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. 18, No. 1 (Fall 2008)

Table of Contents

Letter from the Editors

Dear Subscribers,

We wish to extend a very warm thank you to everyone who worked through our email issues with us for this newsletter issue. We are very sorry for the inconvenience that it caused to all of you. Thank you for working through the use of multiple alternate email addresses and for continuing to submit your work even when the submission process changed.

In the process of updating computers, the RPMNews@msu.edu email account was deleted and reinstating the address took longer than expected. This was due to many factors, including (but not limited to) the Newsletter Coordinator being gone for three months this summer, and I wish to personally thank you for your patience in dealing with the situation.

The RPMNews@msu.edu email address is back up and working, so you can now address your emails to that address instead of the whalonlab@gmail.com, rpmnewsletter@gmail.com, or puvlaow4@msu.edu email addresses. These email addresses will no longer be regularly checked for newsletter email so we ask that you please direct all of your newsletter correspondence to the RPMNews@msu.edu email address. We do not forsee this problem arising again in the future, and thank you for all of your patience and flexibility.

The editors would also like to remind you about the Arthropod Pesticide Resistance Database. This Database can be found at http://www.pesticideresistance.org/DB. It is a database of reported resistance cases from 1914 to the present, citing when resistance is first discovered for a specific time and place. Pesticide resistance is a dynamic, evolutionary phenomena and a record in this database may or may not be indicative of your area. Similarly, the absence of a record in this database does not indicate absence of resistance.

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

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

Sincerely,
Abbra Puvalowski
RPM Newsletter Coordinator
RPNNews@msu.edu

Resistance Management from around the Globe

Studies on Acute Toxicity of Insecticides for Monitoring Insecticide Resistance in the Rice Leaffolder Complex, *Cnaphalocrocis Medinalis* (Guenee) and *Marasmia patnalis* Bradley, in Tamil Nadu, India

ABSTRACT
Acute toxicity tests on fourth instar *C. medinalis* and *M. patnalis* larvae collected from different parts of Tamil Nadu indicated that there was no significant difference in the susceptibility of *C. medinalis* and *M. patnalis* to the insecticides tested. The LD_{50} values of chlorpyriphos, monocrotophos, phosalone, phosphamidon and quinalphos of the F_1 population of *C. medinalis* were 0.26377, 0.05165, 0.44345, 1.44641 and 0.06066 µg/larva respectively and the respective LD_{95} values were 1.43704, 0.47195, 2.52836, 7.11030 and 0.53028 µg/larva. LD_{50} and LD_{95} values of the above insecticides to F_1 population of *M. patnalis* were comparable. Based on the slope function and increased susceptibility, the common tentative discriminating doses (DD) suggested for *C. medinalis* and *M.*

patnalis were chlorpyriphos 1.00 µg, monocrotophos 0.35 µg, phosalone 1.9 µg, phosphamidon 5.5 µg and quinalphos 0.40 µg.

INTRODUCTION

Until 1981, *Cnaphalocrocis medinalis* Guenee was the only species considered as a rice leaffolder. The identification of *Marasmia patnalis* by Bradley in 1981, which caused leaf damage along with *C. medinalis*, led to the realization that there was a leaffolder complex. Four superficially similar species (*C. medinalis, M. patnalis, Marasmia exigua* (Batler) and *Marasmia ruralis* (Walker)) were recorded to be very common in rice growing areas (Reissig *et al*., 1985). In addition, *Marasmia bilinealis* (Hampson), *Marasmia suspicalis* (Walker), *Marasmia trapezalis* (Guenee), and *Marasmia venilialis* (Guenee) (Girst and Lever, 1969; Mathew and Menon, 1984; Barrion *et al*., 1987) were reported from South East Asian countries. Apart from these *Brandia admixtalis* (Walker), from Japan (Lee *et al*., 1973), and *Brachmia aretraea* Mayrick, a Gelichiid leaffolder from Indo-Malaya (Nadarajan and Rajappan, 1987), were also reported as leaffolder pests of rice in India. However, Gunathilagaraj and Gopalan (1986) and Subramanian (1990) indicated the predominance of *C. medinalis* and *M. patnalis* along with the occasional incidence of *M. ruralis* in major rice growing tracts of Tamil Nadu.

Follow up studies on leaf folder species composition by light trap collection by Anbalagan (2001) revealed that two species of leaffolders (*C. medinalis* and *M. patnalis*) occurred together. The population of *C. medinalis* was very high, ranging from 62 to 84 percent, whereas the *M. patnalis* population was 15 and 37 percent. Surveys made on the distribution of the leaffolder complex in different rice growing areas revealed that leaffolders (*C. medinalis* and *M. patnalis*) were found to occur together in both kuruvai and samba seasons. The occurrence of *C. medinalis* was significantly higher than *M. patnalis* irrespective of seasons and places. In general, the kuruvai season harboured a higher *C. medinalis* population than *M. patnalis*. The *M. patnalis* population was higher in the samba season when compared to the kuruvai season. In the kuruvai season, *C. medinalis* constituted 86 and 82 percent in Madurai and Bhavanisagar respectively, while *M. patnalis* accounted for the remaining population. In the samba season, the *C. medinalis* constituted the major component of leaffolder complex in Thirur (72%) and Madurai (71%). The level of *M. patnalis* was higher in Aduthurai (38%) and Madurai (36%) when compared to the 22 and 14 percent in the kuruvai season.

Although the integrated pest management practices indicated the use of resistant varieties, judicious fertilizer application, use of light traps etc., insecticides still remain the major control measure against leaffolder when it exceeds the economic threshold level. In Tamil Nadu, the commonly used insecticides are monocrotophos, phosphamidon, quinalphos, phosalone and chlorpyriphos (Regupathy et. al., 2003; Crop production Guide, 2005). The frequent use of certain insecticides leading to the development of insecticide resistance in rice pests is not uncommon (Endo *et al*., 1987; Endo and Kozano, 1988; Endo *et al*., 1993; Xiao *et al.*, 1994). For estimating the resistance level in the population, knowing the initial base-line level of susceptibility is essential so that future comparisons can be made (Hopkins *et al.*, 1984). Anandan and Regupathy (2007) generated base-line data for *C. medinalis* to certain commonly used insecticides in the rice growing areas of Tamil Nadu. The rice leaffolder complex may exhibit a differential response to insecticide selection pressure as in the case of the co-incident sibling species of *Helicoverpa armigera* (Hub.) and *Helicoverpa punctigera* (Wallengren), which recorded no resistance to any insecticide in Australia (Forrester *et al*., 1993). Hence, the base-line susceptibility to different insecticides was estimated for *C. medinalis* and *M. patnalis* separately.

MATERIALS AND METHODS

Larval populations were collected from the following rice growing regions where the regional/field stations of Tamil Nadu Agricultural University, Coimbatore exist: 1. Aduthurai (Tamil Nadu Rice Research Station), 2. Ambasamudram (Rice Research Station), 3. Bhavanisagar (Agricultural Research Station), 4. Coimbatore (Paddy Breeding Station), 5. Madurai (Agricultural College and Research Institute) and 6. Thirur (Rice Research Station). Collections were made where the insects were reasonably abundant. No special attempt was made to locate and collect from fields where any particular level of control failure was reported. Such collections were reared separately up to the F_1 generation in the green-house insectary at Tamil Nadu Agricultural University, Coimbatore for dosing. The methodology followed by Anandan and Regupathy (2007) was followed for culturing and dosing. Respective cultures of the leaffolders, *C. medinalis* and *M. patnalis*, without exposure to insecticides were maintained on TN-1 potted rice plants, a highly susceptible variety of rice plant in the Paddy Breeding Station (PBS), Tamil Nadu Agricultural University, Coimbatore.

Taxonomic diagnosis: Larval chaetotaxy

To confirm the identity of species, caterpillars of third instar were subjected to chaetotaxy diagnosis at random as per the description made by Yoshiyasu (1980) and Manisegaran(1996). The caterpillars were killed, relaxed in warm water and dissected along the mid-ventral line. Then, they were treated in 10 percent

KOH and glacial acetic acid solution, stained in acid fuchsin and mounted in Canada balsam after clearing in carbol-o-xylol. The mounted specimens were examined and drawings were made using Camera-Lucida (Hinton, 1946). The identity was confirmed by examining under a binocular microscope. Different species of leaffolder were distinguished based on wing markings and colouration as per the description of Barrion and Litsinger (1985) and Reissig *et al.*(1985).

Insecticides: Technical materials with a known purity of five insecticides commonly used on rice (monocrotophos (70%,) quinalphos (69%,), phosphamidon (94.7%) from Syngenta (India) Ltd, Mumbai, chlorpyriphos (97.5%) from Cynamid (India) Ltd., Mumbai and phosalone (98.0 %) from Aventis (India) Ltd Mumbai) were chosen for this study.

Dosing: Fourth instar larvae weighing 20-30 mg selected for assay were placed in a glass vial (9 cm long and 2 cm diameter) and anesthetized by CO_2. The topical application was made with a calibrated 1 µl single needle guided plunger syringe; it was used to apply a 0.5 µl unit of acetone containing a predetermined concentration of a technical insecticide. Application was made to the dorsal prothorax of the insect. The control insects were treated with analytical reagent acetone alone. The treated larvae were transferred to clear plastic cups (8.5 cm diameter at the base) lined with moistened filter paper at 10 larvae/cup and provided with cut leaves. The moisture from the filter paper maintained the turgidity of leaves. The cups were covered with snugly fitted lids and held inside an environmental chamber with a constant temperature range of 25 ± 2°C, 70 ± 5 per cent relative humidity and 12 hours light. Mortality was recorded 24 and 48 hours after treatment (HAT). Larvae were considered dead if they did not move when probed or if they could not right themselves within 14 seconds when placed off their ventors.

This test was conducted on larvae of Coimbatore population for first generation and on each generation, starting from fifth generation, in the greenhouse cultured population without exposure to any insecticide. In the field collected populations, larvae from the first generation were used, and LD_{50} and LD_{95} were estimated for population collected from each location. The corrected percentage mortality was worked out using the Abbot's formula (Abbott, 1925). Log-dose-probit mortality lines by probit analysis, susceptibility indices, the rate resistance decline and the number of generations required for a ten fold decrease in the LC_{50} value were computed (Regupathy and Dhamu, 2001).

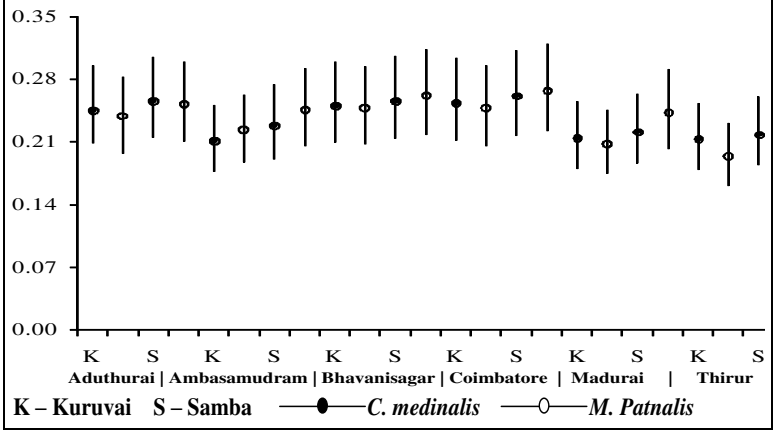
RESULTS AND DISCUSSION

There was no significant difference in the susceptibility of *C. medinalis* and *M. patnalis* to the insecticides evaluated. The LD_{50} values of chlorpyriphos, phosalone, monocrotophos, phosphamidon and quinalphos of the F_1 population of *C. medinalis* were 0.26377, 0.05165, 0.44345,1.44641 and 0.06066 µg/larva respectively (Tables 1-5) and the respective LD_{95} values were 1.43704, 0.47195, 2.52836, 7.11030 and 0.53028 µg/larva. LD_{50} and LD_{95} values of the above insecticides to F_1 population of *M.patnalis* were comparable.

