Resistant Pest Management Newsletter

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

Vol. 21, No. 1 (Fall 2011)

Table of Contents

Resistance Management from Around the Globe

CHOOSING PREDATORS FOR BIOCONTROL OF COLORADO POTATO BEETLE IN THE SOUTH URAL

INTRODUCTION

Invasive species are the most problematic in ecology. Trophic relationships of invasive species are very important for designing methods of pest management and biocontrol. We are researching the adaptation processes in predators proceeding to feed by Colorado potato beetle (*Leptinotarsa decemlineata* Say) in the South Urals. This species is invasive. It has appeared in South Urals 34 years ago. Complex of predators living in potato field is formed since its arrival.

Keywords*: Colorado potato beetle, natural enemy, coccinella septempunctata, trophic stress induction*

MATERIALS AND METHODS

PCR – analysis of predators gut content

Predatory insects were caught in antifreeze (water solution of ethylenglicol) (Weber et al, 2009) and were preserved for a week. We extracted DNA from the gut and its content; also we extracted DNA from other tissues (muscles, fat body). These samples of DNA were used for PCR with specific primers designed from COI of *L. decemlineata* (Greenstone et al, 2007). Predation in the insects can be detected by PCR analysis with species-specific primers even through 24 hours.

Demonstration of stress induction

The level of stress induction is detected by analyzing activity of acetylcholinesterase and the concentration of catecholamynes in the imago's hemolimph at 2 and 18 hours after feeding (aphids, eggs of *L. decemlineata*, and homogenates of larvae *L. decemlineata*).

Activity of acetylcholinesterase is detected by methods of Ellman (Ellman et al., 1961). Concentrations of catecholamynes are determined by modificated method of Ronin (Benkovskaya, 2009).

Survival of imago *C. septempunctata* living in plastic boxes is determined in 14 days.

RESULTS

Several species of Carabidae and Coccinellidae were caught. Seven-spotted lady beetle (*Coccinella septempunctata*), *Pterostichus melanarius, Harpalus rufipes and Calosoma investigator* are the most abundant predators in potato fields. Positive results of PCR – analysis of predators gut content- have been given in the case of *C. septempunctata* (Fig. 1).

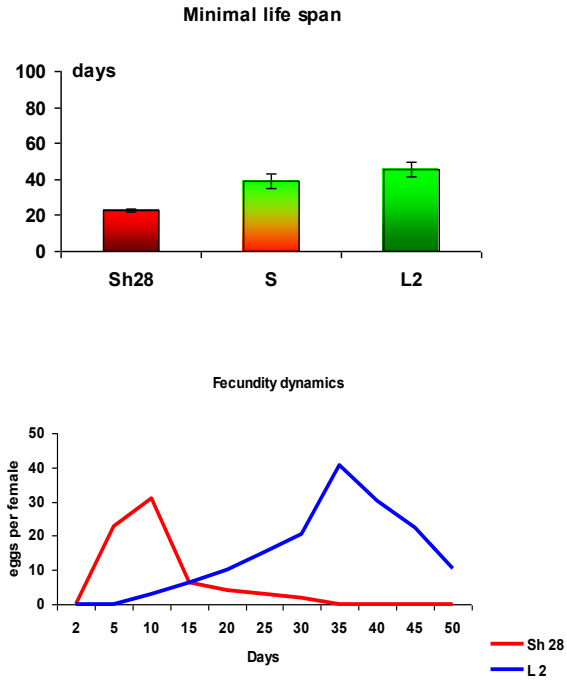

Fig. 1: Differences in life span and reproductive efforts of M. domestica adults (F 20).

The stress dynamics of *C. septem punctata* imago being fed different foods showed an increase of acetylcholinesterase activity after feeding because of an increase in the moving activity of imago. Higher

activity of imago feeding eggs in 2 hours and following decreasing beside in the case of feeding aphids shows fitness for feeding eggs of *L. decemlineata*, but it doesn't use always (Fig.2.).

Concentration of catecholamynes changes a little and differences between variants are unreliably. Increase of repeats is claimed (Fig.2).

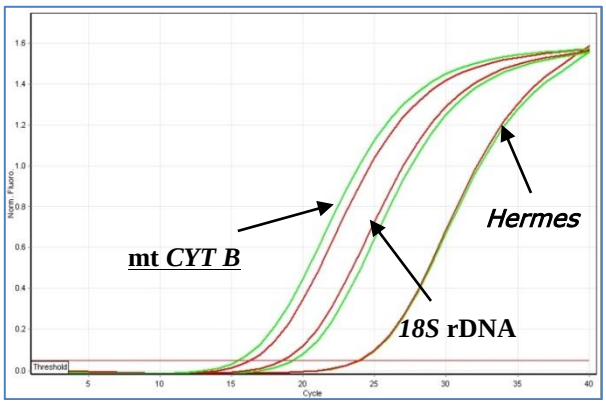

Fig. 2: mtDNA/nuclear DNA copy number ratio in house fly adult's muscles of different strains
Green line: for L 2-strain. Red line: for Sh 28-strain (Hermes ~ 20 copy per cell; rDNA ~ 700-1000; mtDNA ~ 1000 -5000)

Survival of imagoes feeding homogenates of larvae *L. decemlineata* is presented below at 7 – 8 days (Fig.3). Homogenates are an unusual food source for *C. septempunctata* and caused increased mortality.

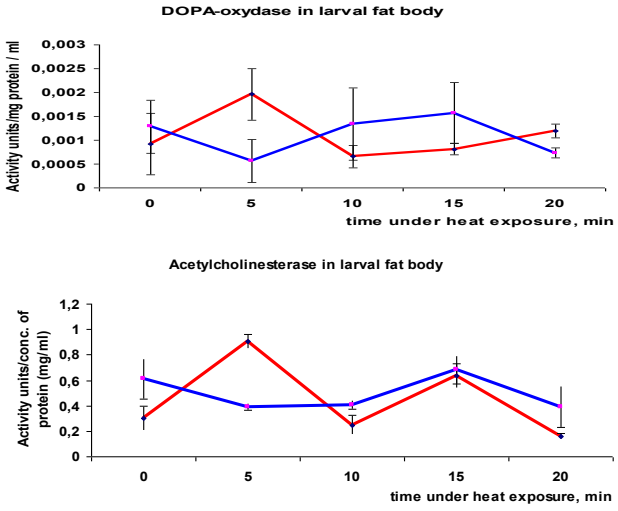

Fig 3: Dynamics of fat body stress enzymes under heat stress (red line – Sh 28, blue line – L 2)

CONCLUSION

Seven-spotted lady beetle adapted for feeding eggs of *L. decemlineata*. But we don't know if this fitness always existed or if it formed recently at the period of invasion of *L. decemlineata*.

We may solve this problem when investigating population genetics of *C. septempunctata* and other predators from different localities.

ACKNOWLEDGEMENTS

Investigations funded by RFBR №09-04-00391-a, №11-04-01886-a and 11-04-97022-a.

REFERENCES

Benkovskaya G.V., 2009. Ecological and physiological features and polymorphism of colorado potato beetle adults in Bashkortostan // Belgorod State University scientific bulletin. Vol.8. No.3 (58). P. 59-67.

Ellman G.L., Courtney K.D., Andres V., Featherstone R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity // Biochem. Pharmacol. Vol.7. No.2. P. 88-95.

Greenstone M.H., Rowley D.L., Weber D.C. *et al.*, 2007. Feeding mode and prey detectability half-lives inmolecular gut-content analysis: an example with two predators of the Colorado potato beetle // Bulletin of Entomological Research. Vol. 97. P. 201 – 209.

Weber D.C., Lundgren J.G., 2009. Detection of predation using qPCR: Effect of prey quantity, elapsed time, chaser diet, and sample preservation on detectable quantity of prey DNA // J. of Insect Science. Vol. 9. N. 41. 12 pp, available online: insectscience.org/9.41

K.A. Kitaev, M.B. Udalov, G.V. Benkovskaya
Institute of biochemistry and genetics of Russian Academy of Science, Russia.
cordek(at)ya.ru

Laboratory and semi-field evaluation of susceptibility of *Anopheles arabiensis* larvae to Temephos in Medani locality, Gezira State, Sudan

Abstract
Although temephos has been widely used throughout the Sudan since 1970s, Elkaraiba *An. arabiensis* population is still susceptible to this chemical. In the outdoor experiment muddy bowels were used to mimic the natural mosquito habitat. The laboratory $LC_{50,}$ LC_{95} and LC_{99} obtained were 0.012, 0.023 and 0.027, respectively while outdoor results were 0.017, 0.034 and 0.040 mg/l, respectively. This preliminary work indicates the necessity for careful, complete and continuing monitoring of resistance: susceptibility status, resistance level and mechanisms, in all areas. Because the national laboratories, if found, all vary enormously in their resources to deal with insecticide resistance, a national resistance surveillance laboratory need to be established here in Sudan. Moreover, the level of effective activity and longevity of temephos in the fields should be evaluated for the most appropriate profitable use.
Keywords: *Anopheles arabiensis,* Larvae, Temephos, Susceptibility, Gezira State Sudan

INTRODUCTION

Malaria is a major vector-borne disease in sub-Saharan Africa that led to approximately 1 million deaths mostly of children under 5 years (WHO**,** 2008**)**. Sudan had about 2.5 million malaria cases in 2006, 62% of the burden in the WHO Eastern Mediterranean Region (WHO, 2006). *Anopheles arabiensis* is considered as the principal malaria vector in Sudan (Petrarca *et al.* 2002 and Ahmed 2007) and *Plasmodium falciparum* and *P. vivax* are the major malaria parasites (WHO, 2008). In Gezira state (center of Sudan) malaria is also a common problem and constitutes 20% of cases seen by the state health facilities (Ministry of health, Gezira state, Sudan, unpublished data). A year round malaria incidence was reported in Gezira-Managil irrigated scheme as a consequence of irrigation and agricultural development (El Gaddal *et al*. 1985). Insecticide resistance has had a profound effect on the reemergence of vector-borne diseases, and threatens disease control (WHO, 1992).

Larviciding could be a method of choice particularly where breeding sites are accessible and relatively limited in number and size. Temephos or temefos (trade name Abate) 0, 0, 0', 0'-tetramerhyl 0, 0'-thiodi-p-phenylene phosphorothioate is an organophosphorus compound developed by American Cyanamid Company (Cyanamid Manual**)**. Temephos (Abate®), has been used for malaria vector control in Gezira State, Sudan, since mid of 1970th. The first efficacy test of Temephos 50% E.C. was performed in 1973 in Alhush city, and since then been recommended for use in mosquito larval control with the dose of 1mg/L for clean water and 1.5 mg/L for the turbid water (Elfatih Abdalla, health official, personal communication). After this long period of Temephos application, there are rare reports on susceptibility status in *An. Arabiensis* population in Gezira State or other places in the Sudan (Ahmed, 2007). In underdeveloped countries especially the remote area, malaria epidemics evolve faster and hence, need proper surveillance. This work aimed at detecting the LC_{50}, LC_{95} and LC_{99} to be considered as the preliminary step to be followed by determining resistance levels and mechanisms.

MATERIALS AND METHODS

Study Area

This study was conducted using population samples from Alkaraiba village (5 kilometers West of Wad Medani, Capital of Gezira State, Sudan) in Wad Medani locality, latitude 14.4 N, longitude 35.5E, and altitude 407 m above the sea level.

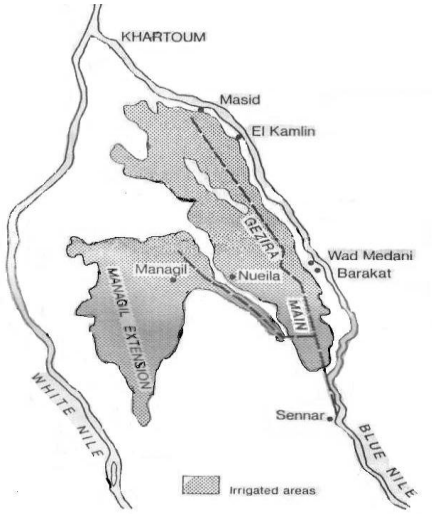

Fig 1: Map of Wad Medani, Central Sudan

Mosquitoes Sampling

A laboratory colony from adult field-collected, *An. Arabiensis* was established at the Blue Nile National Institute for Communicable Diseases (BNICD), University of Gezira, Wad Medani. The mosquitos adults were occasionally collected from the indoor resting sites in Alkaraiba village. The collected mosquitoes were kept, during the transport to the laboratory, in paper cups and sufficient relative humidity was assured by placing small pieces of cotton wool impregnated with water in the cup. Adults were reared in the laboratory, blood fed on rabbits, and F1 eggs were collected the following weeks in wetted

filter papers. F1 eggs were periodically hatched as needed for larval bioassay purpose and larvae were reared to the late 3rd , and early 4th instars. All mosquito larvae used in the laboratory experiments were reared at a room temperature of 28 ±2 ºC, and an approximate 12 hour light: 12 hour dark cycle.

Larvae preparation

Indoor Trials

Larvae were reared in dishes (30 cm. diameter and 9 cm. deep), filled with 1.5 liters of distilled water and covered with nets. Larvae were fed by adding a pinch of crushed fish food, finger pressed before being put and spread evenly on the water surface twice daily.

