., Giebultowicz, J.M., 2009. Does the Clock Make the Poison? Circadian Variation in Response to Pesticides. PLoS ONE. 4(7): e6469.

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