Table 1. Acute toxicity of chlorpyriphos to fourth instars of *C. Medinalis* and *M. patnalis* larvae

	C. Medinalis		*M. patnalis*	
Generation	LD_{50}	LD_{95}	LD_{50}	LD_{95}
1	0.26377	1.43704	0.26140	1.42825
5	0.23904	1.33257	0.23972	1.30423
6	0.23649	1.29221	0.23307	1.22151
7	0.23006	1.18154	0.23199	1.18844
8	0.22664	1.16628	0.22255	1.11571
9	0.22474	1.15946	0.22075	1.09922
10	0.21561	1.06162	0.20832	1.01530

Figure 1. LD_{50} values of chlorpyriphos for *C. Medinalis* and *M. patnalis* populations from different locations in two seasons



The order of toxicity of insecticides to *C. medinalis* and *M. patnalis* was monocrotophos, quinalphos, chlorpyriphos, phosalone and phosphamidon irrespective of generation tested by topical application. The same order of toxicity to *C. medinalis* was reported by Valencia and Heinrichs (1982), Jena *et al*. (1992), Naik *et al*. (1993a) and Anandan and Regupathy (2007).

Table 2. Acute toxicity of monocrotophos to fourth instars of *C. Medinalis* and *M. patnalis* larvae

	C. Medinalis		*M. patnalis*	
Generation	LD50	LD95	LD50	LD95
1	0.05165	0.47195	0.05105	0.46829
5	0.04812	0.45664	0.04686	0.45119
6	0.04755	0.45301	0.04670	0.44763
7	0.04473	0.44533	0.04552	0.44509
8	0.04216	0.41740	0.04289	0.41477
9	0.04164	0.41398	0.04087	0.40884
10	0.03882	0.33280	0.03837	0.33012

Fig 2. LD $_{50}$ values of monocrotophos for *C. Medinalis* and *M. patnalis* populations from different locations in two seasons

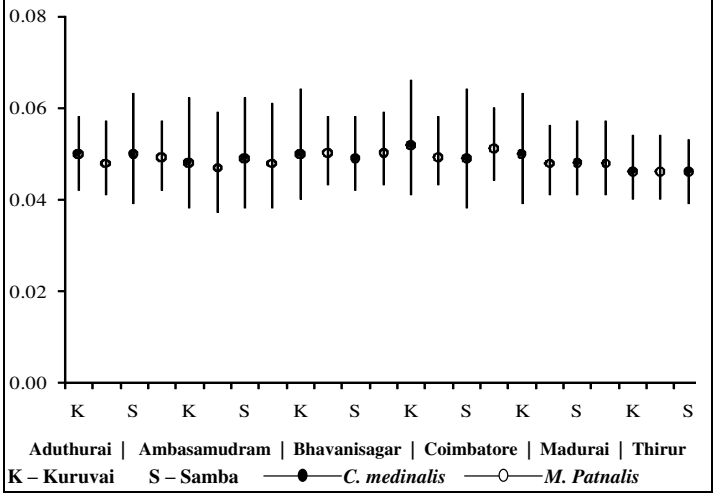


Table 3. Acute toxicity of phosalone to 4th instars of *C. Medinalis* and *M. patnalis* larvae

	C. Medinalis		*M. patnalis*	
Generation	LD50	LD95	LD50	LD95
1	0.44345	2.52836	0.43009	2.49218
5	0.41813	2.37417	0.41446	2.34907
6	0.39128	2.31839	0.38984	2.35388
7	0.38267	2.31061	0.37745	2.27251
8	0.36570	2.24701	0.35975	2.19062
9	0.34810	1.93296	0.33294	1.87953
10	0.31912	1.86620	0.31252	1.85765

Figure 3. LD $_{50}$ values of phosalone for *C. Medinalis* and *M. patnalis* populations from different locations in two seasons

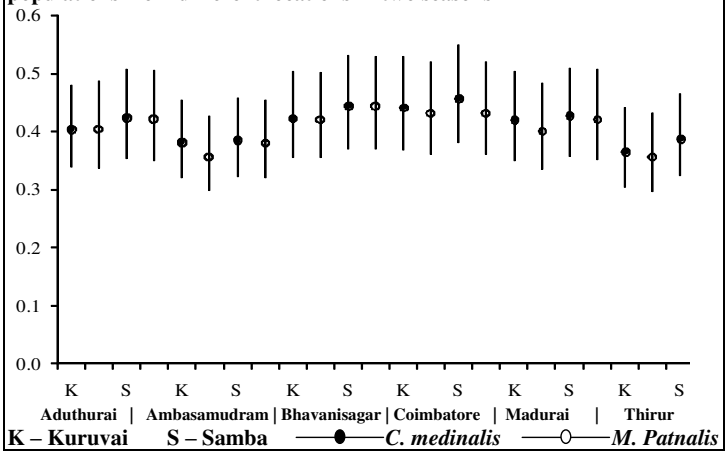


Table 4. Acute toxicity of phosphamidon to fourth instars of *C. Medinalis* and *M. patnalis* larvae

	C. Medinalis		*M. patnalis*	
Generation	LD_{50}	LD_{95}	LD_{50}	LD_{95}
1	1.44641	7.11030	1.43817	7.19949
5	1.40335	6.45702	1.39607	6.87142
6	1.33953	5.8619	1.32559	6.07204
7	1.31629	5.78264	1.30578	5.75221
8	1.29563	5.68339	1.27677	5.51773
9	1.26082	5.53044	1.23582	5.45769
10	1.22207	5.51818	1.21195	5.48810

Figure 4. LD $_{50}$ values of phosphamidon for *C. Medinalis* and *M. patnalis* populations from different locations in two seasons

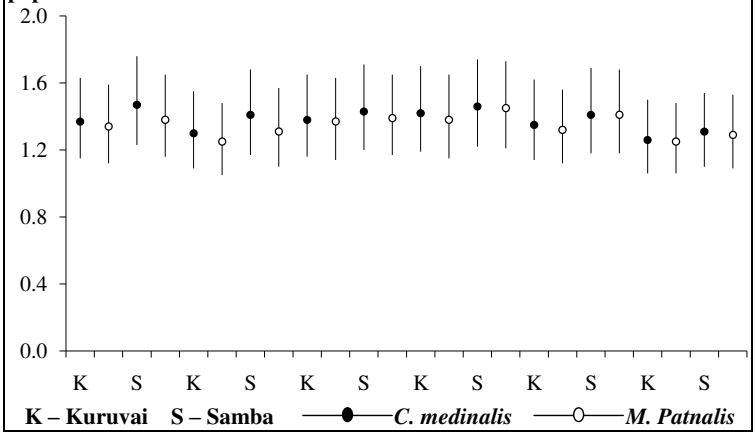
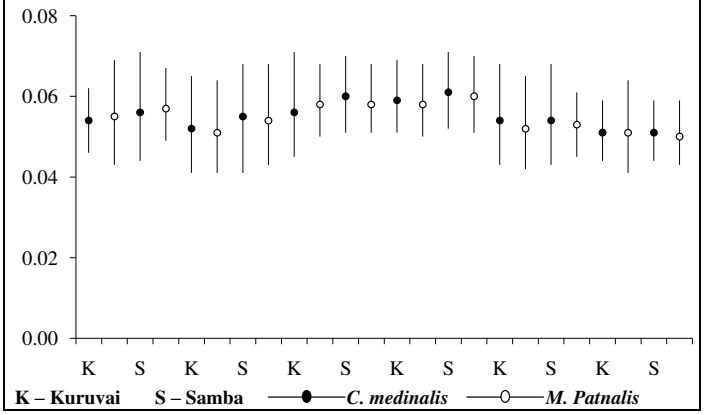


Table 5. Acute toxicity of quinalphos to fourth instair larvae of rice leaffolders

	C. Medinalis		*M. patnalis*	
Generation	LD_{50}	LD_{95}	LD_{50}	LD_{95}
1	0.06066	0.53028	0.05998	0.52018
5	0.05529	0.50542	0.05405	0.49126
6	0.05468	0.49568	0.05343	0.48813
7	0.05339	0.49031	0.05234	0.48111
8	0.05096	0.42771	0.05043	0.42004
9	0.04966	0.42275	0.04817	0.40091
10	0.04819	0.41078	0.04769	0.39033

Figure 5. LD $_{50}$ values of quinalphos for *C. Medinalis* and *M. patnalis* populations from different locations in two seasons



The susceptibility base-line data (LC_{50} in ppm) of *C. medinalis* to chlorpyriphos methyl (0.5), tetrachlorvinphos (5.8), dimethyvinylphos (2.0), diazinon (3.0), isoxanthion (0.28), acephate (4.1), monocrotophos (0.35), cartap (1.8) (Endo *et al*., 1987), monocrotophos (0.003) (Anon, 1970), diazinon (0.13), BPMC (0.13), cypermethrin (0.02) (Tevapunchum, 1991), ethofenprox (0.00063), monocrotophos (0.0068) (Jena *et al*., 1992), quinalphos (0.0074), monocrotophos (0.0025), phosphamidon (0.023) and decamethrin (0.024) (Naik *et al*., 1993) were assessed by various methods. Earlier studies on the *C. medinalis* population of Tamil Nadu by Anandan and Regupathy (2007) reported the LD_{50} values (µg/larva) of F_1 population of *C. medinalis* to monocrotophos (0.0546), quinalphos (0.0712) chlorpyriphos (0.2534) and phosphamidon (1.5074).

After the withdrawal of selection pressure, the susceptibility level of both of the species gradually increased with the advancement of each generation which is evident from decline in the LD_{50} and LD_{95} values to all of the insecticides evaluated except chlorpyriphos (Tables 1-5). The rate of decline of susceptibility base line is very low. For a 10 fold decrease in LD_{50}, the required generations of *C. medinalis* was 114.29 for chlorpyriphos, 80.65 for monocrotophos, 70.03 for phosalone, 136.61 for phosphamidon and 100.10 for quinalphos. In case of *M. patnalis*, it was 101.40 for chlorpyriphos, 80.65 for monocrotophos, 72.10 for phosalone, 134.59 for phosphamidon and 100.40 for quinalphos.

The discriminating dose screen for testing the *C. medinalis* populations estimated by Anandan and Regupathy (2007) was 0.35, 0.50, 1.0 and 5.5 µg. for monocrotophos, quinalphos, chlorpyriphos and phosphamidon respectively. Based on the slope function and increased susceptibility, the common tentative discriminating doses (DD) arrived at based on the LD_{95} values of the insecticides for the F_{10} generation of the laboratory cultured populations of *C. medinalis* and *M. patnalis* were chlorpyriphos 1.00 µg, monocrotophos 0.35 µg, phosalone 1.9 µg, phosphamidon 5.5 µg and quinalphos 0.40 µg.

REFERENCES

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

Anandan, G.K. and A. Regupathy.2007. Assessment of acute toxicity of insecticides for monitoring insecticide resistance in rice leaffolder *Cnaphalocrocis medinalis* (Guenee) in Tamil Nadu. *Resistant pest management.* 16 (2): 3- 5.

Anbalagan, G. 2001. Studies on insecticide resistance in rice leaffolder complex in Tamil Nadu. Ph.D. Thesis, Tamil Nadu Agricultural University, Coimabtore.

Barrion, A.T. and J.A. Litsinger. 1985. Identification of rice leaffolders (LF) by wing markings. **IRRN., 5(4):** 24.

Barrion, A.T., E.B. Medina, R.M. Aguda, J.P. Bandong, P.C. Jr. Pantua, C.R.Vega, V.D. Vrajante, C.G. Dela Cruz, J.S. Jr. Saviano, E.E. Camanag and J.A. Litsinger. 1987. The rice *Cnaphalocrocis* and *Marasmia* (Lepidoptera: Pyralidae) leaffolder complex in the Philippines, Taxanomy, bionomics and control. **XVIII Annual Convention of the Pest Control of the Philippines.** 5-8 May, 1987. Davo City, Philippines.

Bradley, J.D. 1981. *Marasmia patnalis* (Lepidoptera: Pyralidae) on rice in South East Asia. **Bull. Entomol.** Res., 71: 323-327.

Crop Production Guide. 2005. Commissioner of Agriculture, Chepauk. Chennai. pp.148-172.

Endo, S., H. Kozano and T. Masuda. 1987. Insecticide susceptibility of rice leaf roller larvae *Cnaphalocrocis medinalis* Guenee. Appl. **Entomol.** Zool., 22: 145-192.