Outdoor Trials

Artificial small ponds (Fig 2) were created using similar dishes (30 cm. diameter and 9 cm. Deep). Here, the internal surfaces of the dishes were covered first by clay from the *Gambosia* fish rearing pond. The dishes were then filled with pond's water (uncontaminated) before placing the eggs and screened with nylon netting. The rearing dishes were implemented outdoor to allow third and fourth instars larvae to develop. These muddy dishes or artificial ponds were performed to mimic the natural larval habitat so as to identify more accurately the optimum dosages of Temephos 50% EC under field conditions. Water temperatures during the experiments were 27°C.

Fig 2: The muddy dishes or artificial ponds were performed to mimic the natural larval habitat.

Bioassays

Indoor Tests

Cohorts of 25 larvae of 3rd and early 4th instars were distributed into 300 ml beakers filled with 249 ml of water. After an observation for mortality or any abnormalities for 1 h, a preliminary test was conducted to determine the general level of susceptibility. One ml of each of WHO kit series was added to the 249 ml of water to perform the 4 concentrations: 0.005, 0.025, 0.125 and 0.625 mg/L. Then, based on the insecticide concentration giving approximately 10–90% larval mortality a new selected series of 4 concentrations (0.01, 0.015, 0.02, and 0.025 mg/L) were then, used for the test, and the susceptibility status was established based on the larval mortality. The larvae were exposed to the different concentrations of insecticide for 24 hours, during which no food was added. All tests were carried out at temperature of 28 ±2 ºC. Dead and moribund larvae (presenting tremors, rigidity, or inability to reach the water surface when touched) which were considered as dead were combined in the mortality counts. Mortality counts were made after 24 hours of treatment. Mortality in treatments was corrected for control mortality according to Abbott's formula. The data were subjected to a log-dose-probit regression analysis and the final estimation of lethal concentrations (LC_{50} and LC_{95}) was calculated using the Statistical Package for Social Sciences (SPSS) software program.

Outdoor Tests

The indoor test with the aforementioned 4 selected concentrations (0.01, 0.015, 0.02, and 0.025 mg/L) was repeated outdoor. They were dispensed evenly on the surface of the water (not stirred) as naturally applied by the health technicians. Batches of 25 late third and early fourth-instar larvae were distributed into small muddy bowls (16.5 cm. diameter and 9 cm. deep), filled with 249 ml of pond's water. Mortality or any abnormalities was observed for 1 hr. Then, 1 ml of stock solutions were dispensed on the water surface (not stirred) to mimic the natural application and to perform each of this series of four insecticide concentrations. The concentration was repeated three times and the experiment was replicated three times. In the control bowels, 1 ml of ethanol used as diluent (control) was added. The larvae were exposed to the different concentrations of insecticide for 24 h outdoor at 38-40°C, during which no food was added. The live larvae were moved to clean water and moribund and dead larvae were combined in the mortality counts. The results were obtained and analyzed as mentioned before**.**

RESULTS

Indoor and Outdoor Results

WHO kit sets of Temephos concentrations 0.005, 0.025, 0.125 and 0.625 mg/L yielded 3.3, 90.6, 100, and 100% larval mortality of *An. arabiensis*, respectively. Mortality counts obtained from using the four concentrations: 0.01, 0.015, 0.02, and 0.025 mg/L yielded 44.4, 64.9, 77.6 and 96% indoor and 21.3, 53.3, 57.3, and 74.6% outdoor. These counts were used to analyze for the susceptibility status. Table 1, shows *An. arabiensis* larval mortality in the indoor and outdoor experiments, respectively. In the present indoor laboratory test, LC_{50} and LC_{95} were 0.012 and 0.023,

with lower and upper limits at 0.010-0.014 and 0.021-0.026, respectively. Outdoor LC_{50} and LC_{95} were 0.017 mg/L, and 0.034 with lower and upper limits at 0.012-0.020 and 0.026-0.039, respectively. The outdoor LC_{50} and LC_{95} are only about 1.4 times higher than the indoor results. Additionally, the WHO adopted 0.25 mg/L as a diagnostic concentration (double the LC_{99}) for anopheline*s* (WHO, 1986). Persistent survivors at this dose in field populations would be indicative that resistance to temephos has been developed. The LC_{99} obtained from indoor and outdoor tests were 0.027 and 0.041 mg/L with lower and upper limits at 0.025-0.031 and 0.034-0.058, respectively. Hence, all populations tested were susceptible to temephos. The temephos has been used since 1970s. However, the Elkaraiba anopheline population tested remained susceptible to this chemical.

Table 1: Susceptibility to Temephos 50% EC of field-collected larvae of *Anopheles arabiensis*, 24-hr lethal concentration (mg/L).

Type of Test	LC50	LC90	LC95	LC99
Indoor	0.012	0.021	0.023	0.027
outdoor	0.017	0.030	0.034	0.041

Moreover, the result of this work (LC_{95}s) is very close to the results obtained from Khartoum State, Sudan (Ahmed, 2007). *An. arabiensis* in Bahary locality, (200 km from Elkaraiba), is highly susceptible to temephos with LC_{50} and LC_{90} of 0.00271 and 0.025 ppm, respectively. Furthermore, *Anopheles gambiae s. l.* (member of An. *gambiae* complex) larvae from Ouagadougou, Burkina Faso (West Africa) showed complete susceptibility to organophosphorus and carbamates with temephos scoring LC_{50} of 0.0038 mg/L (Majori *et al*. 1986). When comparing the LC_{95}s, the Bahary population is very close to Elkaraiba ones, and the Ouagadougu population is 3 times more susceptible than Elkaraiba population (Table 2).

Table 2: Comparison of *An. arabiensis* susceptibility to Temephos based on lethal concentrations (LCs in mg/L)

Site	LC50	LC90	LC95	LC99	Reference
WHO				0.125 (DC = 0.25)	WHO 1986
Bahary, Sudan	0.00271	0.025			Ahmed, 2007
Ouagadougu, Burkina Faso	0.0038				Majori *et al.*, 1986
Elkaraiba, Indoor	0.012	0.021 (0.019-0.023)	0.023	0.027 (0.025-0.031)	Present work
Elkaraiba, outdoor	0.017	0.030 (0.026-0.039)	0.034	0.041 (0.034-0.0580	Present work

DISCUSSION

Anopheles gambiae and *An. arabiensis* are the world's most efficient vectors of human malaria **(**Levine *et al*. 2004)**.** *An. arabiensis* is thought to be the main malaria vector in Sudan (Himaidan *et al*. 2005). *An. arabiensis* exists the year round in this region. It is known here that the higher *An. arabiensis* numbers are in the rainy season (June to September). During this period, rains favor the formation of breeding sites in addition to the temperature and humidity conditions that favor its biological cycle. This study was carried out during April, the hottest and dries period of the year (>40°C). Vector's resistance to insecticides has impact on both the control of these insect species and on the diseases they transmit. Therefore, Brogdon and McAllister (1998) were stated that close surveillance of vectors' susceptibility is the main defense against resistance which is the objective of this work using Temephos as a larvicide. Temephos (Abate™) is an organophosphorus compound widely used as a larvicide. In the present study the acute toxicity of Temephos (Abate) was studied and compared with the only locally available data of Ahmed (2007), WHO adopted diagnostic concentration (DC) and results from Burkina Faso to obtain a better understanding of the situation since neither susceptible insect strain nor more local reported data are available. For better representation of Medani locality, this work was designed first to be carried out in four sites. For lack of resources, the tests were conducted using population samples from Elkaraiba village only. In the outdoor experiment, the selection of these muddy bowels was to mimic the natural mosquito habitat. Additionally, black-bottomed water dishes are prefered by the gravid females of *An. arabiensis* than the white-bottomed ones for oviposition, and most eggs are laid in pools with muddy and unvegetated edges beside other factor (Chen *et al*. 2006). The procedure followed briefly, was 25 larvae in 249 ml of distilled, and fish rearing waters, for indoor and outdoor experiments, respectively, with

1 ml of each concentration of temephos. Mortality counts were made after 24 hours. The results were analyzed to obtain the LC_{50}, LC_{95} and LC_{99} by probit analysis using a SPSS computer program. The present work is considered as a preliminary step toward accurate and continuing evaluation of *Anopheles arabiensis* susceptibility status, resistance level and mechanism(s) in Sudan.

In the present study and in the first WHO kit test, this concentration (0.125 mg/L) scored 100% death. Therefore, Elkaraiba population is considered susceptible. No laboratory susceptible strain colonized here to perform more detailed work as the resistance ratio based on the median lethal concentration which is the LC_{50} of the resistant/ LC_{50} of the susceptible. However, it would be of great value to obtain conclusive information for such mosquitoes in all Sudan. Furthermore, based on the aforementioned results obtained from using the WHO kit a new series of four concentrations, 0.01, 0.015, 0.02, and 0.025 mg/L were selected to evaluate the susceptibility status indoor and outdoor. Such tests yielded a straight lines relationship between the logarithm of the concentration and probit mortalities (Table 1). Therefore, Elkaraiba population is even about 4.6 and 3 times more susceptible, indoor and outdoor, respectively, than the aforementioned WHO susceptible strain. Temephos 50% E.C. was tested for the first time in Gezira state in Alhush city in 1973 and has been used since then with the dose of 1 mg/L for clean water and 1.5 mg/L for the turbid water (Elfatih Abdalla, Health official, Personal communication). However, susceptibility testing of *An. arabiensis* collected from Elkaraiba during the study period showed that they were more susceptible to temephos when compared with the WHO DC of 1986. Moreover, literature survey revealed that only rare data are reported from this region and from Sudan, in general, in the last decades. Ahmed (2007) stated that despite, continuous use of temephos for more than two decades in control operations, *An. arabiensis* in Bahary locality, Khartoum state, Sudan (200 km from Elkaraiba), is highly susceptible to temephos $LC_{50}$and LC_{90} of 0.00271 and 0.025 ppm, respectively. The result of this work (LCs) is very close to the results obtained from Khartoum State, Sudan (Ahmed, 2007). Additionally, the local health organs have used a dose of 1 and 1.5 mg/l. Hence, the present results supporting Ahmed's field recommendation of using a seven day cyclic application of 0.5 mg/m^2 instead of 1 mg/m^2 (temephos 50% EC) as a cost-effective option. That is because, the indoor, outdoor and field results should be considered when recommending such a dose.

Additionally, *Anopheles gambiae* s. l. larvae from Ouagadougou, showed complete susceptibility to temephos (Majori *et al*. 1986). When comparing the three LC_{95}s, the Bahary population is very close to Elkaraiba ones, and the Ouagadougu population is 3 times more susceptible than Elkaraiba population. Such levels of temephos susceptibility in Sudan and in Africa indicate the necessity for careful and complete monitoring of resistance in all areas. Moreover, studying the vector ecology is important. For instance, the results of Chen *et al*. (2006) stated that the average genetic relatedness for breeding sites tends to be low because *An. arabiensis* female uses multiple breeding sites. Therefore, it is important to create plan to locate, map and monitor larval populations and then implementing targeted larval control based on field-collected data.

Furthermore, the health organs in Sudan use emulsifiable concentrate (EC) which offers more fast release and direct contact with the exact used concentration than sand granules, for example because of its low solubility in water. These factors could contribute to the delay in selection for tolerance up to resistance levels in nature on exposure to insecticide at above-mortality doses. Therefore, level of effective activity and longevity of temephos in the fields should therefore be evaluated for the most appropriate profitable use. It could be useful should a plan be created to collect samples from a range of sites within each locality and from the states within Sudan. In other words, one or few sample points may not provide sufficient information for determining the efficacy of temephos in the laboratory or in the field. That is because, differences in control measures and rate of insecticide application might account for different levels of response to insecticide. In general, resistance could be fluctuating due to differences in the control methods adopted. Therefore, it would of importance, in Sudan case, if correlation to be evaluated between the susceptibility of sub-populations and the distance between different sites within the country. Additionally, the increase in resistance is directly dependent on the selective pressure exerted by the insecticide. Therefore, the correlation between susceptibility and the amount of insecticide applied in the years preceding the study would be of value. If resistance is shown to be directly affecting control, other methods such as rotating the insecticides can be considered. Larviciding should be part of integrated control program, the use of the right insecticides with the right dose, associated with source reduction, biological control and community educational measures should have been able to control mosquito infestations.

Finally, careful, complete and continuing field surveillance for resistance requires simple bioassay, biochemical, and molecular genetics studies (Brogdon and McAllister, 1998). As a result of the present findings, and because the national laboratories, if

found, are varying enormously in their resources to deal with insecticide resistance, a national resistance surveillance laboratory needs to be established here in Sudan.

REFERENCES

Ahmed, B. A. I. (2007). Field evaluation of Temephos and Agnique® (MMF) against immature stages of *Anopheles arabiensis* patton (Diptera: Culicidae) the vector of malaria in Khartoum, Sudan. M. Sc. Thesis, University of Sains, Malaysia.

Brogdon, W. G. and Mc Allister, J .C . (1998). Insecticide Resistance and Vector Control Centers for Disease Control and Prevention, Atlanta, Georgia, USA

Chen, H.; Fillinger, U. and Yan, G. (2006). Oviposition behavior of female *Anopheles gambiae* in western Kenya inferred from microsatellite markers. *Am. J. Trop. Med. Hyg*., 75(2): 246-250.

Cyanamid Manual: Abate, Comprehensive Research and Development Manual

El Gaddal, A.A.; Haridi, A.A.M.; Hassan, F.T. and Hussein, H. (1985). Malaria control in the Gezira-Managil irrigated scheme of the Sudan. J. *Trop. Med. Hyg*., 88: 153-159.

Himeidan, Y. E.; Malik, E. M. and Adam, I. (2005). Epidemiology and Seasonal Pattern of Malaria in an Irrigated Area of Eastern Sudan. *American Journal of Infectious Diseases*, 1(2): 75-78.

Levine, R. S.; Peterson, A. T. and Benedict, M. Q. (2004). Geographic and ecologic distributions of the *Anopheles gambiae* complex predicted using a genetic algorithim. *Am. J. Trop. Med. Hyg*., 70(2): 05-10

Majori, G.; Sabatinelli, G.; Villani, F. and Petrarca, V. (1986). Studies on insecticide susceptibility of *Anopheles gambiae* s. l. and *Culex quinquefasciatus* in the area of Ouagadougou, Burkina Faso (West Africa). *J. Am. Mosq. Control Assoc.,* 2(3): 305-309

Petrarca, V.; Nugud, D. H.; Ahmed, A. E. M.; Haridi, M. A.; Dedeco,A. M. and Coluzzi, M. (2002). Cytogenetic of An. gambiae complex in Sudan, with special reference to An. arabiensis: relationships with East and West African populations. Med. Vet. Entomol., 14: 149-164.

WHO. 1986. Resistance of vector and reservoirs of disease to pesticide. Tenth report of WHO expert committee on vector biology and control. WHO technical report series 737

WHO. 1992. Vector resistance to insecticides. 15th Report of the WHO Expert Committee on Vector Biology and Control. World Health Organ Tech Rep Ser 818 1992;818:1-62.

WHO. 2005. Guidelines of laboratory and field testing of mosquito larvicides.

WHO. 2006. Pesticides and their application for the control of vectors pests of public health importance CDC

WHO. 2008.: http://www.who.int/malaria/wmr2008/MAL2008 CountryProfiles/MAL2008-Sudan-EN.pdf

Hani Mohamadai, Adil E. A. Bala* and Ahmed B. M. Hassan
Blue Nile National Institute for Communicable Disease, University of Gezira, Medani P. O. Box 20, Sudan.
***** Author to whom correspondence and reprint requests should be addressed**.**

Research in Resistance Management

Pyrethroid Resistance in Green Peach Aphid in Southwestern Virginia (USA) and Field Efficacy of Insecticides in Peppers

INTRODUCTION

Green peach aphid (GPA), *Myzus persicae* (Sulzer), is found worldwide and is a pest of many crops (Capinera 2001, van Emden and Harrington 2007). In the summer months, aphids leave their overwintering host, *Prunus* spp., to feed on a wide range of plants including many agronomic crops (van Emden et al. 1969, Blackman and Eastop 2007). The pest can be particularly problematic on pepper [*Capsicum annuum* L.], where aphids feed on young plant tissues causing water stress, wilting, and reduced growth rates. Under heavy infestations, leaves may become curled and distorted, and plants may become stunted (Kuhar et al. 2009). GPA has a remarkable reproductive capability, and populations can quickly reach damaging levels. As these large populations feed on leaves, they excrete large amounts of honeydew, which causes sticky fruit, a decrease in marketability, and also leads to the growth of black sooty mold fungus on the fruit and the leaves (Kuhar et al. 2009). In addition, GPA is also a vector of over 100 different plant viruses (Blackman and Eastop 2007).

The pest significance of GPA in peppers in the USA is elevated because growers frequently apply preventative insecticides on a weekly basis, sometimes resulting in as many as ten applications of synthetic pyrethroids per crop (Welty 1995, Chapman et al. 2009). While these insecticide sprays control most of the important lepidopteran, coleopteran, and hemipteran insect pests (Welty 1995, Kuhar and Speese 2002, Kuhar et al. 2003), they are also highly toxic to beneficial insects (Roush and Hoy 1978, Rock, 1979, Hill and Foster 2000, Baur et al. 2003, Chapman 2009). This loss of natural enemies can intensify secondary pest outbreaks, particularly if the pest insect has developed resistance to the insecticides used. One such example is GPA.

Populations of GPA from around the world have developed resistance to a number of different insecticides, including carbamates, neonicotinoids, organophosphates and pyrethroids (Foster et al. 2007). To our knowledge, insecticide resistance in GPA has not been studied or documented in southwestern Virginia (USA); therefore, the purpose of this study was to assess the efficacy of pyrethroid and other insecticides on GPA in peppers in Virginia.

MATERIALS AND METHODS

In 2011, we evaluated the effects of several insecticides on insect pests of peppers using three separate small-plot field experiments conducted at the Virginia Tech Kentland Research Farm (80° 25' W, 37° 14' N; elevation ≈640 m), near Blacksburg, VA (USA). Each experiment consisted of either 12 or 13 treatments in a randomized complete block design with four replications. Individual plots were 1 row wide by 6-m long with >3-m alleys between plots. Bell peppers (var. 'Aristotle') were established on raised beds on black plastic mulch with drip irrigation, and were maintained using standard agricultural procedures including standard fertilizer, herbicide, and fungicide applications according to a commercial vegetable production manual for Virginia (Kuhar et al. 2006). The insecticides used in the three experiments and their manufacturers are listed in Table 1. Insecticides were applied with a CO_2 backpack sprayer with a one-row boom having 3 hollow-cone nozzles per row (one over the top and one drop nozzle on each side). This sprayer delivered 143 liters ha $^{-1}$ of spray at 2.72 atm. Treatments were applied on 1, 6, 15, and 24 Aug. On 9 Sept, numbers of aphids were counted on 20 randomly-picked leaves per plot. Data were analyzed using analysis of variance (SAS JMP software) to test for significant treatment effects. To stabilize variances, aphid density data were $\log_{10}$ x-transformed. If the treatment source of variation was significant, differences among treatment means were tested using Tukey's HSD at the $P \leq 0.05$ level of significance.

Table 1: Insecticides used in bell pepper experiments conducted near Blacksburg, Virginia in 2011.

Active Ingredient	Trade name (Formulation)	Manufacturer	Insecticide Class	AI (% in product)
Experiment 1				
Acephate	Acephate 97UP	United Phosphorous Inc.	Organophosphate	97.0%
Acetamiprid	Assail 30SG	United Phosphorous Inc.	Neonicotinoid	30.0%
Bifenthrin	Bifenture 2EC	United Phosphorous Inc.	Pyrethroid	25.1%
λ-cyhalothrin	Lambda-Cy 1EC	United Phosphorous Inc.	Pyrethroid	11.4%
Permethrin	Perm-up 3.2EC	United Phosphorous Inc.	Pyrethroid	36.8%
Experiment 2				
Bifenthrin + Avermectin B1	Athena	FMC Corp.	Pyrethroid + Avermectins	8.84 + 1.33%
Bifenthrin + ζ-cypermethrin	Hero	FMC Corp.	Pyrethroid + Pyrethroid	9.72 + 3.24%
Flonicamid	Beleaf	FMC Corp.	Selective homopteran feeding blocker	50.0%
Imidacloprid + Bifenthrin	Brigadier 2SC	FMC Corp.	Neonicotinoid + Pyrethroid	11.3 + 11.3%
β-cyfluthrin	Baythroid XL	Bayer CropScience	Pyrethroid	12.7%
imidacloprid + β-cyfluthrin	Leverage 360	Bayer CropScience	Neonicotinoid + Pyrethroid	21.0 + 10.5%
Methomyl	Lannate LV	Dupont	Carbamate	29.0%
ζ-cypermethrin	Mustang Max	FMC Corp.	Pyrethroid	9.6%
Experiment 3				
Clothianidin	Belay 2.1SC	Valent USA	Neonicotinoid	23.6%
Dinotefuran	Venom 70SG	Valent USA	Neonicotinoid	70.0%
Ethofenprox	Trebon (280 g/liter)	Mitsui Chemicals America, Inc.	Pyrethroid	28.0%
Fenpropathrin	Danitol 2.4SC	Valent USA	Pyrethroid	30.9%
λ-cyhalothrin	Warrior II	Syngenta Crop Protection	Pyrethroid	22.8%
λ-cyhalothrin + Thiamethoxam	Endigo ZC	Syngenta Crop Protection	Pyrethroid + Neonicotinoid	9.48 + 12.6%
Piperonil butoxide	Exponent	MGK	Synergist	91.3%
Thiamethoxam	Actara 25WSG	Syngenta Crop Protection	Neonicotinoid	25.0%

RESULTS

Experiment 1. There was a significant treatment effect on the number of GPA on pepper plants at 15 days after treatment (DAT)($F = 10.8$; df = 9, 27; $p < 0.001$). Aphid densities were lowest in peppers treated with acephate, acetamiprid, or any mixture containing acetamiprid (Table 2). The three pyrethroid treatments, λ-cyhalothrin, bifenthrin, and permethrin had substantially more aphids than all other non-pyrethroid treatments (Table 2).

Table 2: Efficacy of insecticides on *Myzus persicae* in bell peppers; Blacksburg, Virginia; Experiment 1; August 2011.

	Rate kg [AI]/ha	Mean ± SE no. of aphids/ 20 leaves*
Untreated control		10.3 ± 2.5ab
Acetamiprid	0.084	1.5 ± 0.9b
Bifenthrin	0.112	765.5 ± 333.8a
Acephate	1.086	0.0 ± 0.0b
λ-cyhalothrin	0.034	850.8 ± 767.2a
Permethrin	0.224	539.0 ± 470.2a
Acetamiprid + permethrin	0.084 + 0.224	1.3 ± 0.8b
Acetamiprid + λ-cyhalothrin	0.084 + 0.034	3.8 ± 2.4b
Acetamiprid + bifenthrin	0.084 + 0.112	0.3 ± 0.3b
Acetamiprid +acephate	0.084 + 1.086	0.0 ± 0.0b

Numbers within a column followed by the same letter are not significantly different (Tukey's HSD, $P > 0.05$). *At 16 days after four applications of treatments.

Experiment 2. There was a significant treatment effect on GPA density at 15 DAT ($F = 5.11$; df = 8, 24; $p < 0.001$). Aphid densities were lowest in peppers treated with imidacloprid + bifenthrin, imidacloprid + β-cyfluthrin or with flonicamid (Table 3). As in the first experiment, the three pyrethroid-only treatments,

β-cyfluthrin, ζ-cypermethrin, and bifenthrin + ζ-cypermethrin had substantially more aphids than all other non-pyrethroid treatments (Table 3).

Table 3: Efficacy of insecticides on *Myzus persicae* in bell peppers; Blacksburg, Virginia; Experiment 2; August 2011.

Treatment	Rate kg [AI]/ha	Mean ± SE no. of aphids/ 20 leaves*
Untreated control		2.8 ± 1.4 abc
ζ-cypermethrin + bifenthrin	0.064	1014.0 ± 644 a
Imidacloprid + bifenthrin	0.141	0.3 ± 0.3 c
Bifenthrin + avermectin B1	1.220	63.3 ± 55.2 abc
ζ-cypermethrin	0.028	155.0 ± 72.4 ab
ζ-cypermethrin + methomyl	0.028 + 0.336	13.0 ± 5 abc
Flonicamid	0.100	1.0 ± 1.0 bc
β-cyfluthrin	0.025	201.8 ± 161.9 abc
Imidacloprid + β-cyfluthrin	0.074	0.5 ± 0.5 c

Numbers within a column followed by the same letter are not significantly different (Tukey's HSD, P > 0.05). *At 16 days after four applications of treatments.

Experiment 3. Again, there was a significant treatment effect on GPA density at 15 DAT ($F = 4.70$; $df = 10, 30$; $p < 0.0005$). Aphid densities were lowest in peppers treated with clothianidin, thiamethoxam, thiamethoxam + λ-cyhalothrin, or dinotefuran with and without the synergist piperonyl butoxide (PBO) (Table 4). As in the previous experiments, all of the pyrethroid treatments including fenpropathrin, λ-cyhalothrin, and ethofenprox with or without PBO had substantially more aphids than all other non-pyrethroid treatments (Table 4).

Table 4: Efficacy of insecticides on *Myzus persicae* in bell peppers; Blacksburg, Virginia; Experiment 3; August 2011.

Treatment	Rate kg [AI]/ha	Mean ± SE no. of aphids/ 20 leaves*
Untreated control		6.0+2.1abc
Clothianidin	0.075	0.5+0.3bc
Clothianidin + fenpropathrin	0.075 + 0.224	6.3 ± 4.7 abc
Fenpropathrin	0.224	120.0 ± 62.1 ab
Dinotefuran	0.200	2.5 ± 1.2 abc
Dinotefuran + PBO	0.200 + 0.350	0.0 ± 0.0 c
Ethofenprox	0.020	100.0 ± 73.7 abc
Ethofenprox + PBO	0.020 + 0.350 (kg)	925.8 ± 891.5 a
λ-cyhalothrin + thiamethoxam	0.099	1.3 ± 0.8b c
λ-cyhalothrin	0.034	498.5 ± 479.2 ab
Thiamethoxam	0.097	2.5 ± 1.5 abc

Numbers within a column followed by the same letter are not significantly different (Tukey's HSD, P > 0.05). *At 16 days after four applications of treatments.