Endo, S., M. Tsurmachi and K. Tanakka. 1993. Changes in insecticide susceptibility of rice leaffolder *Cnaphalocrocis medinalis* Guenee (Lepideptera : Pyralidae) collected in Kyushu and difference in susceptibility between Japanese and Malaysian colonies. Appl. Entomol. Zool., 28: 125-130.

Endo, S. and H. Kozano. 1988. Insecticides susceptibility of rice leaffolder larvae *Cnaphalocrocis medinalis* Guenee in Kyushu district. Proc. Assoc. Plant Prot., Kyushu. 34: 106-108.

Forrester, N.W., N. Cahill, L.J. Bird and J.K. Layland. 1993. Management of pyrethroid and endosulfan resistance in *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Australia. **Bull.** Ent. Res., Suppl. No. 1.

Girst, D.H. and R.J.A.W. Lever. 1969. Pests of Rice. Longmans, Green and Co., London, U.K.

Gunathilagaraj K and Gopalan M (1986). Rice leaffolder (LF) complex in Madurai, Tamil Nadu, *Intl. Rice Res. Newsl.,* **11**(6): 29.

Hinton, E.A. 1946. On the homology and nomenclature of the setae of lepidopterous larvae with some notes on the phylogeny of the Lepidopteran. Trans. Roy. Ento. Soci., London. 97: 1-36.

Hopkins, A.R., R.F. Moore and W. James. 1984. Contact and residual toxicities of pyretheroids and organophosphate

compounds to the boll weevil (Coleoptera Curculionidae). **J. Georgia Entomol.** Soc., 19: 27-34.

Lee, S.C., Y.H. Song, J.O. Lee and S.C. Han. 1973. Minor pests. Literature Review of Korean Rice Pests, Department of Entomology. Institute of Agricultural Science.Office of Rural Development. Suweon, Korea, pp.30-33.

Manisegaran, S. 1996. A **study on the rice leaffolder complex** *Cnaphalocrocis medinalis* Guenee and *Marasmia patnalis* **Bradley (Lepidoptera: Pyralidae) in Karaikal region,** Ph.D. Thesis. Tamil Nadu Agricultural University, Coimbatore, India, 152p.

Mathew, G. and M.G.R. Menon. 1984. The pyralid fauna (Lepidoptera: Pyralidae Pyralidinae) of Kerala (India). **J. Entomol.** Res., 8(1): 5-13.

Nadarajan, L. and N. Rajappan. 1987. A new rice leaffolder (LF) in Kerala. **IRRN, 12(5):** 23.

Regupathy, A. and K.P. Dhamu. 2001. **Statistics Workbook for Insecticide Toxicology.** Softek Computers, Coimbatore 206 p.

Regupathy, A., S. Palanisamy, N. Chandramohan and K. Gunathilagaraj. 2003. **A Guide on Crop Pests**. (IV edn.0 Sheeba Pprinters, Coimbatore.276p.

Reissig, W.H., E.A. Heinrichs, J.A. Litsinger, K. Moody, L. Fiedler, T.W. Mew and A.T. Barrion. 1985. **Illustrated Guide to Integrated Pest Management in Rice in Tropical Asia.** Int. **Rice Res.** Inst., P.O. Box.933, Manila, Philippines, 411, 214p.

Reissig, W.H., E.A. Heinrichs, J.A. Litsinger, K. Moody, L. Fiedler, T.W. Mew and A.T. Barrion. 1985. **Illustrated Guide to Integrated Pest Management in Rice in Tropical Asia.** Int. **Rice Res.** Inst., P.O. Box.933, Manila, Philippines, 411, 214p.

Subramanian, A. 1990. **Bio-ecology** and **management** of rice **leaffolders *Cnaplzalocrocis medinalis*** Guenee and ***Marasmia patnalis* Bradley (Pyralidae** : **Lepidoptera).** Ph.D. Thesis, Tamil Nadu Agricultural University, Coimabtore, 207p.

Xiao, Z.Y., Z.X. Huang and D.P. Huang. 1994. Resistance of rice leaffolder *Cnaphalocrocis medinalis* to insecticides in Guangdong. **Guangdong Agric.** Sci., 3: 35-37.

Yoshiyasu, Y. 1980. Morphology of immature stages of *Marasmia latimarginalis* (Hampson). **Scientific Report of Kyoto Prefectural University of Agriculture, No.32,** pp.51-55.

G.Anbalagan and A.Regupathy[1]
Department of Entomology, Tamil Nadu Agricultural University, Coimbatore, India-641003, India
[1]Present address: Science Advisor- Entomology, Biofuels Research & Development Centre,
Enhanced Biofuels and Technologies (I)
#5/10-C, Alankar Gardens, G.N. Mills Post,
Coimbatore- 641 029, India,
www.ebtiplc.com
E.mail: regupathya@yahoo.com

A preliminary toxicological study of commonly used acaricides of tea red spider mite (*Oligonychus coffee* Nietner) of North Bengal, India

ABSTRACT

A study was conducted at the University of North Bengal to determine the level of susceptibility of the red spider mite (*Oligonychus coffee* Nietner) population in the Dooars and Terai regions of sub Himalayan West Bengal (North Bengal), India during 2007 – 2008, since the two regions are separated by the mighty Testa River. Six commonly used acaricides were tested against five different populations collected from the Dooars and Terai regions. The study indicated that the level of susceptibility of different acaricides varied by region. The mite populations of western terai showed less susceptibility to all acaricides tested, whereas population of the eastern Dooars region showed high susceptibility to some kinds of acaricides. The mite populations of central and western Dooars and eastern Terai exhibited low to intermediate levels of susceptibility to the acaricides. Based on LC_{50} values, fenpropathrin, fenazaquin and propargite were found to be highly effective whereas ethion, dicofol and micronised sulfur showed less toxicity to the pest. A similar degree of susceptibility was noted for said acaricides in the case of mite eggs. The low susceptibility of the mite population may be due to continued and repeated application of the same acaricides for years together.

Introduction:

Red spider mite (*Oligonychus coffeae*, Nietner: Tetranychidae) is one of the most destructive pests in all the tea growing regions of North East India (Das, 1959 and 1963). Loss in tea crop due to red spider mite attack in India may be as much as 75% (Subramaniam, 1995). Larvae, nymphs and adults of *O. coffeae* cause damage to the mature leaves of tea by sucking from the upper surface. Reddish spots develop at the sucking sites, which subsequently unite to form large brown patches thus reducing the photosynthesis rate of the maintenance leaves. This species breeds throughout the year in Northeast India, and its reproductive rate increases with temperature (Das, 1959). Spherical and reddish eggs are laid on the upper surface of the mature leaves, which hatch in 4 – 6 days during the summer months. The application of pesticide programme in tea in Northeast India is largely guided by the recommendations of Tea Research Association (Das *et al.,* 1992). The commonly recommended dose of ethion, dicofol, propargite, and fenazaquin is 1:400 dilutions (2500 ppm), for micronised sulphur it is 1: 200 (5000 ppm) and for Fenpropathrin it is 1: 1600 (625 ppm).

Tea planters from different pockets of North Bengal have been reporting control failures of the pest with the use of acaricides in recommended dilutions. Experiences of the planters have been supported by reports of the low-efficacy of some new acaricides molecules against red spider mite in North Bengal (Sahoo *el al.,* 2003). Other workers have reported that members of tetranychidae (Acari) have developed a high degree of resistance to a number of acaricides in different agro ecosystems (Rae *et al*., 1995; Funayama and Takahashi, 1995). The sub-Himalayan belt of North Bengal is broadly divided in Treai and the Dooars region by the major river Teasta. Laboratory studies were conducted on susceptibility of *O. coffeae* populations from five tea growing areas (eastern Dooars, western Dooars, central Dooars, eastern Terai and western Terai) of North Bengal to some commonly used acaricides. Eastern Dooars (comprises subdistrict of Kalchini and Jointi), western Dooars (comprises subdistrict of Chulsa and Damdim), central Dooars (comprises subdistrict of Nagrakata, Dalgong and

Binnaguri), all of which are situated on the eastern region of Testa river while the eastern Terai and western Terai are situated on the western region. The weather conditions of these two regions of plantation also show some diversity.

Material and Method:

Red spider mites (*O. coffeae*) were collected from the nine tea growing regions of the North Bengal. They were reared in the laboratory for one generation before bioassay tests. Acaricides used in the studies were ethion (Ethion 50 EC); dicofol (Colonel-S 18.5 EC); propargite (Omite 57 EC); fenazaquin (Magister 10 EC); fenpropathrin (Meothrin 30 EC) and micronised sulphur (Thiovit 80 W/W). Graded concentrations of acaricides were prepared in distilled water from commercial formulations of the acaricides. The relative toxicity was determined by following IRAC method No. 4 (i.e. modified leaf disc method) named as 'Whole leaf residual contact assay' developed by Dr. T. Dennehy of Cornell University (Regupathy *et al.,* 2007). One half of a Petri dish, of 9 cm in diameter, was toweled by a layer of cotton wool. Water was added to it only to the point of saturation. Individual leaves were treated by dipping for 5 seconds in test liquids. Surface liquid was allowed to dry from leaves before placing dorsoventrally them in Petri dishes. Cotton wool strips, 1 cm in width, soaked in tap water were laid around the margin of each treated leaf with half over the leaf and half over the cotton wool bed. A small piece of damp cotton wool has placed around the petiole of each leaf. A population of at least 10 adult mites per leaf was released. With the help of a binocular microscope or hand lens, it was necessary to ensure that there are no gaps between the leaves and cotton wool strips. Each treatment was replicated five times and kept in a B.O.D. incubator at 26 ± 1° C temperature and 80% RH. Mortality was recorded 24 hours after treatment. The absolute mortality data was converted to percent mortality and subjected to probit analysis (Finney, 1973 and Busvine, 1971). LC_{50} and LC_{95} values for each acaricides were computed.

For the assessment of ovicidal properties of the acaricides fifteen gravid females of red spider mite were introduced on TV 1 mature fourth leaf from the top of the shoot and kept over night in a Petri dish for oviposition. The mature leaves were padded with water soaked cotton. After 18 hours, the introduced mites were removed with the help of fine brush. The eggs laid on tea leaves were counted under microscope as pre-treatment count and maintained at 30 eggs/leaf by removing excess eggs by help of a fine needle. Each ovicidal treatment of the acaricides was done on 30 eggs with five replicates. Each batch of eggs was treated by spraying different acaricides at three different concentrations (0.05%, 0.1% and 0.25%). The control eggs were treated with water under similar setup. Hatchability was determined both for experimental and control batches of eggs for a period of 12 days after oviposition. Those eggs that did not hatch after this period were regarded as non-viable. From the observed egg mortality corrected percent mortality was calculated using Abott's formula (1925).

Result and Discussion:

Based on percent LC_{50} values of different acaricides against the five populations, eastern Dooars population of *O. coffeae* was found to be the most susceptible to all the acaricides, while western Terai population exhibited a highest level of tolerance. Of all the synthetic organic acaricides synthetic pyrethroids (fenpropathrin and fenazaquin) were found to be the most effective in all the five location. The mite population of central Dooars was found to develop 2.61, 1.94, 2.79, 2.17, 1.72, and 2.55 fold resistance to ethion, dicofol, propargite, fenazaquin, fenpropathrin, and micronised sulphur respectively, and the population of the western Dooars developed 2.89, 1.01, 2.71, 2.90, 1.07 and 1.40 fold resistance respectively when compared to eastern Dooars population; the latter, therefore, showing the minimum resistance and thus forming the base line reference. Furthermore, eastern Terai population exhibited 6.11, 1.66, 5.16, 4.74, 2.65 and 2.38 fold resistance and western Terai registered 6.44, 2.71, 3.93, 7.70, 4.13 and 5.57 fold to ethion, dicofol, propargite, fenazaquin, fenpropathrin, and micronised sulphur respectively as compared to the base line data from eastern Dooars (Table 1). Using LC_{95} values as threshold acaricides such as fenpropathrin, fenazaquin and propargite were found to have high killing efficacy, where as acaricides such as ethion, dicofol and micronised sulphur were ineffective at commonly recommended dilutions.

Table 1. Acaricidal susceptibility of field collected *O. coffeae* from different tea growing areas in North Bengal.