DISCUSSION

Our experiments demonstrated that GPA populations from Kentland Research Farm near Blacksburg, VA (USA) are not susceptible to pyrethroid insecticides, and that repeated use of these insecticides including β-cyfluthrin, ζ-cypermethrin, bifenthrin, fenpropathrin, λ-cyhalothrin, permethrin, or even the novel pyrethroid, ethofenprox, will result in substantial increases in aphid numbers. To our knowledge, this is the first report of pyrethroid resistance in a GPA population from southwestern Virginia (USA); however, in eastern Virginia and other states, Chapman et al. (2009) demonstrated significant increases in GPA populations on peppers that were sprayed with λ-cyhalothrin. The mechanism for pyrethroid resistance in the GPA is not completely known (Foster et al. 2007). In one of our experiments, addition of the synergist PBO to ethofenprox did not increase efficacy against GPA, but rather caused a noticable increase in aphid densities. Thus, the mechanism for resistance may not involve cytochrome P450 or non-specific esterase inhibition, which should be suppressed by the addition of PBO (Moores et al. 2009). However, more detailed research in this area is needed.

Our experiments also showed that if pyrethroids are used on crops such as peppers for control of other pest insects, then the addition of or the use of a pre-mixture containing a neonicotinoid insecticide such as imidacloprid, acetamiprid, clothianidin, thiamethoxam, or dinotefuran will prevent outbreaks of GPA populations. Other effective insecticides for GPA control in Virginia include acephate, methomyl, and flonicamid. The latter would have the least deleterious effects on natural enemies or other nontarget organisms.

REFERENCES

Baur, M. E., J. Ellis, K. Hutchinson, and D. J. Boethel. 2003. Contact toxicity of selected insecticides for non- target predaceous hemipterans in soybeans. J. Entomol. Sci. 38: 269-7 277.

Blackman, R. L., and V. F. Eastop. 2007. Taxonomic issues, pp. 1–22 in H. F. van Emden and R. Harrington, *Aphids as Crop Pests*. CABI, U.K.

Capinera, J. L. 2001. *Handbook of Vegetable Pests*. Academic Press. San Diego, California. 729 pp.

Chapman, A. V., T. P. Kuhar, P. B. Schultz, T. W. Leslie, S. J. Fleischer, J. P. Dively, and J. Whalen. 2009. Integrating chemical and biological control of European corn borer in bell pepper. J. Econ. Entomol. 102:287–295.

Foster, S. P., G. Devine, and A. L. Devonshire. 2007. Insecticide resistance, pp. 261–285 in H. F. van Emden and R. Harrington, *Aphids as Crop Pests.* CABI, U.K.

Hill, T. A., and R. E. Foster. 2000. Effect of insecticides on the diamondback moth (Lepidoptera: Plutellidae) and its parasitoid *Diadegma insulare* (Hymenoptera: Ichneumonidae). J. Econ. Entomol. 93: 763-768.

Kuhar, T. P., and J. Speese. 2002. Evaluation of foliar insecticides for control of European corn borer in peppers, 2001. Arthropod Manag. Tests. 27: E56.

Kuhar, T. P., J. Speese, R. J. Cordero, R. Y. Venkata, V. M. Barlow, and R. Cordero. 2003. Evaluation of foliar insecticides for control of lepidopterous pests in peppers, 2002. Arthropod Manag. Tests 28: E37.

Kuhar, T. P., S. B. Phillips, R. A. Straw, C. M. Waldenmaier, and H. P. Wilson. 2006. Commercial vegetable production recommendations. Va. Coop. Ext. Pub.456–420

Kuhar, T. P., S. Reiter, and H. Doughty. 2009. Green peach aphid on vegetables. Virginia Coop. Ext. Pub. No. 2902.1081.

Moores, G. D., D. Philippou, V. Borzatta, P. Trincia, P. Jewess, R. Gunning, and G. Bingham. 2009. An analogue of piperonyl butoxide facilitates the characterisation of metabolic resistance. Pest Manag. Sci. 65: 150–154. doi:10.1002/ps.1661. PMID 18951417.

Rock, G. C. 1979. Relative toxicity of two synthetic pyrethroids to a predator *Amblyseius fallacis* and its prey *Tetranychus urticae*. J. Econ. Entomol. 72: 293- 294.

Roush, R. T., and M. A. Hoy. 1978. Relative toxicity of permethrin to a predator, *Metaseiulus occidentalis*, and its prey, *Tetranychus urticae*. Environ. Entomol. 7: 287-288.

JMP. 2011. Version 8. SAS Institute Inc., Cary, NC, 1989-2007

van Emden, H. F., V. F. Eastop, R. D. Hughes, and M. J. Way. 1969. The ecology of *Myzus persicae*. Annu. Rev. Entomol. 14: 197-270.

van Emden, H. F., and R. Harrington. 2007. *Aphids as Crop Pests.* CABI North American Office, Cambridge, Massachusetts.

Welty, C. 1995. Monitoring and control of European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae), on bell peppers in Ohio. J. Agric. Entomol. 2: 145-161.

Thomas P. Kuhar, Christopher R. Philips, Katherine Kamminga, and Anna Wallingford
Department of Entomology, Virginia Tech, 216 Price Hall, Blacksburg, VA 24061-0319; e-mail: tkuhar@vt.edu

Baseline susceptibility of five classes of insecticides on Bihar hairy caterpillar *Spilosoma obliqua (*Walk.) (Lepidoptera: Arctiidae)

ABSTRACT
Bihar hairy caterpillar *S. obliqua* is a sporadic pest of groundnut in India. It causes severe damage to the groundnut productivity. Chemical pesticides of various classes are used for controlling caterpillars in the field. The present study is focused on understanding the baseline susceptibility of five classes of chemical insecticides namely imidacloprid, cypermethrin, Emamactin, Neem and Flubendiamide on third instar larvae of *S.obliqua*. Based on the LC_{50}, LC_{90} and LC_{99} values results shows emamectin benzoate as the most potent insecticide (LC_{50}: 2.459g a.i/ha), followed by cypermethrin (LC_{50}: 41.72g a.i/ha). This information can be used for designing IPM programs in groundnut.

INTRODUCTION

Groundnut (*Arachis hypogaea L*.) is an important oilseed cash crop, which accounts for 45% of oil production. In India, about 75% of the groundnut cultivated area lies in low to moderate rainfall zones that are part of peninsular region and western and central regions with a short period of distribution (Kumar *et al*., 1997). Groundnut is attacked by several insects at different stages of plant growth (Wightman *et al*., 1994). The Bihar hairy caterpillar, *S.obliqua* (Walk.) is a voracious feeder, feeding gregariously on ground nut and soybean leaves in early instars. In the case of severe infestation, the entire crop is damaged badly causing 40% defoliation of leaf area (Thippaiah, 1997). Present study is aimed to obtain the lethality estimates data on toxicity of five classes of insecticides against *S. obliqua*. The pesticides chosen for this study are used in groundnut and other associated crops grown in south Asia.

MATERIALS AND METHODS

Insects

S. obliqua population was collected on untreated groundnut field in Salem District, Tamilnadu, India. Larvae were brought to the laboratory and maintained at 25°C with 75% relative humidity. Larvae are fed on groundnut leaves. Larval bioassay was performed on third instar larvae.

Chemicals

The active ingredients, trade name and manufacturers of the chemical pesticides used in this study are: imidacloprid (17.8% SL, Bayer), cypermethrin (10% EC, SIDCO), Emamactin (dispersible granule WG, Syngenta, Turkey), Neem (1% EC, Parry) and Flubendiamide, (480 SC, Bayer). Chemical insecticides were used at the recommended field dose and two higher and lower than recommended doses were also taken for testing in the bioassay. The test concentrations were varying for each pesticide, for imidacloprid 5, 10, 25, 50 and 75g a.i. / ha, cypermethrin 5, 25, 50, 75, 100g a.i. / ha, Emamactin 0.1, 1, 5 11, 25g a.i. / ha and Flubendiamide 20, 40, 60, 80 and 100g a.i. / ha, neem 100, 250, 500, 1000, 1500 (ppm).

Topical bioassay

Topical bioassay was conducted to determine the toxicity of the pesticide to *Spilosoma obliqua.* For the

topical bioassay, 3rd instar larvae were treated with five chemical insecticides. Chemicals were diluted with deionized water to prepare the required dosage. Emamectin granules were dissolved in deionized water from the dilution of 2µl of chemicals delivered at the ventral side of the larvae using micropipette; larvae treated with deionized water served as a control. Groundnut leaves were provided as the food source. Three replicates per treatment and ten larvae per replicate were taken. The mortality was determined 24 hours after treatment.

Statistical Analysis

The mortality data were subjected to probit analysis, using the SPSS software (version 10.0). The LC_{50}, LC_{90} and LD_{99} values of the insecticides and the slopes of dose-mortality were generated and p values were calculated. Pearson's chi-square test was used to determine the fit of the statistical model (Finney, 1971). Data from all bioassays were corrected using Abbott's formula (Abbotts, 1925).

RESULT AND DISCUSSION

The bioassay was carried out to observe the response of third instar *S. obliqua* to the selected insecticide. LC_{50}, LC_{90} and LC_{99} estimates shows that emamectin benzoate followed by cypermethrin were most effective against this pest (Table 1). This may be due to the differences in the mode and site of action of such compound than conventional insecticides (Salgado, 1997). Emamectin benzoate, semi synthetic derivatives of natural products acts as an antagonist of neurotransmitter (GABA and glutamate) gated chloride channels. They act in the insect nervous system resulting in strong chloride ion influx into the cell followed by disruption of nerve impulses, paralysis and finally death (Nauen, 2006). Emamectin targets various lepidopteron pests of field crops and vegetables (Ishaaya *et al*., 2002; Gupta *et al* 2004). The high toxicity of emamectin benzoate has been studied against brinjal shoot and fruit borer, *P. xylostella* and other lepidopteron (Kumar and Devappa, 2006; Janson *et al*., 1998; Murugaraj *et al*., 2006). Among all the insecticides tested emamectin benzoate was highly toxic to *S. obliqua*, with respect to our findings (Shakeban *et al*., 2010) reported that emamectin benzoate was the most toxic insecticides between the lepidopteron.

Table 1: Toxicological response of *S. obliqua* to different insecticides of different chemical classes: imidacloprid (neonicotinoid) cypermethrin (synthetic pyrethroid), emamectin (avermectin), flubendiamide (diamide group), Neem (botanical pesticide).

Pesticides	LC_{50} g a.i/ha	LC_{90} g a.i/ha	LC_{99} g a.i/ha	Slope	SE	$\chi 2$	df	Significance Value
Imidacloprid	84.7971	3996.6	92442.56	0.765878	0.255949	1.110783	3	P=0.7745
Cypermethrin	41.72666	920.9024	11475.17	0.953677	0.243518	3.948549	3	P=0.2671
Emamectin	2.459378	26.08629	178.8676	1.249578	0.317486	9.391	3	P=0.0245
Flubendiamide	67.98103	179.3717	395.6187	3.041419	0.556344	3.232465	3	P=0.3572
Neem (ppm)	533.99	2494.888	8767.623	1.914141	0.303926	0.612137	3	P=0.8936

$\chi 2$: chi^2 **df:** degree of freedom **LC_{50}, LC_{90}** and **LC_{99}**: lethal doses for 50%, 90% and 99% of larvae **SE:** standard error **g a.i/ha:** gram active ingredients per hectare **ppm:** parts per million.

The present findings are in accordance with the findings of above, showing emamectin benzoate more effective. The data obtained in the present investigation can be taken as a baseline susceptibility estimate for larvae *S. obliqua,* which can be utilized in insect pest management programs.

REFERENCES

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomology. 18: 265-267.

Finney, D.J. 1971. Probit analysis: A statistical treatment of the sigmoid response curve. Cambridge university press, London, New York: Melbourne. pp. 333.

Gupta, G.P., Rani, S., Birah, A. and Raghuraman, M., 2004. Relative toxicity of certain new insecticides against *Spodoptera litura* (Fab.) J. pest. Research. 16: 45-47.

Ishaaya, I., Kontsedalov, S., Horowitz, A.R., 2002. Emamectin, a novel insecticide for controlling field crop pests. Pest Manag. Sci. 58, 1091-1095.

Jansson, R.K., Dybas, R.A., 1998. Avermectins: biochemical mode of action, biological activity and agricultural importance, pp. 153-170. In: Ishaaya, I., Degheele, D. (Eds.), Insecticides with Novel Modes of Action mechanisms and Application. Springer, Berlin, Heidelberg, New York.

Kumar and Devappa, V., 2006, Bioefficacy of Emamectin benzoate 5% SG (Proclaim) against diamond back moth in cabbage. Pestology, 30: 23-25.

Kumar, Vijaya, P., Ramakrishna, Y.S., Krishna Murty, K., Ashok Kumar, B. and Sheikh, A.M.1997. Identifying the climatic constraints for optimum production of groundnut and delineating the areas with highest production potential on climatic basis. In: Proc. of the Symposium on Tropical Crop Research and Development India-International, Trissur, India.

Murugaraj, P., Nachiappan, R.M. and Selvanarayanan, V., 2006, Efficacy of Emamectin benzoate (Proclaim 05 SG) against tomato fruit borer, *Helicoverpa armigera* (Hubner). Pestology. 30: 11-16.

Nauen, 2006. Insecticide Mode of Action; return to the ryanodine receptor. Pest Manag. Sci. 62: 690-692.

Salgado, V. L. 1997.The mode of action of spinosad and other insect control products. Down To Earth. 52: 35-43.

Shekeban, Magdy, M.K., Saad, A.S.A., Magdy, A., Meharb R., 2010. The latent effect of certain Insecticides on pink bollworm, *Pectinophora gossypiella*, (Saund.). J. Adv. Agric. Res. (Fac. Ag. Saba Basha), 15 (1): 57-75.

Thippaiah, M., 1997. Bioecology of semilooper, *Thysanoplusia orichalcea* (Fabric us) (Noctuidae: Lepidoptera) with observation on other pest complex of soybean and their management *M. Sc (Agri). Thesis,* Univ. Agric. Sci., Bangalore (India).

Wightman, J. A., Ranga Rao, G. V., 1994. Groundnut pests. In: The Groundnut Crop: A Scientific Basis for Improvement. Ed. by Smartt, J. London: Chapman & Hall, 395–479.

Muthusamy R, Karthi S, and M.S. Shivakumar
Molecular Entomology Lab
Department of Biotechnology
Periyar University
Salem – 636011, India
E-mail: skentomol@gmail.com

Insecticide Resistance of *Helicoverpa armigera* (Hubner) to Cypermethrin and Methomyl

ABSTRACT
The *H. armigera* larvae of the Mahaboobnagar district recorded a LD_{50} of 29.125 µg/larva and 59.609 µg/larva at LD_{90} for cypermethrin. The LD_{50} and LD_{90} values of cypermethrin for Raichur population of *H armigera* were 32.481 and 38.172 µg/larva, respectively. Toxicity of cypermethrin to Nagpur population of *H. armigera* showed that the LD_{50} and LD_{90} values were 20.069 and 54.708 µg/larva, respectively, The chi-square test revealed that the population used in the study was homogenous ($p < 0.05$ %). The *H. armigera* larvae of the Mahaboobnagar district recorded a LD_{50} of 3.651µg/larva and 10.287µg/larva at LD_{90} for methomyl. The LD_{50} and LD_{90} values of methomyl for Raichur population of *H armigera* was 3.630 and 10.417 µg/larva, respectively, while Toxicity of methomyl to Nagpur population of *H. armigera* showed that the LD_{50} and LD_{90} values were 2.652 and 7.214 µg/larva, respectively, when the chi-square test revealed that the population used in the study was homogenous ($p < 0.05$ %).
Key words: *Helicoverpa armigera*, resistance, cypermethrin and methomyl

INTRODUCTION

The bollworm, *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) is a polyphagous pest of worldwide occurrence inflicting annual crop damage in India worth US $1 billion. In India this insect occurs as a major pest in many economically important crops, including cotton, pigeonpea, chickpea, tomato, okra, blackgram, maize, sorghum and many other crops, inflicting substantial crop losses every year (Reed and Pawar 1982; Manjunath 1990). Understanding the genetic variation among the *H. armigera* populations occurring on host plants has become essential to understanding the variation in their susceptibility to different insecticides. The ability of insect species to thrive on diverse host plants is an adaptive advantage for their better survival in the ecosystem. *H. armigera* is also characterized by its high mobility and fecundity and it has shown great capacity to develop resistance to synthetic insecticides used in its management (Armes et al. 1996; Kranthi 1997; Ramasubramaniam and Regupathy 2004b). The versatility of this species may be due to the presence of a strong genetic variability governing the behavior of *H.armigera* making it a serious pest on several crops (Zhou et al. 2000).

The occurrence of insecticide resistant strains can be reduced or delayed by reducing the selection pressure, by using alternate insecticides with novel mode of action. The pyrethroids and organophosphorus combination insecticides were found to be effective against the resistant insect pest population of *H. armigera* (Martin *et al*., 2003).

MATERIALS AND METHODS

Determination of the insecticide resistance in *H. armigera*

Collection of *H. armigera*

The larvae of *H. armigera* were collected on red gram, cotton and bengal gram crops during October 2006 from Raichur, December-2006 and January 2007 from Mahaboobnagar and February2010 from Nagpur for conducting the investigation. The work was carried out in the Department of Entomology, College of Agriculture, Rajendranagar, Hyderabad, Andhra Pradesh

Test Insecticides

The degree of resistance acquired by *H. armigera* to test insecticides *viz.,* cypermethrin representing synthetic pyrethroids and methomyl representing carbamates group of insecticides (Table 1) were tested.

Table 1: Insecticides used for the determination of insecticide resistance in *H. armigera*

S.No	Common name	Formulation	Trade name	Chemical name	Source of supply
1	Methomyl	40 SP	Lannate	S-methylN-(methyl carbamoxyloxy) Thioacetimidate	M/S Dupont Chemical (India) Limited, Mumbai-400076
2	Cypermethrin	10 EC	Cypra	(RS)-a-cyno-3-phenoxybenzyl-(1RS)-cis,trans-3-(2,2 dichlorovinyl)-2,2-dimethyl cyclopropanecarboxylate	M/S Hyderabad chemicals supplies Ltd, A-24/25, APIE, Balanagar, Hyderabad-500 037

Test Insect Population

The larvae collected from Mahaboobnagar, Raichur and Nagpur were reared separately in the laboratory to obtain pupae. Male, female pupae were separated and kept for single pair mating. The eggs obtained from single pair were reared to get first generation larvae. Third instar *H.armigera* larvae from (1^{st} generation) F_1 with an average weight of 30 mg ± 0.011 S.E. of Mahaboobnagar, Raichur and Nagpur strains were subjected separately to different concentrations of the test insecticides. The survivals at LD_{50} concentration in each test insecticide at F_1 (1^{st} generation) were further used for resistance studies.

Bioassay

Bioassay was done by topical application method using Hamilton micro applicator to evaluate the toxicity of all the test insecticides (FAO, 1971).

Topical Application Method

Initially 1.0 per cent stock solution of the test insecticide was prepared from the formulated products by dissolving the required quantities after accurate weighing in double distilled water. The stock solution thus prepared was preserved in the refrigerator for further use. Individual working concentrations for each of the test insecticides were prepared from the 1.0 per cent stock solution through serial dilution technique using double distilled water as solvent. One micro litre of the respective insecticidal solution was applied on the dorsum of second thoracic segment by micro applicator. Three replications were maintained for each insecticidal concentration with 10 larvae in each replication.

Data Collection

Mortality of the larvae was recorded at 24, 48 and 72 hours after treatment. The mortality at 72 hours after treatment was considered as end point for the assessment of toxicity of test insecticides as reported by Fisk and Wright (1992). Thus, concentrations of wide range initially and narrow range subsequently were tested so as to get mortality data in the range of 5-90 %. The moribund larvae also were considered as dead while recording the mortality data. The amount of insecticide present in one micro litre of test concentration was calculated and expressed as (LD_{50}) dose in µg/µl.

Assessment of the Degree of Resistance Acquired by *H. armigera* to the Test Insecticides

The mortality data of third instar *H. armigera* larvae of Mahaboobnagar, Raichur and Nagpur populations of all the test insecticides were subjected to probit analysis (Finney, 1971) using POLO-PC software (Ross, 1987) to calculate LD_{50}, LD_{90}, Heterogeneity (χ^2), intercept (a), slope of the regression line (b), regression equation and fudicial limits. The degree of resistance acquired by *H. armigera* was calculated by dividing the higher LD_{50} value of a strain with the lower LD_{50} value of another strain among the three populations for each test insecticide and thus the relative degree of resistance was assessed (Resistance factor = LD_{50} of the resistant strain / LD_{50} of the susceptible strain).

The degree of resistance acquired by all the three strains was also calculated by comparing the present data with the available baseline data at LD_{50} and LD_{90} levels. The degree of resistance to methomyl and cypermethrin were calculated by using the baseline data of Nagpur susceptible strain (Kranthi, 2005) (Table 2).

Table 2: Particulars of base line data used to calculate the degree of insecticide resistance in the larvae of *H.armigera*

S. No	Name of the insecticide	Name of strain	LD_{50} µg/larva	LD_{90} µg/larva	Reference
1	Cypermethrin	Nagpur susceptible	0.007	0.028	Kranthi, 2005
2	Methomyl	Nagpur susceptible	0.030	0.165	Kranthi, 2005

Resistance factor = LD_{50} of the F_1 resistant strain / LD_{50} of the Nagpur susceptible strain

RESULTS AND DISCUSSION

The larvae of *H.armigera* collected from Mahaboobnagar, Raichr and Nagpur selected for cypermethrin and methomyl insecticides at F_1 generation were reared to F_2 by single pair mating. They further proceeded to F_3 by the same single pair method. Larvae were subjected to these insecticides of different doses at F_2 and F_3 and the results were discussed here under.

The *H. armigera* larvae of the Mahaboobnagar district recorded a LD_{50} of 29.125 µg/larva which rose sharply to 59.609 µg/larva at LD_{90} for cypermethrin at F_1, which later still increased to 32.123 and 37.109 µg/larva at LD_{50} and LD_{90} respectively at F_2. Resistance increased to 35.115 and 42.124 at LD_{50} and

LD_{90} respectively at F_3 (Table 3). The LD_{50} and LD_{90} values of cypermethrin for Raichur population of *H armigera* was 32.481 and 38.172 µg/larva, respectively at F_1, which rose to 35.467 and 41.875 µg/larva at LD_{50} and LD_{90}, respectively at F2, further they were increased to 38.906 and 74.202 at LD_{50} and LD_{90}, respectively at F_3 (Table 4). Toxicity of cypermethrin to Nagpur population of *H. armigera* showed that the LD_{50} and LD_{90} values were 20.069 and 54.708 µg/larva, respectively at F_1, further they were sharply increased to 23.383 and 32.233 µg/larva at LD_{50} and LD_{90}, respectively at F_2, later they were increased to 25.054 and 32.224 µg/larva at LD_{50} and LD_{90}, respectively (Table 5) at F_3.

Table 3: Resistance pattern in Mahaboobnagar population of *H. armigera*

S. No.	Strain	Generation	LD_{50} µg/larva (95%FL)	LD_{90} µg/larva (95%FL)	Slope ± S.E (b)	Heterogeneity (χ^2)	Regression equation
1	Cypermethrin	F_1	29.125 (23.867 – 33.325)	59.609 (51.126-76.562)	4.120 ± 0.669	0.809	Y = -1.033 + 4.120 X
2	Cyper - Cyper	F_2	32.123 (12.508 – 35.456)	37.109 (33.641 – 97.573)	20.452 + 9.654	0.777	Y = -25.818 + 20.452 X
3	Cyper- Cyper – Cyper	F_3	35.115 (28.231 – 38.382)	42.124 (38.525 – 55.876)	16.213 + 5.669	0.828	Y = -20.058 + 16.213 X
4	Methomyl	F_1	3.651 (2.693 – 4.460)	10.287 (8.259-14.714)	2.849 + 0.471	3.333	Y = 3.398+ 2.849 X
5	Metho - Metho	F_2	3.872 (0.569 – 4.824)	5.829 (4.667 – 29.441)	7.215 + 3.274	0.190	Y = 0.758 + 7.215 X
6	Metho – Metho - Metho	F_3	3.934 (3.285 – 4.261)	4.668 (4.305 – 5.971)	17.250 ± 5.818	0.658	Y = -5.261 + 17.250 X

Table 4: Resistance pattern in Raichur population of *H. armigera*

S. No.	Strain	Generation	LD_{50} µg/larva (95%FL)	LD_{90} µg/larva (95%FL)	Slope ± S.E (b)	Heterogeneity (χ^2)	Regression equation
1	Cypermethrin	F_1	32.481 (15.858 – 35.904)	38.172 (34.615 – 87.453)	18.279 + 8.376	1.091	Y = -22.630 + 18.279 X
2	Cyper - Cyper	F_2	35.467 (23.520 – 38.971)	41.875 (38.106 – 62.869)	17.767 + 7.332	0.611	Y = -22.537 + 17.767 X
3	Cyper - Cyper - Cyper	F_3	38.906 (32.472 – 43.748)	74.202 (64.183-96.176)	4.571 + 0.811	0.911	Y = -2.267 + 4.571 X
4	Methomyl	F_1	3.630 (2.719 – 4.408)	10.417 (8.357-14.905)	2.799 + 0.450	3.321	Y = 3.433+ 2.799 X
5	Metho - Metho	F_2	3.659 (2.259 – 4.365)	5.471 (4.566 – 10.348)	7.337 ± 2.611	1.081	Y = 0.866 + 7.337 X
6	Metho – Metho - Metho	F_3	3.851 (3.058 – 4.208)	4.708 (4.297 – 6.485)	14.684 ± 5.189	0.891	Y = -3.598 + 14.684 X

Table 5: Resistance pattern in Nagpur population of *H. armigera*

S. No.	Strain	Generation	LD_{50} µg/larva (95%FL)	LD_{90} µg/larva (95%FL)	Slope ± S.E (b)	Heterogeneity (χ^2)	Regression equation
1	Cypermethrin	F_1	20.069 (15.312 – 24.138)	54.708 (44.089-78.011)	2.943+ 0.477	2.602	Y = 1.167 + 2.943 X
2	Cyper - Cyper	F_2	23.383 (16.024 – 26.927)	32.233 (27.885 – 53.609)	9.193 + 3.261	1.004	Y = -7.585 + 9.193 X
3	Cyper- Cyper – Cyper	F_3	25.054 (18.153 – 28.330)	32.224 (28.485 – 47.966)	11.725 + 4.165	0.931	Y = -11.401 + 11.725 X
4	Methomyl	F_1	2.652 (2.051 – 3.174)	7.214 (5.583 - 11.827)	2.949 + 0.555	1.675	Y = 3.751+ 2.949 X
5	Metho - Metho	F_2	2.844 (2.083 – 3.199)	3.716 (3.292 – 5.682)	11.032 ± 3.906	0.953	Y = -0.007 + 11.032 X
6	Metho – Metho - Metho	F_3	2.944 (2.607 – 3.088)	3.279 (3.122 – 3.900)	27.373 ± 9.661	0.805	Y = -7.836 + 27.373 X

Amongst the three populations of *H. armigera*, the population of Raichur has developed 1.115 and 0.640 fold relative resistance at LD_{50} and LD_{90}, respectively as compared with the Mahaboobnagar population. The same Raichur population has developed higher levels of relative resistance by 1.168 and 0.698 fold when compared with the Nagpur population at LD_{50} and LD_{90}, respectively, while Mahaboobnagar population recorded 1.451 and 1.090 fold resistance at LD_{50} and LD_{90}, respectively in comparison with Nagpur. In comparison with base line data of Nagpur susceptible population reported by Kranthi (2005) the three populations of Raichur, Mahaboobnagar and Nagpur acquired 4640.143, 4160.714, 2867.000 folds at LD_{50} and 1363.286; 2128.893 and 1953.857 fold resistance to cypermethrin at LD_{90} levels, respectively (Table 6).