Acaricides	Location				
	Jalpaiguri District			Darjeeling District	
	Eastern Dooars	Central Dooars	Western Dooars	Eastern Terai	Western Terai
	LC_{50} (ppm)				
Ethion 50 EC	123.01 (S)	321.47 (2.61)	366.69 (2.89)	751.02 (6.11)	791.99 (6.44)
Dicofol 18.5 EC	199.58 (S)	388.16 (1.94)	201.17 (1.01)	330.58 (1.66)	541.44 (2.71)
Propargite 57 EC	22.94 (S)	64.10 (2.79)	62.19 (2.71)	118.48 (5.16)	90.26 (3.93)
Fenpropathrin 30 EC	1.63 (S)	3.53 (2.17)	4.73 (2.90)	7.72 (4.74)	12.55 (7.70)
Fenazaquin 10 EC	4.06 (S)	6.968 (1.72)	4.35 (1.07)	10.76 (2.65)	16.77 (4.13)
Micro. sulphur 80 W/W	466.67 (S)	1188.66 (2.55)	654.85 (1.40)	1110.45 (2.38)	2599.06 (5.57)

Figures within parenthesis represent resistance factors (RFs) which were determined at LC_{50} relative to the corresponding lowest LC_{50}s of the respective acaricides in the respective regions (Chatrvedi, 2004).
"S" indicates susceptible population.

Table2. Ovicidal action of certain acaricides against Tea Red spider mite (*Oligonychus coffeae*) on tea.

Acaricides used	Mean percent mortality of eggs and corrected percent mortality with different concentrations					
	0.05%		0.1%		0.25%	
	MPM	CPM	TPM	CPM	TPM	CPM
Ethion	18.23 (4.77)	0.00	34.33 (6.36)	19.59	50.67 (7.62)	39.60
Dicofal	18.00 (4.74)	0.00	35.00 (6.42)	20.41	46.00 (7.28)	33.88
Propergite	54.00 (7.85)	43.68	71.00 (8.93)	64.49	88.33 (9.90)	85.71
Fenazaquin	89.00 (9.93)	86.53	92.00 (10.09)	90.20	97.67 (10.38)	97.15
Fenpropathrin	35.00 (6.42)	20.41	55.33 (7.94)	45.30	78.67 (9.37)	73.88
Control (water spray)	18.33 (4.78)	0.00	18.33 (4.78)	0.00	18.33 (4.78)	0.00
SEM(±)	0.345		0.417		0.373	
CD (0.05)	1.048		1.252		1.118	
CV	0.165		0.135		0.105	

Figures in parenthesis are the $\sqrt{Y + 0.05}$ transformed value. Where Y = mean percentage mortality. MPM indicates Mean percent mortality; CPM indicates corrected percent mortality.

The higher tolerance reaction of *O. coffeae* may possibly be attributed to repeated and long term application of the as compared to the relatively newer acaricides like propargite, fenazaquin, fenpropathrin. However, the exact cause for differential susceptibility levels to different acaricides remains to be a matter of further investigation using biochemical and genetic level.

Studies on ovicidal toxicity to *O. coffeae* eggs revealed that fenazaquin at all different concentrations showed high killing properties in the tune of 86.53% to 97.15%. But dicofol (0.05%) and ethion (0.05%) were found to have little or no ovicidal action. The propargite at 0.05 %, 0.1% and 0.25% concentrations effected 43.68, 64.49 and 85.71 percent mortality of eggs (table 2).

However, at higher concentrations (0.01 and 0.25 percent) ethion and dicofol caused 20 to 40 percent egg mortality. By and large the order of ovicidal properties of these selected acaricides could be arranged as: fenazaquin > propargite > fenpropathrin > ethion > dicofol. Similar findings of ovicidal action of the pesticides in question on were reported in *Tetranychus urticae* by Kumar and Singh (2004). In view of the differential susceptibility of the populations from the five different regions, area wise control strategy can be

formulated, so that mite pest can be managed efficiently to obtain profitable yields. Additionally, acaricides with high ovicidal action may be incorporated in the control programmes keeping in mind the peak oviposition period of this pest.

REFERENCES:

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

Busvine, J.R., 1971. A Critical review of techniques for testing insecticides. *C.A.B. Publication*, London, pp. 271-276.

Chaturvedi, I., 2004. A Survey of Insecticide Resistance in *Helicoverpa armigera* in Central and South Indian Cotton Ecosystems, 1999 to 2003. *Resistant Pest Management Newsletter,* **14**(1):23- 26.

Das, G.M., 1959. Bionomic of the tea Red Spider, *O. coffea* (Nietner). *Bull. of Entomological Res*, **50** (2):265 -275.

Das, G.M., 1963. Important pest of tea. *Two and a bud*, **10**(2): 4 – 8.

Das, S.C., Kakoty, N.N., Borthakur, B.K. and Barua, G.C.S., 1992. *Latest pesticidal recommendations. Tocklai Experimental Station, Jorhat, Assam*. pp. 9.

Finney, D.J., 1973. Probit Analysis, *Cambridge University Press, Cambridge*, pp.333.

Funayama, K. and Takahashi, Y., 1995. Resistance to fenpyroximate and pyridaben in Two – spotted spider mite, *Tetranychus urticae* (Acari : Tetranychidae) in southern Akita. *Prefecture Bulletin of the Akita fruit tree Experimental Station*. **25**:19-30.

Rae, C. J., Kim Y. J., Ahn, Y. J., Yoo J. and Lee, J.O., 1995. Monitoring of acaricides resistance in field collected population of *Tetranychus urticae* (Acari: Tetranychidae) in Korea. *Korean Journal of Applied Entomology*, **34** (1): 40– 45.

Regupathy, A., Radhakrishnan, B. and Ayyasamy, R., 2007 Need for Acaricide Resistance Monitoring in Mites Affecting Tea in South India. *Resistant Pest Management Newsletter* **17**: 1 8-12.

Sahoo, B., Sahoo, S.K. and Somchowdhury, A.K. 2003. Studies in the toxicity of newer molecules against Tea Red spider mite, *proceeding of National Symposium on Frontier Areas of Entomological. Research 3-7 November*, 2003. pp.301-302.

Sen, A.R. and Chakraborty, P.P., 1964. Estimation of loss of crop from Pests and Diseases of tea from sample surveys. *Biometrics*, **2**: 472 -504.

Subaharan, K. and Regupathy, A. 2006. Effect of neem formulations on the management of purple mite, *Calacarus carinatus* L. infesting tea. *J. Plantn. Crops.* **34** (3): 435 -438.

Subramaniam, B., 1995. Tea in India. *P.I.D. and Viley Eastern LTD*. Pp. 38-40.

Somnath Roy[1], G.Gurusubramanian[2] and Ananda Mukhopadhyay [1]

[1]Entomology Research Unit, Department of Zoology, University of North Bengal,
Darjeeling-734 430, West Bengal, India.
E-mail: entosomnath@yahoo.co.in; dr_amukherjee_nbu@rediffmail.com;
Fax No.0353-2581546

[2]Department of Zoology, Mizoram Central University, Tanhril,
Aizawl -796 009, Mizoram, India.
E-mail: gurus64@yahoo.com; Fax No.0389-2330642/644

Susceptibility status of *Helopeltis theivora* Waterhouse (Heteroptera: Miridae) to the commonly applied insecticides in the tea plantation of the Sub-Himalayan Dooars area of North Bengal, India

ABSTRACT

Among various biotic stresses that tea plants face, insect attack, especially from the tea mosquito bug (*Helopeltis theivora*), has been a major challenge in recent years. Difference in relative toxicity of different commonly used insecticides to *Helopeltis theivora* was observed in the plantation of the tea subdistrict of the Dooars, located in the region of northern West Bengal, India. The study indicated that relative susceptibility values (LC_{50}) of *H.theivora* to different insecticides varied region wise. The populations of Kalchini tea subdistrict showed less susceptibility to all insecticides tested as compared to the high susceptibility of those from the Damdim and Chulsa subdistricts to most of the tested insecticides. The populations of the Nagrakata, Dalgong and Binnaguri subdistrict represented intermediate level of susceptibility to different insecticides tested. Endosulfan showed the lowest susceptibility against *H.theivora* in all tea growing sub-districts in the Dooars with a high LC_{50} value in all locations in the Dooars tea plantation. The effective field dosages of these insecticides were computed based on LC_{50} values, and when compared with the recommended dosages, it suggested a significant decrease in the susceptibility of the test population was observed against six insecticides like endosulfan, deltamethrin, λ-cyhalothrin, imidaclopyrid, quinalphos and oxydemeton methyl. The decrease in susceptibility of *H. theivora* to endosulfan (12.33 to 72.26 fold), deltamethrin (4.02 - 22.40 folds) and imidaclopyrid (13.61 to 29.16 folds) was the highest. However, there was not much change in case of monocrotophos and fenpropathrin, which therefore was found effective even at a lower dose than the recommended dose.

KEY WORDS: *Helopeltis theivora*, insecticides, LC_{50}, relative toxicity, resistance factors, effective field dose.

INTRODUCTION

The tea mosquito bug (*Helopeltis theivora*) is considered as one of the major pests of tea in Assam, Dooars, Terai and Darjeeling because it attacks only the young shoots that is the actual crop of tea. Planters from different parts of the Dooars tea plantation have been reporting control failure of the notorious pest with the use of insecticides. All Tocklai released tea clones, garden released clones and seed jats are found susceptible to *H. theivora* attack at varying degrees. It was estimated that 80% of the tea plantations area in India is affected by this pest alone, which often result in crop loss to the tune of 10-50% (Bora and Gurusubramanian 2007). Among the tea growing region of India, pest activity has always been reported to be high in the Dooars (Borbora & Biswas 1996 and Sannigrahi and Talukdar 2003). The climatic conditions of this region and monoculture of tea over vast stretches of land contribute largely to high pest incidence. With increases in the quantity of pesticide being applied every passing year, the problem has been aggravated and the cost of pest control is increasing day by day (Sannigrahi and Talukdar 2003). A survey conducted during 1998 to 2004 by Roy *et al.* (2008a) reported that on average 7.499 l/kg of insecticide was

used per hectare per year in the Dooars, of which the organo-chlorine, organo-phosphate and carbamate (Non-pyrethroid) accounted for 73.5% and pyrethroids 36.6%. Among the different subdistrict in the Dooars, the lowest consumption was noted in Damdim subdistrict (5.799 kg/L per hectare) followed by Chulsa (6.433 kg/L per hectare), Binnaguri (7.399 kg/Lper hectare), Nagrakata (7.655 kg/L per hectare), and Dalgong (7. 920 kg/L per hectare) with the highest consumption noted in Kalchini (9.793kg/L per hectare). The requirement of synthetic pyrethorid gradually increased with every passing year in the Dooars. Endosulfan, monocrotophos, deltamethrin and cypermethrin were extensively used in the entire subdistrict.

In spite of regular application of insecticides, *H. theivora* has turn into a menace all around the year. Decrease in the susceptibility to different classes of insecticides may be one of the causes for their resurgence and persistence on tea crops (Sarker and Mukhopadhyay, 2003, 2006, 2006a; Rahman *et al*., 2006; Sarmah *et al*., 2006; Bora *et al*., 2007, 2007a and 2008; Roy *et al*., 2008,).

In order to confirm the above fact, a study was conducted to assess the relative toxicity at effective concentrations of twelve commonly used pesticides: organochlorine (endosulfan); organophosphates (monocrotophos, quinalphos, profenophos and oxydemeton methyl); synthetic pyrethroids (deltamethrin, cypermethrin, λ- cyhalothrin, alphamethrin and fenpropathrin,); and neonicotinoids (imidaclopyrid, thiomethoxam) against *H. theivora* populations of six tea growing subdistricts of the Dooars.