Table 6: Relative degree of resistance among the three populations of *H. armigera* to cypermethrin at F_1

Population	LD_{50} µg/larva	LD_{90} µg/larva	Resistance factor in comparison with Mahaboobnagar population(folds)		Resistance factor in comparison with Nagpur population (folds)		Resistance factor in comparison with Baseline Data	
			at LD_{50}	at LD_{90}	at LD_{50}	at LD_{90}	at LD_{50}	at LD_{90}
Raichur	32.481	38.172	1.115	0.640	1.618	0.698	4640.143	1363.286
Mahaboobnagar	29.125	59.609	-	-	1.451	1.090	4160.714	2128.893
Nagpur	20.069	54.708	-	-	-	-	2867.000	1953.857
Baseline data (Kranthi, 2005)	0.007	0.028	-	-	-	-	-	-

The *H. armigera* larvae of the Mahaboobnagar district recorded a LD_{50} of 3.651µg/larva which rose sharply to 10.287µg/larva at LD_{90} for methomyl at F_1. Further the values rose to 3.872 and 5.829 µg/larva at LD_{50} and LD_{90}, respectively at F_2. Interestingly at F_3 the resistance recoded was 3.934 and 4.668 at LD_{50} and LD_9, respectively at F_3 (Table 3). The LD_{50} and LD_{90} values of methomyl for Raichur population of *H armigera* were 3.630 and 10.417 µg/larva, respectively at F_1. Further the values rose to 3.659 and 5.471 µg/larva at LD_{50} and LD_{90}, respectively at F_2. Interestingly at F_3 the resistance recoded was 3.851 and 4.708 at LD_{50} and LD_{90}, respectively at F_3 (Table 4). Toxicity of methomyl to Nagpur population of *H. armigera* showed that the LD_{50} and LD_{90} values were 2.652 and 7.214 per cent, respectively at F_1. Further the values rose to 2.844 and 3.716 µg/larva at LD_{50} and LD_{90}, respectively at F_2. Interestingly at F_3 the resistance recoded was 2.944 and 3.279 at LD_{50} and $LD_{90,\text{ respectively}}$ at F_3 (Table 5).

Amongst the three populations of *H. armigera*, the population of Mahaboobnagar has developed 1.006 and 0.988 fold relative resistance at LD_{50} and LD_{90}, respectively as compared with the Raichur population. The same Mahaboobnagar population has developed higher levels of relative resistance by 1.377 and 1.426 fold when compared with the Nagpur population at

LD_{50} and LD_{90}, respectively, while Raichur population recorded 1.369 and 1.444 fold resistance at LD_{50} and LD_{90}, respectively in comparison with Nagpur. In comparison with base line data of Nagpur susceptible population reported by Kranthi (2005) the three populations of Mahaboobnagar, Raichur and Nagpur acquired 121.700, 121.000, 88.400 folds at LD_{50} and 62.345, 63.133, 43.721 folds resistance to methomyl at LD_{90} levels, respectively (Table 7).

Table 7: Relative degree of resistance among the three populations of H. armigera to methomyl at F_1

Population	LD_{50} µg/larva	LD_{90} µg/larva	Resistance factor in comparison with Raichur population (folds)		Resistance factor in comparison with Nagpur population (folds)		Resistance factor in comparison with Baseline Data	
			at LD_{50}	at LD_{90}	at LD_{50}	at LD_{90}	at LD_{50}	at LD_{90}
Mahaboobnagar	3.651	10.287	1.006	0.988	1.377	1.426	121.700	62.345
Raichur	3.630	10.417	-	-	1.369	1.444	121.000	63.133
Nagpur	2.652	7.214	-	-	-	-	88.400	43.721
Baseline data (Kranthi, 2005)	0.03	0.165	-	-	-	-	-	-

The present results of cypermethrin resistance are in proximity with (Kranthi *et al*., 2001) who reported >1000 folds resistance to cypermethrin in four strains of *H. armigera* of Central India. Nirmal Singh and Mahal (2005) reported that 2087 fold resistance to cypermethrin. Rao *et.al.,* (2005) reported that the synthetic pyrethroids showed high resistance frequencies (>80%) with a LD_{50} value of 8.51µg/larva and it had developed highest resistance (946 folds) against cypermethrin. Chaturvedi (2007) was reported that the *H. armigera* exhibited widespread resistance (RF= 48-919) to cypermethrin. Suryawanshi *et al.* (2008) revealed that the synthetic pyrethroid cypermethrin showed high resistance frequencies (84-88%) with a LD_{50} values of 1.399 g/larva and it had developed higher resistance (279.80 fold) against cypermethrin. Nimbalkar *et al.* (2009) were reported that the LD_{50} value for cypermethrin was highest, during October (1.459 mg/larvae in Aurangabad) and lowest during August in Parbhani (0.157 µg/larva) during 2008, which might be due to continuous use of cypermethrin to manage this pest. Achaleke *et al.* (2009) indicated high resistance to cypermethrin (RF=67-1771) among *H. armigera* field populations and laboratory-selected strains. From the present findings the resistance ratio appears to be high because of the comparison with most susceptible strain as base line (LD_{50}=0.007 µg/larva), but in reality it is indicated that there is decrease in the levels of resistance to cypermethrin in *H. armigera* compared to latest reports of Suryawanshi *et al.* 2008 and Nimbalkar *et al.* 2009 which may be probably due to decreased selection pressure with significant decrease in the use of cypermethrin and increasing the area under *Bt* cotton.

The present results of methomyl resistance are in proximity with Jhansi *et al*., 2004 according to whom, during early part of the season on cotton the per cent resistance was found almost constant (31.34 to 64.00) and 6 -30 folds of resistance to methomyl from Guntur region. Resistance level against methomyl in Khandwa district of Madhya Pradesh was found quite low (2.50%) in the first week of October and then varied from 2.50 per cent to 18.06 per cent during the remaining period of the month. However, the highest resistance level (44.52%) was recorded during the third week of November (Choudhary *et al.,* 2004). From the present investigations it is evident that there is decrease in the levels of resistance to methomyl in *H. armigera* compared to latest reports of Suryawanshi *et al.* 2008 which may be probably due to significant decrease in the use of methomyl in managing the pest due to reduced bollworm incidence and due to increased area under *Bt* cotton.

REFERENCES

Achaleke J, Martin T, Ghogomu R T, Vaissayre M and Brevault T 2009a Esterase mediated resistance to pyrethroids in field populations of *Helicoverpa armigera* (Lepidoptera: Noctuidae) from Central Africa. Pest Management Science 65(10): 1147-1154.

Armes N J, Jadhav D R and Desouza K R 1996 A survey of insecticide resistance in *Helicoverpa armigera* in the Indian subcontinent. Bulletin of Entomological Research 86: 499-514.

Chaturvedi I 2007 Status of insecticide resistance in the cotton bollworm, *Helicoverpa armigera* (Hubner). Journal of Central European Agriculture 8(2): 171-182.

Chowdhary R K, Yadav A S, Shrivastava V K, Tomar S P S and Patel Y 2004 Studies on insecticide resistance in *Helicoverpa armigera* (Hubner) on cotton in Madhya Pradesh. In: International Symposium on Strategies for Sustainable Cotton Production – A global vision 3. Crop Protection, University of Agricultural Sciences, Dharwad, Karnataka, India, 23-25 November.

FAO (Food and Agricultural Organization) 1971 Recommended methods for the detection and measurement of pest resistance to pesticides. Tentative method for larvae of Egyptian cotton leafworm (*Spodoptera littoralis* Biosd.) F.A.O method No.8 Food and Agricultural Organization. Plant Protection Bulletin 19:32-35.

Finney D J 1971 Probit analysis, Cambridge University, London Pp. 333.

Fisk T and Wright D J 1992 Speed of action and toxicity of acylurea insect growth regulators against *Spodoptera exempta* (Walk.) and *Spodoptera*

littoralis (Biosd.) larvae: effect of inter moult age. Pesticide Science 35:331-337.

Jhansi K, Radhika P and Subbaratnam G V 2004 Insecticide resistance in insect pests of cotton in Andhra Pradesh. In: International Symposium on Strategies for Sustainable Cotton Production – A global vision 3. Crop Protection, University of Agricultural Sciences, Dharwad, Karnataka, India, 23-25 November.

Kranthi K R 2005 Insecticide resistance monitoring, mechanisms and management manual. Central Institute for Cotton Research, Nagpur Pp: 80-94.

Kranthi K R, Armes N J, Rao N G V, Raj S and Sundaramurthy V T 1997 Seasonal dynamics of metabolic mechanisms mediating pyrethroid resistance in *Helicoverpa armigera* in central India. Pesticide Science 50:91-98.

Kranthi K R, Jadhav D R, Wanjari R R, Ali S S and Russell D 2001 Carbamate and organophosphate resistance in cotton pests in India 1995 to 1999. Bulletin of Entomological Research 91(1): 37-46.

Manjunath T M 1990 Controlling cotton pests. Deccan Herald, January 4,1990 Science and society Pp: 1-3.

Martin T, Ochou G O, Vaissayre M and Fournier D 2003 Monitoring of insecticide resistance in *Helicoverpa armigera* (Hubner) from 1998 to 2002 in Cote d'Ivoire, West Africa. Resistant Pest Management Newsletter 12(2): 51-55.

Nimbalkar R K, Shinde S S, Tawar D S and Muley S P 2009 Response of cotton bollworm *Helicoverpa armigera* (Hubner) (Lepidoptera: noctuidae) to different insecticides in Maharashtra, India. World Journal of Agricultural Sciences 5(2): 250-255.

Nirmal Singh and Mahal M S 2005 Acute toxicity of different insecticides against *Helicoverpa armigera* (Hubner) in Punjab. Pesticide Research Journal 17(2): 52-54.

Ramasubramanian T and Regupathy A 2004 Magnitude and mechanism of insecticide resistance in *Helicoverpa armigera* (Hub.) population of Tamil Nadu, India. Asian Journal of Plant Sciences 3(1): 94-100.

Rao G M V P, Rao N H and Raju K 2005 Insecticide resistance in field population of American bollworm, *Helicoverpa armigera* (Hub.) (Lepidoptera:Noctuidae). Resistant Pest Management Newsletter 15(1): 15-17.

Reed W and Pawar C S 1982 *Heliothis*, a global problem. In: Proceedings of International Workshop on *Heliothis* Management, ICRISAT, Patancheru, Andhra Pradesh, India pp: 9-14.

Ross G J S 1987 Maximum likely hood program. The numerical Algorithms Group. Rothamsted Experimental Station, Harpenden, UK.

Suryawanshi D S, Bhede B V, Bhosale S V and More D G 2008 Insecticide resistance in field population of American bollworm, *Helicoverpa armigera* (Hub.) (Lepidoptera: Noctuidae). Indian Journal of Entomology 70(1): 44-46.

Zhou X, Fartor O, Applebaum S W and Coll M 2000 Population structure of the pestiferous moth *Helicoverpa armigera* in the Eastern Mediterranean using RAPD analysis. Heredity 85: 251-256.

S Upendhar, J Satyanarayana and T V K Singh
Department of Entomology, College of Agriculture, Acharya N.G. Ranga
Agricultural University, Rajendranagar, Hyderabad –500 030, Andhra Pradesh, India.

Toxicity of Certain Novel Insecticides against Chilli Thrips, *Scirtothrips Dorsalis* (Hood)

ABSTRACT
All the novel and new insecticides evaluated for managing the resistance in *S.dorsalis* indicated that the toxicity of the insecticides was in order of spinosad > pymetrozine > diafenthiuron > imidacloprid > fipronil > clothianidin > vertimec > indoxacarb > chlorfenapyr > flubendiamide > emamectin benzoate at LC_{50}. Spinosad, pymetrozine, diafenthiuron, imidacloprid and fipronil were highly effective and proved very successful in suppression of resistance.