Materials and Methods

Insecticide Susceptibility

Helopeltis theivora adults were collected from the tea plantations of the six tea subdistrict of the Dooars region. They were placed in rearing jars (20cm x 15 cm) in laboratory for conditioning at a temperature of 27± 2°C, 70-80% RH and a 16:10 LD photoperiod for seven days. Insecticides used in the studies were imidaclopyrid 17.5 SL, thiomethoxam 25 WG, deltamethrin 2.8 EC, alphamethrin 10 EC, cypermethrin 25EC, λ-cyhalothrin 5 EC, fenpropathrin 30 EC, monocrotophos 37SL, endosulfan 35 EC, quinalphos 25 EC, profenophos 50 EC and oxydemeton methyl 25 EC. Graded concentrations of insecticides were prepared in distilled water from commercial formulations of the insecticides. Toxicity assays were conducted as per the standard method Leaf Dipped Method recommended by FAO Method No. 10a (FAO, 1980). Healthy shoots of TV1 were collected from the experimental garden. The leaves were washed thoroughly with distilled water and air-dried. Fifteen tea shoots for each treatment were dipped up-to five seconds in the pesticides solutions to ensure complete welting and stem part of the sprayed shoot was inserted in a glass tube containing water and wrapped with cotton. The treated tea shoots were kept under ceiling fans for 15 minutes to evaporate the emulsion. This arrangement was caged in a glass chimney. The mouth of which was covered with muslin cloth. The whole set up was kept at 27 ± 2°C in culture room. Ten field-collected and preconditioned *H. theivora* were released separately into each glass chimney containing tea shoots. Observations of adult mortality were recorded in all the three replications of each concentration after 24 hours of the treatment. Moribund insects were counted as dead (Bora and Gurusubramanian, 2007). Five to seven concentrations of each insecticide were tested to obtain a concentration – probit mortality curve. The mortality data was converted to percent mortality and subjected to probit analysis (Finney, 1973; Busvine, 1971) to obtain LC_{50} values. Resistance factors (RFs) were determined based on LC_{50} values of an insecticides in reference to the lowest LC_{50} value of the same insecticides from a subdistrict. This method was adopted due to the unavailability of a suitable reference susceptible strain. normally used to calculate resistance factors (Chaturvedi, 2004).

The expected effective concentration of each insecticide was calculated by doubling the LC_{50} value to attain a LC_{100} value, and then effective field dosages of these insecticides were computed based on the following formula and compared with recommended dosages as per the standard method of Misra (1989).

$$\text{Expected effective concentration } (LC_{100})\ (\%) = 2 \times LC_{50}\%$$

$$\text{Expected effective dose (g a.i./ ha)} = ED /100 \times EC \times 20 \text{ fold}$$

$$ED = \%\ \text{concentration} / EC \times 1000 \times 400 \text{ liters of spray fluid/ha.}$$

Result and Discussion:

Resistance factors (RFs) were determined based on LC_{50} values relative to the corresponding lowest LC_{50} value of monocrotophos, oxydemeton methyl, thiomethoxam, imidaclopyrid, fenpropathrin and alphamethrin for the Damdim strain's and of endosulfan, deltamethrin, cypermethrin, λ-cyhalothrin, quinalphos, profenophos for the Chulsa strain, due to the unavailability of a suitable reference, a susceptible strain was normally used to calculate resistance factors.

Endosulfan:

The Kalchini population recorded the highest LC_{50} value to endosulfan (1580.77 ppm) followed by the population from Dalgong (952.715 ppm), Binnaguri (938.213 ppm), Nagrakata (884.95 ppm) and Damdim (544.722 ppm). The lowest LC_{50} value was observed in the population from Chulsa (269.744 ppm). While the Kalchini strain showed the highest resistance to endosulfan (5.86 folds), followed by Dalgong (3.53 folds), Binnaguri (3.47 folds), Nagrakata (3.28 folds) and the least resistance was observed in the population of Damdim (2.02-folds) (Table 1).

Table 1. Relative toxicity values of Endosulfan 35 EC against *Helopeltis theivora* in different tea growing Sub district in Dooars.

Location – Sub- district in Dooars	Endosulfan 35 EC					Resistance Factors (RF)
	χ^2	Regression equation	LC_{50} (ppm)	S.E.	Fiducial limits (95%)	
Damdim	2.16	y = 3.902 x - 17.384	544.72	0.011	607.753 480.324	2.02
Chulsa	4.95	y = 2.375 x - 7.899	269.744	0.010	328.181 221.712	1.00
Nagrakata	5.51	y = 4.270 x - 20.369	884.95	0.010	988.899 791.936	3.28
Binnaguri	2.38	y = 5.621 x - 28.568	938.213	0.006	1017.63 864.995	3.47
Dalgong	2.45	y = 3.501 x -15.934	952.715	0.012	1099.37 825.689	3.53
Kalchini	2.38	y = 4.428 x - 22.455	1580.77	0.009	1756.22 1422.48	5.86

- Susceptible Chulsa Population.

Monocrotophos:

The Dalgong population recorded the highest LC_{50} value to monocrotophos (18.046 ppm) followed by the population from Nagrakata (17.903 ppm), Kalchini (16.270 ppm), Chulsa (7.378 ppm), and Binnaguri (4.592 ppm). The lowest LC_{50} value was observed in the population from Damdim (3.025 ppm). The Dalgong strain showed the highest resistance to monocrotophos (5.97-folds) and the least resistance was observed in the population of Binnaguri (1.52-folds) (Table 2).

Table 2. Relative toxicity values of Monocrotophos 37SL against *Helopeltis theivora* in different tea growing Sub district in Dooars.

Location - Sub- district in Dooars	Monocrotophos 37SL					Resistance Factors (RF)
	χ^2	Regression equation	LC_{50} (ppm)	S.E.	Fiducial limits (95%)	
Damdim	4.97	y = 3.942 x - 8.722	3.025	0.008	3.382 2.706	1
Chulsa	4.26	y = 7.026 x - 22.179	7.378	0.006	7.895 6.895	2.44
Nagrakata	5.30	y = 4.230 x - 12.991	17.903	0.010	20.172 15.890	5.92
Binnaguri	1.31	y = 1.723 x - 1.309	4.592	0.025	6.101 3.457	1.52
Dalgong	0.49	y = 3.976 x - 11.926	18.046	0.011	20.421 15.946	5.97
Kalchini	2.99	y = 2.004 x - 3.441	16.270	0.017	20.045 12.056	5.38

- Susceptible Damdim Population.

Profenophos:

The Kalchini population recorded the highest LC_{50} value to profenophos 50 EC (29.713 ppm) followed by the population from Damdim (12.328 ppm), Dalgong (12.112ppm), Nagrakata (11.786 ppm) and Binnaguri (11.235 ppm). The lowest LC_{50} value was observed in the population from Chulsa (7.633 ppm). The Kalchini strain showed the highest resistance to profenophos (3.89 folds) and the least resistance was observed in the population of Binnaguri (1.47 folds) (Table 3).

Table 3. Relative toxicity values of Profenophos 50 EC against *Helopeltis theivora* in different tea growing Sub district in Dooars.

Location - Sub- district in Dooars	Profenophos 50 EC					Resistance Factors (RF)
	χ^2	Regression equation	LC_{50} (ppm)	S.E.	Fiducial limits (95%)	
Damdim	5.79	y = 3.973 x - 11.255	12.328	0.011	13.972 10.878	1.61
Chulsa	6.23	y = 4.007 x - 10.56	7.6336	0.011	8.654 6.733	1.00
Nagrakata	4.72	y = 5.359 x - 16.818	11.786	0.007	12.856 10.805	1.54
Binnaguri	0.69	y = 6.142 x - 19.88	11.235	0.008	12.215 10.332	1.47
Dalgong	5.68	y = 3.806 x - 10.541	12.112	0.011	13.760 10.661	1.59
Kalchini	1.37	y = 3.568 x - 10.963	29.713	0.012	33.967 25.991	3.89

- Susceptible Chulsa Population.

Quinalphos:

On the basis of LC_{50} values, the descending order of toxicity of quinalphos 25 EC was observed in six different subdistricts in the Dooars was Chulsa (6.560 ppm), Damdim (18.335 ppm), Dalgong (29.051 ppm), Nagrakata (38.836 ppm), Binnaguri (43.764 ppm) and Kalchini (214.471 ppm). The Kalchini strain showed the highest resistance to quinalphos (32.69-folds) followed by Binnaguri (6.67 folds), Nagrakata (5.92 folds), Dalgong (4.42 folds). The least resistance ratio was observed in the population of Damdim (2.79 folds) (Table 4).

Table 4. Relative toxicity values of Quinalphos 25 EC against *Helopeltis theivora* in different tea growing Sub district in Dooars.

Location - Sub- district in Dooars	Quinalphos 25 EC					Resistance Factors (RF)
	χ^2	Regression equation	LC_{50} (ppm)	S.E.	Fiducial limits (95%)	
Damdim	2.94	y = 3.048 x - 7.995	18.335	0.011	21.140 15.903	2.79
Chulsa	4.04	y = 2.267 x - 3.654	6.560	0.016	7.943 5.418	1.00
Nagrakata	4.41	y = 3.581 x - 11.435	38.836	0.010	44.005 34.274	5.92
Binnaguri	8.03	y = 3.595 x - 11.688	43.764	1.119	49.927 38.362	6.67
Dalgong	2.99	y = 2.927 x - 8.061	29.051	0.012	33.754 25.003	4.42
Kalchini	7.10	y = 2.564 x - 8.672	214.471	0.014	254.542 180.708	32.69

* Susceptible Chulsa Population.

Oxydemeton Methyl:

The population of *H.theivora* collected from Kalchini sub-district was comparatively less susceptible to oxydemeton methyl 25 EC which registered a LC_{50} value of 74.076, whereas the Damdim population showed a relatively higher degree of susceptibility to the same insecticides. Considering the LC_{50} values of oxydemeton methyl 25 EC, the order of susceptibility was as Damdim (18.309 ppm) > Chulsa (19.362 ppm) > Binnaguri (30.051 ppm) > Nagrakata (30.997 ppm) > Dalgong (58.346 ppm) > Kalchini (74.076 ppm). The resistance factor (RF) against susceptible strain was found to be highest for population of Kalchini (4.05 folds) followed by Dalgong (3.19 folds), Nagrakata (1.69 folds), Binnaguri (1.64 folds). The least resistance ratio was observed in the population of Chulsa (1.06 folds) (Table 5).

Table 5. Relative toxicity values of Oxydemeton Methyl 25 EC against *Helopeltis theivora* in different tea growing Sub district in Dooars.

Location - Sub- district in Dooars	Oxydemeton Methyl 25 EC					Resistance Factors (RF)
	χ^2	Regression equation	LC_{50} (ppm)	S.E.	Fiducial limits (95%)	
Damdim	2.27	y = 7.088 x - 25.216	18.309	0.006	19.571 17.128	1
Chulsa	3.42	y = 6.816 x - 24.219	19.362	0.006	20.735 18.081	1.06
Nagrakata	8.48	y = 2.122 x - 4.5324	30.997	0.015	37.973 25.303	1.69
Binnaguri	4.00	y = 2.831 x - 7.678	30.051	0.014	35.212 25.646	1.64
Dalgong	1.27	y = 3.083 x - 9.696	58.346	0.010	66.358 51.301	3.19
Kalchini	3.57	y = 3.968 x - 14.325	74.076	0.008	82.082 66.850	4.05

- Susceptible Damdim Population.

Imidaclopyrid:

The Kalchini population recorded the highest LC_{50} value to imidaclopyrid (19.907 ppm) followed by the population from Binnaguri (18.496 ppm), Nagrakata (15.052 ppm), Dalgong (15.173 ppm), and Chulsa (15.052 ppm) and the lowest LC_{50} value was observed in the population from Damdim (10.162 ppm). The values of relative resistance to imidaclopyrid when calculated taking Damdim populaton as base showed that the resistance factor (RF) to be highest in population of Kalchini (1.99 folds) followed by Binnaguri (1.82 folds), Nagrakata (1.52 folds), Dalgong (1.49 folds). The least resistance ratio was observed in the population of Chulsa (1.48 folds) (Table 6).

Table 6. Relative toxicity values of Imidaclopyrid 17.8 SL against *Helopeltis theivora* in different tea growing Sub district in Dooars.

Location - Sub- district in Dooars	Imidaclopyrid 17.8 SL					Resistance Factors (RF)
	χ^2	Regression equation	LC_{50} (ppm)	S.E.	Fiducial limits (95%)	
Damdim	3.75	y = 4.032 x - 11.155	10.162	0.008	11.313 9.1284	1.00
Chulsa	0.88	y = 5.180 x - 16.640	15.052	0.009	16.510 13.722	1.48
Nagrakata	6.65	y = 5.186 x - 16.737	15.537	0.007	16.998 14.202	1.52
Binnaguri	2.70	y = 7.378 x - 26.483	18.496	0.005	19.7394 17.3323	1.82
Dalgong	3.59	y = 2.955 x - 7.353	15.173	0.012	17.654 13.041	1.49
Kalchini	1.52	y = 3.641 x - 10.654	19.907	0.010	22.499 17.616	1.99

- Susceptible Damdim Population.

Thiomethoxam:

Populations from Kalchini, Nagrakata, Binnaguri and Dalgong showed more or less the same LC_{50} value against thiomethoxam that ranged from 5.346 to 5.761, but Damdim and Chulsa showed relatively lower LC_{50} (i.e. 4.599 ppm and 4.737 ppm respectively). The values of relative resistance when calculated taking the LC_{50} of thiomethoxam in Damdim as base ranged from 1.03 folds to 1.25 folds (Table 7).

Table 7. Relative toxicity values of Thiomethoxam 25 WG against *Helopeltis theivora* in different tea growing Sub district in Dooars.

Location - Sub- district in Dooars	Thiomethoxam 25 WG					Resistance Factors (RF)
	χ^2	Regression equation	LC_{50} (ppm)	S.E.	Fiducial limits (95%)	
Damdim	4.14	y = 2.248 x - 3.234	4.599	0.828	5.631 3.756	1.00
Chulsa	2.92	y = 2.087 x - 2.671	4.737	0.022	5.924 3.788	1.03
Nagrakata	1.50	y = 2.247 x - 3.449	5.761	0.016	6.996 4.744	1.25
Binnaguri	6.64	y = 1.883 x - 2.052	5.557	0.023	7.139 4.326	1.21
Dalgong	1.27	y = 1.941 x - 2.237	5.346	0.022	6.834 4.182	1.16
Kalchini	2.73	y = 2.032 x - 2.638	5.738	0.021	7.284 4.520	1.25

- Susceptible Damdim Population

Deltamethrin:

On the basis of the LC_{50} value (Table 8), the descending order of toxicity of deltamethrin 2.8 EC in six different subdistricts in the Dooars was Chulsa (0.131ppm), Damdim (0.289 ppm), Binnaguri (0.326 ppm), Dalgong (0.678 ppm), Nagrakata (0.691 ppm), and Kalchini (0.731 ppm). It was noticed that the relative resistance factor (RF) of deltamethrin against *H.theivora* from Kalchini, Nagrakata, Dalgong, Binnaguri and Damdim populations were found to be 5.58, 5.27, 5.17, 2.49 and 2.21 folds in respect to Chulsa population (Table 8).

Table 8. Relative toxicity values of Deltamethrin 2.8 EC against *Helopeltis theivora* in different tea growing Sub district in Dooars.

Location - Sub- district in Dooars	Deltamethrin 2.8 EC					Resistance Factors (RF)
	χ^2	Regression equation	LC_{50} (ppm)	S.E.	Fiducial limits (95%)	
Damdim	5.13	y = 2.555 x - 1.288	0.289	0.016	0.346 0.241	2.21
Chulsa	0.32	y = 2.240 x + 0.255	0.131	0.018	0.162 0.106	1
Nagrakata	4.75	y = 4.042 x - 6.476	0.691	0.010	0.775 0.616	5.27
Binnaguri	5.67	y = 1.774 x + 0.539	0.326	0.024	0.426 0.250	2.49
Dalgong	6.40	y = 4.324 x - 7.241	0.678	0.009	0.756 0.608	5.17
Kalchini	3.65	y = 5.509 x - 10.781	0.731	0.008	0.818 0.683	5.58

* Susceptible Chulsa Population.

Cypermethrin:

The Kalchini population recorded the highest LC_{50} value to cypermethrin (7.475 ppm) followed by the population from Nagrakata (3.813 ppm), Dalgong (3.026 ppm), Binnaguri (2.222 ppm), and Damdim (1.276 ppm) and the lowest LC_{50} value was observed in the population from Chulsa (0.802 ppm). Resistance to cypermethrin was very variable, ranging from 1.59-fold in the Damdim strain to 9.32-fold in the Kalchini (Table 9).

Table 9. Relative toxicity values of Cypermethrin 25 EC against *Helopeltis theivora* in different tea growing Sub district in Dooars.

Location - Sub- district in Dooars	Cypermethrin 25 EC					Resistance Factors (RF)
	χ^2	Regression equation	LC_{50} (ppm)	S.E.	Fiducial limits (95%)	
Damdim	2.59	y = 2.510 x - 2.796	1.276	0.212	1.581 1.029	1.59
Chulsa	6.32	y = 2.688 x - 2.691	0.802	0.016	0.966 0.666	1.00
Nagrakata	2.75	y = 1.971 x - 2.057	3.813	0.020	4.795 3.032	4.75
Binnaguri	4.35	y = 2.147 x - 2.186	2.222	0.017	2.739 1.805	2.77
Dalgong	7.77	y = 2.368 x + 3.245	3.026	0.018	3.714 2.466	3.77
Kalchini	6.57	y = 2.896 x - 6.218	7.475	0.014	8.756 6.381	9.32

* Susceptible Chulsa Population

Alphamethrin:

The Kalchini population recorded the highest LC_{50} value to alphamethrin 10 EC (1.532 ppm) followed by the population from Nagrakata (0.759 ppm), Binnaguri (0.593 ppm), Dalgong (0.435 ppm) and Chulsa (0.287 ppm). The lowest LC_{50} value was observed in population from Damdim (0.231 ppm). Resistance to alphamethrin ranged from 1.24 fold in the Chulsa strain to 6.63-fold in the Kalchini strain (Table 10).

Table 10. Relative toxicity values of Alphamethrin 10 EC against *Helopeltis theivora* in different tea growing Sub district in Dooars.

Location - Sub- district in Dooars	Alphamethrin 10 EC					Resistance Factors (RF)
	χ^2	Regression equation	LC_{50} (ppm)	S.E.	Fiducial limits (95%)	
Damdim	1.58	y = 5.558 x - 11.071	0.231	0.009	0.853 0.711	1.00
Chulsa	5.06	y = 3.391 x - 3.333	0.287	0.013	0.332 0.248	1.24
Nagrakata	0.73	y = 6.995 x - 15.151	0.759	0.006	0.814 0.708	3.29
Binnaguri	4.35	y = 2.893 x - 3.206	0.593	0.015	0.702 0.502	2.57
Dalgong	1.58	y = 3.423 x - 4.032	0.435	0.131	0.503 0.376	1.88
Kalchini	1.59	y = 4.748 x - 10.124	1.532	0.007	1.673 1.404	6.63

- Susceptible Damdim Population

Lamda cyhalothrin:

The Kalchini population recorded the highest LC_{50} value for lamda cyhalothrin (5.324 ppm) followed by the population from Nagrakata (3.175 ppm), Dalgong (2.405 ppm), Binnaguri (1.335 ppm), and Damdim (0.560 ppm). The lowest LC_{50} value was observed in the population from Chulsa (0.474 ppm). The Kalchini strain showed the highest resistance to λ- cyhalothrin (11.23-fold) followed by Nagrakata (6.70), Dalgong (5.07) and Binnaguri (2.82), The least resistance ratio was observed in the population of Damdim (1.18) (Table 11).

Table 11. Relative toxicity values of Lamda cyhalothrin 5 EC against *Helopeltis theivora* in different tea growing Sub district in Dooars.

Location - Sub- district in Dooars	Lamda cyhalothrin 5 EC					Resistance Factors (RF)
	χ^2	Regression equation	LC_{50} (ppm)	S.E.	Fiducial limits (95%)	
Damdim	4.45	y = 3.284 x - 4.025	0.560	0.012	0.646 0.487	1.18
Chulsa	0.93	y = 4.023 x - 5.765	0.474	0.010	0.532 0.422	1.00
Nagrakata	3.71	y = 2.038 x - 2.136	3.175	0.007	3.945 2.556	6.70
Binnaguri	2.01	y = 2.396 x -2.491	1.335	0.402	1.600 1.114	2.82
Dalgong	3.39	y = 2.129 x - 2.198	2.405	0.017	2.951 1.959	5.07
Kalchini	2.80	y = 2.441 x - 4.107	5.324	0.017	6.438 4.402	11.23

* Susceptible Chulsa Population

Fenpropathrin:

Populations from Chulsa, Nagrakata and Binnaguri showed more or less same LC_{50} value against fenpropathrin that ranged from 0.040 to 0.048 ppm, but Kalchini and Dalgong showed relatively higher LC_{50} (i.e. 0.064 ppm and 0.058 ppm respectively). The lowest LC_{50} value was observed in Damdim (i.e. 0.033 ppm). The values of relative resistance when calculated taking LC_{50} of fenpropathrin in Damdim as base showed that the resistance factor (RF) against susceptible ranged from 1.23 fold to 1.98 fold (Table 12).

Table 12. Relative toxicity values of Fenpropathrin 30 EC against *Helopeltis theivora* in different tea growing Sub district in Dooars.

Location - Sub- district in Dooars	Fenpropathrin 30 EC					Resistance Factors (RF)
	χ^2	Regression equation	LC_{50} (ppm)	S.E.	Fiducial limits (95%)	
Damdim	2.70	y = 1.007 x + 3.473	0.0328	0.040	0.051 0.020	1.00
Chulsa	1.105	y = 0.976 x + 3.382	0.0454	0.043	0.074 0.028	1.38
Nagrakata	1.947	y = 0.827 x + 3.606	0.0485	0.317	0.085 0.028	1.48
Binnaguri	4.19	y = 0.842 x + 3.647	0.0404	0.044	0.069 0.024	1.23
Dalgong	2.02	y = 1.044 x + 3.155	0.0582	0.067	0.093 0.036	1.77
Kalchini	7.53	y =0.796 x +3.557	0.0648	0.045	0.112 0.037	1.98

- Susceptible Damdim Population

The data on the dosage-mortality response of *H. theivora* collected from different subdistricts in the Dooars revealed that chi-square values indicated a good fit of probit responses in all the bioassays showing that there was no heterogeneity between the observed and expected responses.

The present study suggested that the LC_{50} value of *H.theivora* at Chulsa and Damdim (low pesticide applied area) were low as compared to other locations. Kalchini subdistrict (high pesticide applied area) registered the highest LC_{50} value for almost all tested insecticides except monocrotophos and thiomethoxam. This differential response to the insecticides in the populations of the Dooars could be due to indiscriminate use of pesticides. It is clear from the table that susceptibility was comparatively low in all subdistricts that used endosulfan.

Generally, it is accepted that field application rates of insecticides should at least be 20 fold or more of the LC_{50} value (determined through bioassay methods) to achieve satisfactory control of the pest in agriculture (Misra, 1989). Following this simple logic, the expected effective dosages of various insecticides were worked out in subdistrict wise and are presented in Table 13. Among the chosen insecticides the comparison of expected effective dosages of seven insecticides (endosulfan, Oxydemeton methyl, λ-cyhalothrin, quinalphos, imidaclopyrid, thiomethoxam and deltamethrin) based on their LC_{50} values with the recommended dosages revealed a pronounced shift in the level of susceptibility of *H. theivora* in all different subdistricts in the Dooars. In case of endosulfan when the computed expected dosages was compared with the recommended dosages of the insecticide, it was observed that 11.05 – 72.27 times more of the recommended dosage of endosulfan might be required to achieve desirable control of the pest. The change in susceptibility of *H. theivora* against deltamethrin in the order of 4.02, 8.75, 9.99, 20.75, 21.15 and 22.40 fold for Chulsa, Damdim, Binnaguri, Dalgong, Nagrakata, and Kalchini subdistrict populations respectively; similarly against imidaclopyrid the resistance levels were recorded as 13.61, 20.16, 20.32, 20.81, 24.77 and 26.66 fold for Damdim, Chulsa, and Dalgong, Nagrakata, Binnaguri and Kalchini regions respectively. In case of oxydemeton methyl, λ-cyhalothrin, quinalphos, thiomethoxam when compared with the recommended dosages, a 1.17 to 4.74, 1.06 to 11.93, 1.17 to 13.73 and 1.47 to 1.84 fold decrease in the susceptibility were evident. The usual recommended dose of synthetic pyrethroids (deltamethrin and λ-cyhalothrin), neonicotinoids (Imidaclopyrid, thiomethoxam), organophosphates (quinalphos) and organochlorines (endosulfan), however, was practically ineffective against this pest.