Keywords: Toxicity, novel insecticides, resistance management

INTRODUCTION

India has emerged today as the foremost producer and exporter of chillies contributing to almost 1/4th of the world's production. In India, chilli is grown in an area of 8.06 lakh ha, with a production of 12.98 lakh tonnes (Agricultural Statistics at a glance, 2009). The important chilli growing states in India are Andhra Pradesh, Orissa, Maharashtra, Karnataka and also in a number of other states as a round the year crop. In Andhra Pradesh, chilli is cultivated in an area of 1.89 lakh hectares with a production of 2.08 lakh tonnes. Guntur district in Andhra Pradesh alone contributes to over 35 per cent in area under chilli crop in India.

The important pests are thrips, *Scirtothrips dorsalis* (Hood), white mite, *Polyphagotarsonemus latus* (Banks), aphids, *Aphis gossypii* Glover and *Myzus persicae* Sulzer as sucking complex and tobacco caterpillar, *Spodoptera litura* (Fabricius) and pod borer, *Helicoverpa armigera* (Hubner) as pod borers (Rao and Ahmed, 1985). Chilli thrips, *Scirtothrips*

dorsalis (Hood) (Thysanoptera: Thripidae) is a serious pest of *Capsicum annuum* L. in India, responsible for leaf curling (Ananthakrishnan, 1971). It multiplies appreciably at a faster rate during dry weather periods and the yield loss caused by the thrips is reported to range from 30-90 per cent (Borah, 1987 and Varadharajan, 1994).

Guntur district in Andhra Pradesh is traditionally a chilli growing district with an area of 63,573 ha with high input usage under monocropping conditions. Further, intensive cultivation of input responsive high yielding varieties and hybrids and sole reliance on insecticides are the common features of chilli cultivation in Guntur district. The excessive dependence on insecticides, their over use and abuse has accelerated insect control problems through development of insecticide resistance (Reddy *et al*., 1992), pest resurgence, pesticide residues (Joia *et al*., 2001), reduction in natural enemy population and environmental contamination. Moreover, several of the chilli consignments meant for export were rejected stating higher insecticide residues being the culprit, thus lots of foreign exchange lost by way of rejections. Further there were several reports from farmers experiencing difficulties in pest control. Many conventional insecticides are being used to manage these pests with which many folds of resistance was reported in pests like *S. litura* (Prasad *et al*., 2008), *Spodoptera exigua* (Hubner) (Wang *et al.*, 2002), *H. armigera* (Kranthi *et al*., 2002) *etc*. Studies on seasonal occurrence of thrips on chillies provide information on the initiation and extent of damage at different growth stages of the crop and its relation to weather parameters which will be of great help to plan appropriate management. The occurrence of insecticide resistance strains can be reduced or delayed by reducing the selection pressure and by adopting insecticide resistance management strategies and alternate insecticides with novel mode of action. In view of the above constraints in chilli cultivation, it is felt high time to estimate the current status of insecticide resistance so as to corrologate with field control problems besides evaluating newer insecticides with novel mode of action both under laboratory and field conditions so as to have better option on hand that could mitigate the present control failures and residue problems plausing the farming community.

MATERIALS AND METHODS

Among the available new synthetic organic insecticides, *viz*., fipronil, imidacloprid, spinosad, diafenthiuron, indoxacarb, pymetrozine, vertimec, chlorfenapyr, clothianidin, flubendiamide and emamectin benzoate were selected to test the relative toxicity against *S. dorsalis*. The *S. dorsalis* population of Guntur district which was found relatively resistant than Vizianagaram population was selected for this study. The toxicity of these eleven insecticides to the resistant population of Guntur district was determined by conducting leaf dip method of bioassay (FAO, 1979) as detailed in 3.3.5.1 and LC_{50} and LC_{90} values were determined.

The relative toxicity of the insecticides was calculated by dividing the LC_{50} and LC_{90} values of each of the conventional insecticides *viz,* monocrotophos, acephate, dimethoate, triazophos, phosalone and carbaryl with the corresponding LC_{50} and LC_{90} values of each of the new insecticides tested and thus the toxicity of new insecticides compared to the conventional ones were determined for the management of the resistant population of *S. dorsalis*.

RESULTS AND DISCUSSION

Toxicity of Insecticides

Fipronil

It is clear from the data (Table 1) that the LC_{50} and LC_{90} values of fipronil were 0.0120 and 0.0390 percent respectively to the resistant population of *S. dorsalis* of Guntur district. The chi-square test indicated that the thrip population used in this study was homogeneous ($P \leq 0.05$). The slope value (b) of log concentration probit (lcp) line was 2.492. When the toxicity of fipronil was compared with monocrotophos, dimethoate, acephate, phosalone, carbaryl and triazophos, it was found to be 11.17, 14.92, 12.17, 14.17, 10.50 and 9.33 times more toxic at LC_{50} and 9.15, 9.03, 11.18, 9.10, 11.90 and 7.13 times more toxic at LC_{90} than the respective insecticides (Table 2 & 2a).

Table 1: Toxicity of new insecticides against resistant population of *S. dorsalis* of Guntur district

S. No.	Insecticides	LC_{50} (%) (95%FL)	LC_{90} (%) (95%FL)	Slope ± S.E (b)	Heterogeneity (χ^2)	Regression equation
1	Fipronil	0.0120 (0.0080-0.0160)	0.0390 (0.0260-0.1280)	2.492 ± 0.68	1.777	Y = 9.785 + 2.492 X
2	Imidacloprid	0.0090 (0.0030-0.0260)	0.6120 (0.1330-29.7870)	0.703 ± 0.17	0.709	Y = 6.432 + 0.703 X
3	Spinosad	0.0050 (0.0020-0.0090)	0.0920 (0.0340-1.1410)	1.002 + 0.24	3.802	Y = 7.319 + 1.002 X
4	Diafenthiuron	0.0080 (0.0020-0.0220)	0.7760 (0.1450-71.0480)	0.637 ± 0.16	1.103	Y = 6.352 + 0.637 X
5	Indoxacarb	0.0200 (0.0110-0.0300)	0.1170 (0.0630-0.6150)	1.676 + 0.43	3.316	Y = 7.846 + 1.676 X
6	Emamectin Benzoate	0.0220 (0.0080-0.0510)	0.7260 (0.2320-8.1690)	0.845 ± 0.18	1.822	Y = 6.399 + 0.845 X
7	Vertimec	0.0160 (0.0090-0.0210)	0.0470 (0.0310-0.1960)	2.652 ± 0.81	2.804	Y = 9.795 + 2.652 X
8	Flubendiamide	0.0210 (0.0110-0.0320)	0.1140 (0.0620-0.6210)	1.745 + 0.47	3.221	Y = 7.929 + 1.745 X
9	Chlorfenapyr	0.0200 (0.0110-0.0300)	0.1360 (0.0690-0.9290)	1.528 ± 0.40	2.540	Y = 7.604 + 1.528 X
10	Pymetrozine	0.0060 (0.0030-0.0130)	0.1120 (0.0380-2.1520)	1.015 + 0.26	3.072	Y = 7.247 + 1.015 X
11	Clothianidin	0.0130 (0.0060-0.0230)	0.1400 (0.0560-3.5610)	1.233 ± 0.37	4.259	Y = 7.332 + 1.233 X

Table 2: Relative toxicity of new insecticides to *S. dorsalis* on chillies in comparison with conventional insecticides

Insecticides	Number of folds toxic at LC_{50} compared to					
	Monocrotophos	Dimethoate	Acephate	Phosalone	Carbaryl	Triazophos
Fipronil	11.17	14.92	12.17	14.17	10.50	9.33
Imidacloprid	14.89	19.89	16.22	18.89	14.00	12.44
Spinosad	26.80	35.80	29.20	34.00	25.20	22.40
Diafenthiuron	16.75	22.38	18.25	21.25	15.75	14.00
Indoxacarb	6.70	8.95	7.30	8.50	6.30	5.60
Emamectin Benzoate	6.09	8.14	6.64	7.73	5.73	5.09
Vertimec	8.38	11.19	9.13	10.63	7.88	7.00
Flubendiamide	6.38	8.52	6.95	8.10	6.00	5.33
Chlorfenapyr	6.70	8.95	7.30	8.50	6.30	5.60
Pymetrozine	22.33	29.83	24.33	28.33	21.00	18.67
Clothianidin	10.31	13.77	11.23	13.08	9.69	8.62

Table 2a: Relative toxicity of new insecticides to *S. dorsalis* on chillies in comparison with conventional insecticides

Insecticides	Number of folds toxic at LC_{90} compared to					
	Monocrotophos	Dimethoate	Acephate	Phosalone	Carbaryl	Triazophos
Fipronil	9.15	9.03	11.18	9.10	11.90	7.13
Imidacloprid	0.58	0.58	0.71	0.58	0.76	0.45
Spinosad	3.88	3.83	4.74	3.86	5.04	3.02
Diafenthiuron	0.46	0.45	0.56	0.46	0.60	0.36
Indoxacarb	3.05	3.01	3.73	3.03	3.97	2.38
Emamectin Benzoate	0.49	0.48	0.60	0.49	0.64	0.38
Vertimec	7.60	7.49	9.28	7.55	9.87	5.91
Flubendiamide	3.13	3.09	3.82	3.11	4.07	2.44
Chlorfenapyr	2.63	2.59	3.21	2.61	3.41	2.04
Pymetrozine	3.19	3.14	3.89	3.17	4.14	2.48
Clothianidin	2.55	2.51	3.11	2.54	3.31	1.99

Imidacloprid

The data presented in Table 1 revealed that the LC_{50} and LC_{90} values of imidacloprid were 0.0090 and 0.6120 percent respectively to the resistant population of *S. dorsalis* of Guntur district. The chi-square test indicated that the thrip population used in this study was homogeneous ($P \leq 0.05$). The slope value (b) of log concentration probit (lcp) line was 0.703. Imidacloprid was found to be 14.89, 19.89, 16.22, 18.89, 14.00 and 12.44 times more toxic at LC_{50} and 0.58, 0.58, 0.71, 0.58, 0.76 and 0.45 times more toxic at LC_{90} than monocrotophos, dimethoate, acephate, phosalone, carbaryl and triazophos at LC_{50} and $LC_{90,}$ respectively (Table 2 & 2a).

Indoxacarb

It is evident from the data (Table 1) that the LC_{50} and LC_{90} values of indoxacarb were 0.0200 and 0.1170 percent respectively to the resistant population of *S. dorsalis* of Guntur district. The chi-square test indicated that the thrip population used in this study was homogeneous ($P \leq 0.05$). The slope value (b) of log concentration probit (lcp) line was 1.676. Indoxacarb was found to be 6.70, 8.95, 7.30, 8.50, 6.30 and 5.60 times more toxic at LC_{50} and 3.05, 3.01, 3.73, 3.03, 3.97 and 2.38 times more toxic at LC_{90} than monocrotophos, dimethoate, acephate, phosalone, carbaryl and triazophos at LC_{50} and $LC_{90,}$ respectively (Table 2 & 2a).

Spinosad

It is clear from the data (Table 1) that the LC_{50} and LC_{90} values of spinosad were 0.0050 and 0.0920 per cent respectively to the resistant population of *S. dorsalis* of Guntur district. The chi-square test indicated that the thrip population used in this study was homogeneous ($P \leq 0.05$). The slope value (b) of log concentration probit (lcp) line was 1.002. When the toxicity of spinosad was compared with monocrotophos, dimethoate, acephate, phosalone, carbaryl and triazophos, it was found to be 26.80, 35.80, 29.20, 34.00, 25.20 and 22.40 times more toxic at LC_{50} and 3.88, 3.83, 4.74, 3.86, 5.04 and 3.02 times more toxic at LC_{90} than the respective insecticides (Table 2 & 2a).

Emamectin benzoate

It is evident from the data (Table 1) that the LC_{50} and LC_{90} values of emamectin benzoate were 0.0220 and 0.7260 percent respectively to the resistant population of *S. dorsalis* of Guntur district. The chi-square test indicated that the thrip population used in this study was homogeneous ($P \leq 0.05$). The slope value (b) of log concentration probit (lcp) line was 0.845. Emamectin benzoate was found to be 6.09, 8.14, 6.64, 7.73, 5.73 and 5.09 times more toxic at LC_{50} and 0.49, 0.48, 0.60, 0.49, 0.64 and 0.38 times more toxic at LC_{90} than monocrotophos, dimethoate, acephate, phosalone, carbaryl and triazophos at LC_{50} and $LC_{90,}$ respectively (Table 2 & 2a).

Diafenthiuron

It is clear from the data (Table 1) that the LC_{50} and LC_{90} values of diafenthiuron were 0.0080 and 0.7760 percent respectively to the resistant population of *S. dorsalis* of Guntur district. The chi-square test indicated that the thrip population used in this study was homogeneous ($P \leq 0.05$). The slope value (b) of log concentration probit (lcp) line was 0.637. When the toxicity of diafenthiuron was compared with monocrotophos, dimethoate, acephate, phosalone, carbaryl and triazophos, it was found to be 16.75, 22.38, 18.25, 21.25, 15.75 and 14.00 times more toxic at LC_{50} and 0.46, 0.45, 0.56, 0.46, 0.60 and 0.36 times more toxic at LC_{90} than the respective insecticides (Table 2 & 2a).