However, there was no major development of resistance in the case of monocrotophos (0.13 to 0.78 fold) and fenpropathrin (0.010 to 0.012 fold), which therefore held promise to being effective even at a lower dose than the recommended dose. In some subdistricts (Chulsa, Damdim, Nagrakata, Dalgong, Binnaguri) profenophos, cypermethrin and alphamethrin proved effective even at a lower dose than the recommended dose, but in Kalchini sub-district, these insecticides required 1.22 to 2.39 times more of than the recommended dosage for effective control of the pest (Table 13).

Table 13. Comparison of effective field dosage with recommended dosage of different insecticides against *Helopeltis theivora* in different tea growing sub districts in Dooars.

Insecticide	Recom-mended dose (g a.i/ha)	Location					
		Nagrakata	Binnaguri	Damdim	Chalsa	Kalchini	Dalgong
		Expected effective dose (g a.i/ha) Recommended dose					
Endosulfan 35 EC	350.00	40.45	42.89	24.90	12.33	72.26	43.55
Monocrotophos 37SL	370.00	0.77	0.20	0.13	0.32	0.70	0.78
Quinalphos 25 EC	250.00	2.49	2.80	1.17	0.42	13.73	1.86
Profenophos 50 EC	200.00	0.94	0.90	0.99	0.61	2.38	0.97
Oxydemeton Methyl 25 EC	250.00	1.98	1.92	1.17	1.24	4.74	3.73
Thiomethoxam 25 WG	50.00	1.84	1.78	1.47	1.52	1.84	1.71
Imidaclopyrid 17.8 SL	23.49	20.81	24.77	13.61	20.16	26.66	20.32
Deltamethrin 2.8 EC	5.60	21.15	9.99	8.57	4.02	22.40	20.75
Fenpropathrin 30 EC	75.00	0.01	0.01	0.01	0.01	0.01	0.012
Alphamethrin 10EC	20.00	0.61	0.47	0.19	0.23	1.23	0.35
Cypermethrin 25 EC	50.00	1.22	0.71	0.41	0.42	2.39	0.97
λ-cyhalothrin 5EC	10.00	7.11	2.99	1.25	1.06	11.93	5.39

A comparison of LC_{50} values of most insecticides used against the *H. theivora* population of the Dooars with other tea growing parts of the North East India

(Darjeeling and Assam) revealed that the insecticides were 2 to 350 times less toxic to the Dooars population than other regions (Bora *et al.,* 2007 and Bora and Gurusubramanian, 2007). *H. theivora* showed the lowest susceptibility to endosulfan in all tea growing subdistrict of the Dooars with high LC_{50} value ranging from 269.744 ppm to 1580.77 ppm. The poor performance of endosulfan has also been reported by Roy *et al.* (2008), and on other insect pests by Singh and Deol (1998), Peter and Sundararajan (1990), Kalra *et al.,* (1997) and Singh *et al.* (2005), who observed the falling efficacy of this insecticide against the larvae of *Mythimna separata, Heliothis armigera*, *Plutella xylostella* and *Henosepilachna vigintioctopunctata* respectively. The present study suggests that the usual recommended doses of organochlorines (endosulfan), synthetic pyrethroids (deltamethrin and λ-cyhalothrin), neonicotinoids (imidaclopyrid) and organophosphates (quinalphos and oxydemeton methyl) were practically ineffective against *H. theivora* population of the Dooars. The change towards less susceptibility of *H. theivora* against endosulfan was found to be remarkable. Similar findings were reported against *H. theivora* population from Jorhat tea plantations of South Assam, India, where 1.54 – 82.85 fold increases in resistance caused control problems on tea (Bora and Gurusubramanian, 2007 and Bora *et al.,* 2008). According to Borbora and Biswas (1996), Sannigrahi and Talukdar (2003) and Roy *et al.* (2008), organochlorines (endosulfan), synthetic pyrethroids (deltamethrin) and organophosphates (quinalphos) were extensively used for tea pest management in Dooars for a long period of time whereas molecules like imidaclopyrid and λ-cyhalothrin were introduce in tea very recently. Such high levels of resistance to these compounds may be mediated through different mechanisms. Mechanisms of pyrethroid resistance in pests include reduced penetration (Armes *et al.,* 1992; Kranthi *et al*., 2000 and 2001), decreased nerve sensitivity and enhanced metabolism (Ahmad and McCaffery, 1991). The absence of a common resistance mechanism that could confer cross-resistance between these compounds suggests that the use of the compounds in rotations or sequences for resistance management should be explored. However, there was no change in the case of monocrotophos, profenophos and fenpropathrin that may prove effective even at a lower dose than the recommended dose.

However, there is a great deal of variation in insecticide resistance from location to location within the Dooars tea ecosystems. The variations in insecticide susceptibility status from different geographical populations of pest were also reported by Kranth *et al.,* (2000), Chaturvedi (2004) and Fakrudin *et al.,* (2004) with Cotton Bollworm, *H. armigera* from Central and South Indian Cotton Ecosystem and Zhou *et al.,* (2000) from Northern China with same pest.

The resistance levels in Kalchini, Dalgong, Binnaguri and Nagrakata region is high due to heavy dependence on insecticides. The synthetic pyrethroids are being used widely in tea plantations, and their consumption is about 3-5 liters/ha in North East India (Gurusubramanian *et al.,* 2005), and a recent survey by Roy *et al.* (2008) revealed that on average 7.499 l/kg of insecticides were used per hectare per year in Dooars of which the organochlorine, organophosphate and carbamate (nonpyrethroid) accounted 73.5% and pyrethroid represent 36.6% during 1998 to 2004. Endosulfan, monocrotophos, deltamethrin and cypermethrin were extensively used in all the regions of the Dooars and it was noted that the requirement of synthetic pyrethorid gradually increased with every passing year in all subdistricts. The reasons are i) per hectare requirement was less (100 ml/ha), ii) having knockdown effect and iii) cost effectiveness. Against the tea mosquito bug, planters using insecticides as prophylactic, due to it being a wet season pest and their peak season (May-July) coinciding with the rainy season (June-July), caused the consumption of pesticides to increase, with about 8-16 applications per year of synthetic pyrethroids on top of other chemical applications.

This clearly explains that resistance levels were proportionate with the usage of pesticides. The study conducted by Forrester (1990) also clearly revealed that resistance levels rose when pyrethroids were used but fell significantly when they were withheld. Thus the pesticides were creating very high selection pressure for resistant genotypes. This suggests that indiscriminate use and heavy dependence on pesticides will further complicate the already worsened situation and this hints at aiming for insecticide resistance management strategies.

Thus, *H. theivora* insecticide-resistance issues in the Dooars are becoming ever more acute. Pyrethroid resistance is widespread in populations in almost all geographic populations. It is likely that few refugia of susceptible populations remain to dilute the build-up of resistant populations. Resistant management strategies appropriate for the region should be implemented immediately. These should include greater control over insecticide application and use. Unless this happens, the areas affected by resistant *H. theivora* populations will continue to increase and could ultimately result in the abandonment of tea growing in large areas of North east India. By some strategy, the allelic frequencies for major insecticide resistance genes need to be diluted.

Since the shift in level of susceptibility to insecticides was noticed in *H. theivora* certain measures can be initiated for combating and delaying the problem of resistance so that it does not assume unmanageable proportions. The measures may be: a) restricted use of deltamethrin, imidaclopyrid, quinalphos and endosulfan since their effective dose was higher than the recommended dose, in *H. theivora* prone areas, and reviewing of the recommended dose b) Judicious use of these insecticides only if there use is essential, d) no prophylactic spraying of chemicals, e) timing and frequency of applications should be such which does not create selection pressure, and f) altering of the insecticides in such a way that their modes of action are different. Further in-depth studies are needed to explore the possibility of determining the resistance level by using resistance enzyme studies and biotypes identification through molecular techniques besides the log dose probit assays.

REFERENCE

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

Ahmad, M. and McCaffery, A.R., 1991. Elucidation of detoxication mechanisms involved in resistance to insecticides in the third instar larvae of a field selected strain of *Helicoverpa armigera* with the use of synergists. *Pesticide Biochemistry and Physiology*, **41**: 41-52.

Ahmed, K., Rao, V.H. and Rao, P.P., 2001. Promising new insecticides as potential ovicides against eggs of *Spodoptera litura* Fab. *Pestology*, **25** (4): 20-21.

All, J., Lance, K. and Lohmeyer, K., 2001. Ovicidal properties of Leverage for bollworm in cotton, pp. 802-803. *In Proceedings of the Beltwide Cotton Conference*. 9-13 January 2001, Anaheim, CA. Natl. Cotton Counc. Am., Memphis, TN.

Anonymous., 1978. Management of *Helopeltis theivora*. *Ann. Sci. Rep*., Tocklai Experimental Station, TRA, Jorhat, **78**: 50.

Armes, N.J., Jadhav, D.R., Bond, G.R. and King, A.B.S., 1992. Insecticides resistance in *Helicoverpa armigera* in South India. *Pesticide Science*, **34**: 335-364.

Barbora, B.C. and Biswas, A.K., 1996. Use pattern of pesticides in Tea Estates of North East India. *Two and a bud*, **47** (2): 19-21.

Bora, S. and Gurusubramanian, G., 2007. Relative toxicity of some commonly used insecticides against adults of *Helopeltis theivora* Waterhouse (Miridae: Hemiptrra) collected from Jorhat area tea Plantations, South Assam, India. *Resistance Pest Management Newsletter*, 17(1): 8- 12.

Bora, S., Borah, T., Rahman, A., Sarmah, M. and Gurusubramanian, G., 2008. Susceptibility change in male *Helopeltis theivora* Waterhouse (Jorhat Population) to different classes of insecticides. *Pesticide Research Journal*, (*in press).*

Bora, S., M. Sarmah, A.Rahaman and Gurusubramanian, G., 2007a. Relative toxicity of pyrethroid and non-pyrethroid insecticides against male and female tea mosquito bug, *Helopeltis theivora* Waterhouse (Darjeeling strain). *J. Ent. Res.,* 31(1): 37-41.

Busvine, J.R., (1971). *A critical review of the techniques for testing insecticides*, C.A.B. Publication, London, pp. 271-276.

Chaturvedi, I., 2004. A Survey of Insecticide Resistance in *Helicoverpa armigera* in Central and South Indian Cotton Ecosystems, 1999 to 2003. *Resistant Pest Management Newsletter,* 14(1):23- 26.

Elzen, G.W., 1997. Changes in resistance to insecticides in tobacco budworm populations in Mississippi, 1993-1995. *Southwestern Entomologist,* **22**: 61-72.

Fakrudin, B., Vijaykumar., Krishnareddy, K. B., Patil, B. V. and Kuruvinashetty, M.S., 2004. Status of Insecticide Resistance in Geographical Populations of Cotton Bollworm, *Helicoverpa armigera* in South Indian Cotton Ecosystem During 2002-03. *Resistant Pest Management Newsletter*, **13**(2): 12-15.

Finney, D.J., 1971. *Probit Analysis*, Cambridge University Press, Cambridge, pp. 333.

Forrester, N.W., 1990. Designing, implementing and servicing an insecticide Resistance Management Strategy. *Pesticide Science,* **28**: 167-179.

Gope, B. and Handique, R., 1991 Bio-Ecological Studies on the Tea Mosquito Bug, *Helopeltis theivora waterhouse* in North-East India. *Two and a Bud,* **38 (1&2)**: 21-28.

Gurusubramanian, G., Borthakur, M., Sarmah, M. and Rahman, A., 2005. Pesticide selection, precautions, regulatory measures and usage. In Dutta, A., Gurusubramanian, G. and Barthakur, B.K. (Eds.), Plant Protection in tea: Proc. Plant Protection Work shop. Tocklai Experimental Station, TRA, Jorhat Assam Printing Works Private Limited, Jorhat, Assam, India: 81-91.

Horowitz, A. R., Mendelson, Z., Weintraub, P. G. and Ishaaya, I., 1998. Comparative toxicity of foliar and systemic application of acetamiprid and imidaclopyrid against the cotton whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Bull. Ent. Res*. **88**: 437-442.

Kalra, V.K., Sharma, R.C. and Bhanot, J.P., 1997. Shift in the level of resistance together with relative toxicity of some commonly used and important insecticides to diamond black moth, *Plutella xylostella* (L.) in Haryana (India). *J.ent. Res.,* **21**(4): .351-354.