Pymetrozine

The resistant *S. dorsalis* population of Guntur district when exposed to the new insecticide, pymetrozine the LC_{50} and LC_{90} values were 0.0060 and 0.1120 percent, respectively (Table 1). The chi-square test indicated that the thrip population used in this study was homogeneous ($P \leq 0.05$). The slope value (b) of log concentration probit (lcp) line was 1.015. Comparison of the toxicity of pymetrozine with monocrotophos, dimethoate, acephate, phosalone, carbaryl and

triazophos revealed that it was 22.33, 29.83, 24.33, 28.33, 21.00 and 18.67 times more toxic at LC_{50} and 3.19, 3.14, 3.89, 3.17, 4.14 and 2.48 times more toxic at $LC_{90,}$ respectively (Table 2 & 2a).

Vertimec

It is clear from the data (Table 1) that the LC_{50} and LC_{90} values of vertimec were 0.0160 and 0.0470 per cent respectively to the resistant population of *S. dorsalis* of Guntur district. The chi-square test indicated that the thrip population used in this study was homogeneous ($P \leq 0.05$). The slope value (b) of log concentration probit (lcp) line was 2.652. Vertimec was found to be 8.38, 11.19, 9.13, 10.63, 7.88 and 7.00 times more toxic at LC_{50} and 7.60, 7.49, 9.28, 7.55, 9.87 and 5.91 times more toxic at LC_{90} than monocrotophos, dimethoate, acephate, phosalone, carbaryl and triazophos at LC_{50} and $LC_{90,}$ respectively (Table 2 & 2a).

Clothianidin

It is clear from the data (Table 1) that the LC_{50} and LC_{90} values of clothianidin were 0.0130 and 0.1400 percent respectively to the resistant population of *S. dorsalis* of Guntur district. The chi-square test indicated that the thrip population used in this study was homogeneous ($P \leq 0.05$). The slope value (b) of log concentration probit (lcp) line was 1.233. When the toxicity of clothianidin was compared with monocrotophos, dimethoate, acephate, phosalone, carbaryl and triazophos, it was found to be 10.31, 13.77, 11.23, 13.08, 9.69 and 8.62 times more toxic at LC_{50} and 2.55, 2.51, 3.11, 2.54, 3.31 and 1.99 times more toxic at LC_{90} than the respective insecticides (Table 2 & 2a).

Chlorfenapyr

It is clear from the data (Table 1) that the LC_{50} and LC_{90} values of chlorfenapyr were 0.0200 and 0.1360 percent respectively to the resistant population of *S. dorsalis* of Guntur district. The chi-square test indicated that the thrip population used in this study was homogeneous ($P \leq 0.05$). The slope value (b) of log concentration probit (lcp) line was 1.528. Comparison of the toxicity of chlorfenapyr with monocrotophos, dimethoate, acephate, phosalone, carbaryl and triazophos revealed that it was 6.70, 8.95, 7.30, 8.50, 6.30 and 5.60 times more toxic at LC_{50} and 2.63, 2.59, 3.21, 2.61, 3.41 and 2.04 times more toxic at $LC_{90,}$ respectively (Table 2 & 2a).

Flubendiamide

It is clear from the data (Table 1) that the LC_{50} and LC_{90} values of flubendiamide were 0.0210 and 0.1140 percent respectively to the resistant population of *S. dorsalis* of Guntur district. The chi-square test indicated that the thrip population used in this study was homogeneous ($P \leq 0.05$). The slope value (b) of log concentration probit (lcp) line was 1.745. Flubendiamide was found to be 6.38, 8.52, 6.95, 8.10, 6.00 and 5.33 times more toxic at LC_{50} and 3.13, 3.09, 3.82, 3.11, 4.07 and 2.44 times more toxic at LC_{90} than monocrotophos, dimethoate, acephate, phosalone, carbaryl and triazophos at LC_{50} and $LC_{90,}$ respectively (Table 2 & 2a).

Spinosad was superior to all other novel insecticides tested and also to the commonly used insecticides *viz*., monocrotophos, acephate, dimethoate, phosalone, triazophos and carbaryl at LC_{50}. The superior toxicity of spinosad was also reported against several lepidopteran caterpillars. Since spinosad belongs to a new class of natural insecticide which has spinosyn as an active principle with different mode of action. It acts on the central nervous system by activation of nicotinic acetylcholine receptors and also effects on GABA gated chloride channels. Pymetrozine can be used as an effective tool for the management of resistant population of *S. dorsalis* on chilli as it belongs to a new group of insecticides with different mode of action which blocks the stylet penetration. Imidacloprid can also be used as an effective tool for the management of resistant population of *S. dorsalis* on chilli as it belongs to a new group of insecticides *i.e*., neonicotinyl compounds with different mode of action which act by binding with nicotine acetyl choline receptors.

LITERATURE CITED

Agricultural statistics at a glance 2009. http/.www. agricoop.nic.in

Ananthakrishnan T N 1971. Thrips (Thysanoptera) in agriculture, horticulture, forestry -Diagnosis, bionomics and control. Journal of Scientific and Industrial Research 30: 113-146.

Borah D C 1987. Biology of *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) and *Scirtothrips dorsalis* (Thysanoptera : Thripidae) infesting chilli and their natural enemies. Ph.D. Thesis submitted to University of Agricultural Sciences, Dharwad, Karnataka.

FAO (Food and Agricultural Organization) 1979. FAO methods recommended method for detection and measurement of resistance in agricultural pests to pesticides. FAO Plant Protection Bulletin 127:2-5.

Joia B S, Jaswinder Kaur and Udean A S 2001. Persistence of ethion residues on/in green chilli. Proceedings of 2nd National Symposium on Integrated Pest Management (IPM) in Horticultural Crops, New molecules, Biopesticides and Environment. Bangalore, 17-19 October: 174-175.

Kranthi K R, Jadhav D R, Kranthi S, Wanjari R R, Ali S S and Russell D 2002. Insecticide resistance

in five major insect pests of cotton in India. Crop Protection 21: 449-460.

Prasad D K, Madhumathi T, Arjunarao P and Srinivasarao V 2008. Status of OP and Pyrethroid resistance in *Spodoptera litura* (Fab). on cotton. Pesticide Research Journal 20 (1):103-106.

Rao D and Ahmed K 1985. Evaluation of certain insecticides for the control of the pest complex on chilli in Andhra Pradesh. Pesticides 19(2): 41-44.

Reddy G P V, Prasad V D and Rao R S 1992. Relative resistance in chilli thrips, *Scirtothrips dorsalis* (Hood) populations in Andhra Pradesh to some conventional insecticides. Indian Journal of Plant Protection 20(2): 218-222.

Varadharajan S 1994. Studies on host plant resistant and biology of chilli thrips, *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae). M.Sc. (Ag.) Thesis submitted to Annamalai University, Annamalainagar pp.150.

Wang K Y, Jiang X Y, Yi M Q, Chen B K and Xia X M 2002. Insecticide resistance of *Spodoptera exigua*. Acta – Phytophylacica – Sinica 29(3): 229-234.

K. Vanisree, S. Upendhar and P. Rajasekhar,
Department of Entomology, Agricultural College,
Bapatla 522 101, Andhra Pradesh

Abstracts in Resistance Management

Reproduction and life span in house fly strains with different displacement of reproductive efforts

INTRODUCTION

Musca domestica L. is a perspective model in gerontological experiments because of fast synchronous development of individuals as well as the facilities of their rearing. Lack of information about *M. domestica* genome sequencing makes it necessary to reveal genes responsible for life span control. Taking into account the phenotypical particularities of individuals from these strains we can suppose the participation in life span control of gene systems which are responsible for power supply of metabolic processes and protein biosynthesis.

METHODS AND MATERIALS

We produced, from strain Cooper (S), two house fly strains with early and late reproductive efforts and then two homogenic strains with short (*Sh28*, 23 days) and long (*L2*, 42 days, fig.1) minimal life span have been isolated. Q-PCR had been used for comparing of nuclear and mitochondrial genes copy number ratio in thorax muscles of adults from *Sh28* and *L2* strains.

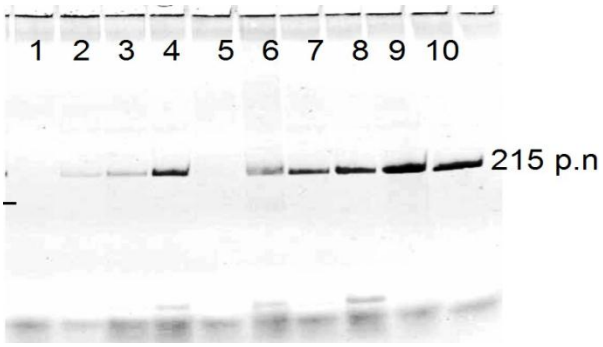

Figure 1. Gel show result of PCR – analysis of *C. septempunctata* gut content. 1-9 DNA samples of gut of *C. septempunctata*, 10 – DNA sample of larvae *L. decemlineata*.

RESULTS

The results of quantitative polymerase chain reaction (Q-PCR) with species-specific primers for ribosomal RNA genes and mitochondrial DNA showed two-fold decreasing of mtDNA copy number in 24 hrs-old adults of *Sh28* strain compared with individuals from *L2* strain. However, in both females and males from *Sh28* strain has been shown the 2.1-2.4-fold increasing of ribosomal DNA copy number (fig.2).

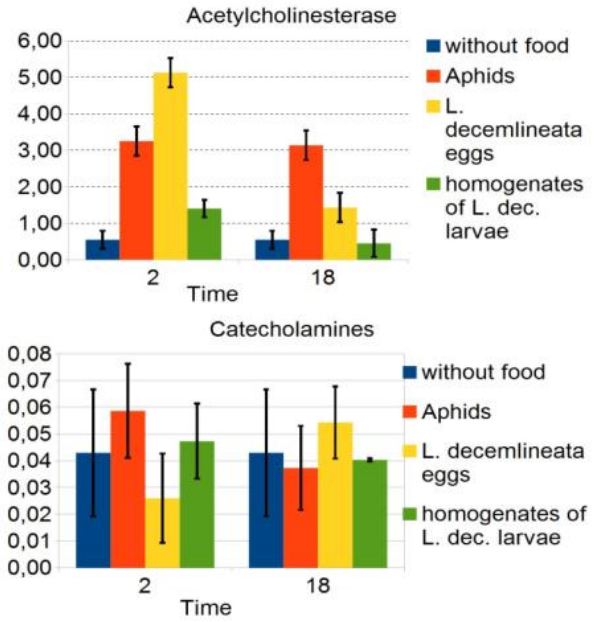

Figure 2. The level of activity acetylcholinesterase and catecholamynes in the hemolymph after 2 and 18 hours. Imago C. septempunctata have been fed aphids, eggs of *L. decemlineata*, homogenates of larvae *L. decemlineata* before analysis.

Evidently a higher content of mtDNA allows *L2* strain house flies to maintain a stronger resistance to ROS and other detrimental agents due to intense mitochondrial biogenesis and/or induction of mtDNA replication. The assumption has been confirmed by higher resistance levels to heat stress and intoxication in larvae and adults from *L2* strain. High content of ribosomal DNA in *Sh28* houseflies promotes higher protein biosynthesis level by more ribosomes number. This assumption is consistent with increased level of protein content during vitellogenesis and has been verified by early reproductive maturing, frequent reproductive cycles and displacement of oviposition spike to the first decade of adult's life. Another confirmation of our assumption is increased reactivity of fat body phenoloxidases and acetyl cholinesterase (fig. 3) under the heat stress in *Sh28* strain larvae**.**

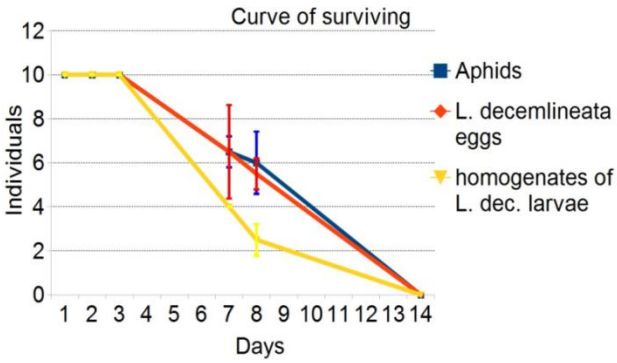

Figure 3. Surviving of *C. septempunctata* imagoes fed with different food.

CONCLUSION

Selection in *M. domestica* strains regarding to reproductive efforts and life span is attended by changes of mt/nuclear DNA contents ratio in muscle tissues that is evidence of essential significance of this index for life strategies determination.

ACKNOWLEDGEMENT

Investigations funded by Russian Federation for Basic Research 11-04-97005-r_povolzye_a.

G.V. Benkovskaya, Y.M. Nikonorov, R.S. Mustafina
Institute of biochemistry and genetics,
Ufa Scientific Centre of Russian Academy of Science, Russia,
bengal2 @yandex.ru

Announcements and Submission Deadlines

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

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

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

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

The next two **submission deadlines** are:

Monday, March 12, 2012
Monday, September 10, 2012

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

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

rpmnews@msu.edu, or

Newsletter Coordinator
Resistant Pest Management Newsletter
B-11 Center for Integrated Plant Systems
Michigan State University
East Lansing, MI 48824-1311
USA

Please visit us online today at**http://whalonlab.msu.edu/Newsletter/index.html**