Khodzhaev, S.H.T. and Eshmatov, O. T., 1983. The ovicidal action of pyretheoids. *Zashchita Rastenii*, **7**: 24-25.

Kranthi, K. R., Jadhav, D., Wanjari, R., Kranthi, S. and Russell, D., 2001. Pyrethroid resistance and mechanism of resistance in field strains of *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Journal of Economic Entomology*, **94**: 254-263.

Kranthi, K.R. Shakir Ali, S. and Banerjee, S.K., 2000. Baseline toxicit of spinosad on the cotton bollworm, *Helicoverpa armigera* (Hub.), in India. . *Resistant Pest Management Newsletter* **11**(1): 9-12.

Misra, R.K., 1989. Toxicity of various insecticides against *Heliothis armigera* Hub. Guntur strain. *Pesticide Research Journal*, **1**: 105-109.

Parrish, M. D., Ayad, H. and Holmes, K., 2001. Ovicidal activity of acetamiprid (Assail TM brand 70WP insecticide) on economic pests of cotton. pp. 904-906. . *In Proceedings of the Beltwide Cotton Conference* 9-13 January 2001, Anaheim, CA. Natl. Cotton Counc. Am., Memphis, TN.

Peter, C. and Sundararajan, R., 1990. Evaluation of toxicity of insecticides to the larvae of *Heliothis armigera* (Hubner) by topical application method. *J. Insect. Sci.*, **3**(2): 202-203.

Rahaman, A., M. Sarmah., Phukan, A. K. and Gurusubramanian, G., 2007. Varietal preference and Chemical control Strategies for management of *Helopeltis theivora* Waterhouse. *Uttar Pradesh J. Zool.,* **27 (1)**: 1-11

Rahman, A., Sarmah, M., Phukan, A.K., Roy, S., Sannigrahi, S., Borthakur, M. and Gurusubramanian, G., 2006. Approaches for the management of tea mosquito bug, *Helopeltis theivora* waterhouse (Miridae: Heteroptera). *In Strategies for Quality: Proc. 34th Tocklai Conference*. Tocklai Experimental Station, Tea Research association, Jorhat, Assam, India, pp. 146-161.

Rao, N. H. P., Rao, V. H. and Gopalaswamy, S. V. S., 2001. Utility of insecticides as ovicides against *Helicoverpa armigera* (Hub.) on cotton in Andhra pradesh. *Pestology*, **25 (2)**: 7-11.

Roy, S., Mukhopadhyay, A. and Gurusubramanian, G., 2008a. Use Pattern of Insecticides in Tea Estates of the Dooars in North Bengal, India. *NBU J. Anim. Sc.,* **2** (1): 35-40.

Roy, S., Mukhopadhyay, A. and Gurusubramanian, G., 2008b. Insecticide persistence and residual toxicity monitoring in tea mosquito bug, *Helopeltis theivora* Waterhouse (Heteroptera: Hemiptera: Miridae) in Dooars, West Bengal. Resistance Pest Management Newsletter, (in press).

Sannigrahi, S. and Talukdar, T., 2003. Pesticide use patterns in Dooars tea industry. *Two and a Bud*, 50: 35-38.

Sarker, M. and Mukhopadhyay, A., 2003. Expression of esterases in different tissues of the tea pest, *Helopeltis theivora* exposed and unexposed to synthetic pesticide sprays from Darjeeling foothills and plains. *Two and a Bud*, **50:** 28-30.

Sarker, M. and Mukhopadhyay, A., 2006. Studies on salivary and midgut enzymes of a major sucking pest of tea, *Helopeltis theivora* (Heteroptera : Miridae) from Darjeeling plains, India. *Journal of Entomological Research Society*, **8** (1): 27-36.

Sarker, M. and Mukhopadhyay, A., 2006a. Studies on some enzymes related to Insecticide resistance in Helopeltis theivora Waterhouse (Insecta : Heteroptera : Miridae) from Darjeeling foothills and plains. *Journal of Plantation Crops*, **34 (3)**: 423-428

Sarmah, M., Rahman, A., Phukan, A.K. and Gurusubramanian, G., 2006. Bioefficacy of insecticides in combination with acaricides and nutrients against *Helopeltis theivora* Waterhouse in tea. *Pesticide Research Journal*, **18 (2)**: 141-145.

Sing, S., Kumar, A. and Pandey, N.D. 2005. Relative toxicity of some used insecticides against Hadda beetle in brinjal. *Indian J. of Entomology*, **67**(1): 22-23.

Singh, D. and Deol, G.S. 1988. Efficacy of different insecticides for the control of armyworm, *Mythimna separata* (Walker) under field and laboratory conditions. *Pesticide*, **22** (2): 19-21.

Vastrad, A.S., Lingappa, S. and Basavangoud, K. 2004 Ovicides for Managing Resistant Populations of Diamondback Moth, *Plutella xylostella* (L.) *Resistant Pest Management Newsletter* **14**(1): 15-16.

Vekaria, M.V. and Vyas, H.N., 1985. Studies on ovicidal toxicity of certain insecticides against the eggs of *Heliothis armigera* (Hübner). *Pesticides,* **19**(10): 43-45.

Zhou J.Z. Rui, C., Lu, M., Fan, X. Ru., L. and Meng, X., 2000 Monitoring and Management of *Helicoverpa armigera* Resistance to Transgenic Bt Cotton in Norntern China. *Resistant Pest Management Newsletter* **11**(1): 28-31.

Somnath Roy[1], Ananda Mukhopadhyay [1] and G.Gurusubramanian[2]

[1]Entomology Research Unit, Department of Zoology, University of North Bengal,
Darjeeling-734 430, West Bengal, India.
E-mail: entosomnath@yahoo.co.in; dr_amukherjee_nbu@rediffmail.com; Fax No.0353-2581546

[2]Department of Zoology, Mizoram Central University, Tanhril, Aizawl -796 009, Mizoram, India.
E-mail: gurus64@yahoo.com; Fax No.0389-2330642/644

Detection of Insecticide Resistance in *Helicoverpa armigera* (Hubner) larvae collected from two locations in Madhya Pradesh, India

Abstract
The levels of resistance developed in a field stain of *Helicoverpa armigera* (Hubner) against five insecticides was assessed in two locations in Madhya Pradesh in laboratory conditions using a discriminating dose assay. The larvae of the test insect exhibited a wide spread resistance from 100 percent in cypermethrin and fenvalerate at the Khandwa location to above 50 percent at the Chhindwara location. High resistance of 46 percent to quinalphos was found in the field strains of Khandwa as compared to the Chhindwara strains (12.51%). The level of resistance against methomyl and Endosulfan was found to be lowest. Development of insecticide resistance in *H. armigera* is an important feature among the most commonly used group of insecticides.
Key words: Discriminating dose assay, *Helicoverpa armigera*, insecticides resistance

Introduction

Over the past one and a half decades, insecticide resistance in cotton pests has emerged as a key area of concern in cotton pest management in India. Most of the pest management difficulties in recent times, especially in the year of the bollworm outbreak, have been traced to insecticide resistance. The problem of resistance has rendered insecticides a less useful and reliable tool. If cotton pest management is to be effective, it is necessary to address the problem of resistance to insecticides and devise appropriate proactive management strategies to ensure that it does not continue to impair pest management in the field.

Helicoverpa armigera Hubner (Lepidoptera; Noctuidae), generally known as cotton bollworm or American bollworm, is the most dreaded pest of many agricultural crops worldwide. It has been recorded on more than 200 hosts in India (Pawar, 1998). It causes 24 to 68 percent losses of seed cotton yield at a national level (Vadodaria *et al* 1998). Cotton occupies only 5 percent of the total cultivable area in India but consumes more than 55 percent of the total insecticide used in the country (Puri, 1995). Dhingra *et al.* (1988) and Mc Caffery *et al.* (1989) first reported on the development of resistance by *H. armigera* to pyrethroids and attributed field control failures to the resistance. Subsequently, high levels of pyrethroid resistance were reported in several cotton and pulse growing regions of the country (Mehrotra and Phokela, 1992). Kranthi *et al* (2001) and Choudhary *et al.* (2004) also reported increased resistance of *H. armigera* to conventional insecticides such as methomyl, endosulfan and quinalphos in India.

Materials and methods

The experiment was carried out in a laboratory at the JNKVV Research Stations at Khandwa and Chhindwara during the 2003-04 crop season. The stock solutions of technical grade insectide (namely cypermethrin, fenvalerate, endosulfan, quinalphos and methomyl) were obtained from CICR, Nagpur. Eggs of *Helicoverpa armigera* were collected on a weekly basis from cotton fields. A discriminate dose assay was performed on the larvae at the third instar stage with 30-40 mg of body weight. The discriminate dose used was cypermethrin 0.1 µg, fenvalarate 0.2 µg, endosulfan 10 µg, quinalphos 0.75 µg and methomyl 1.2 µg/larva for the monitoring of resistance. An insecticide dose of 1µl was applied on thorasic dorsam of the third instar larvae by a Hamilton hand micro-applicator (Armes *et al*; 1996). Larvae treated with acetone only were used as a control. Larvae were held individually in 12-well tissue culture plates containing

a chickpea based artificial diet, at 25±2°C. Larval mortality was observed after every 24 hours up to 6 days (144 hours).

Results and discussion

Studies conducted in the present investigation in two locations of Madhya Pradesh indicated that larval survival (%) of Helicoverpa armigera treated with cypermethrin 0.1µg and fenvalerate 0.2µg exhibited a range from 44.50 to 65.22 percent with a high mean value of 56 percent, and from 44.45 to 63.34 percent with a mean value of 53.63 percent in the field strains of *H. armigera* at Khandwa, whereas, the survivability ranged between 44.50 to 65.22 percent in cypermethrin and 44.45 to 63.34 percent with a mean value of 53.63 percent at the Chhindwara location. However, 100 percent survival was noted for both treatments at the Khandwa location. Survival frequencies to discriminating doses of cypermethrin and fenvalerate were reported to be generally above 75 percent in the country. High levels of resistance were found in the population of *Helicoverpa armigera* larvae against cypermethrin and fenvalerate, a pyrethroid group of insecticides, which may be due to the extremely high use of these chemicals in the area over the years. A similar trend was also observed in field population of *H. armigera* against pyrethroids (Kranthi *et al*., 2002).

In endosufan, the 10.0µg treated larval population exhibited a moderately high mean value (36.95%) of survival in the Khandwa location which ranged from 28.03 to 45.87 percent. The larval survival in the population tested at the Chhindwara location expressed a range from 0.0 to 14.29 percent with a moderately low mean value of 6.3 percent. The survival frequencies were observed to be slightly low in the beginning of the season (i.e. from September to mid October). Armes *et al*. (1996) and McCaffery *et al.* (1989) reported 28 and 13 fold Endosulfan resistance in *H. armigera* strains from Andhra Pradesh. They observed that even the low level (5 to 10 fold) resistance to endosufan was sufficient to render the chemical ineffective for *H. armigera* control under field conditions. Hanumanth rao *et al*. (2000) identified an erratic pattern in the level of resistance to endosulfan until the middle of December, which then increased up to 77.8 percent by the end of January.

Survivality of larval population in quinalphos 0.75µg treatment varied from 35.50 to 56.50 percent in Khandwa and from 0.0 to 29.50 percent in Chhindwara locations during the cropping season with 46.0 percent and 12.51 percent as their respective mean values. Slightly low larval survival (%) was recorded in the beginning which gradually increased during the season. Armes *et al*. (1996) found 59 fold resistance to quinalphos in the field strain of *H. armigera* in India, with the highest level (10-15 fold) in strains collected from the south India. Kranthi *et al*. (2002) reported 30-35 percent survival (range, 16-46%) at the end of November during 1994-95 and 1995-96 cropping season and an average survival of 44 percent during November, 1997-98. In the 1998-99 season, quinalphos resistance in *H. armigera* gradually increased from the beginning of November reaching 83.9 percent by the 1st week of February (Hanumanth Rao *et al*., 2000).

The survival values for *H. armigera* larvae in the methomyl 1.2µg treated population were found to be consistent over the season at Khandwa location with a mean value of 35.48 percent, which ranged from 26.97 to 44.0 percent. The survival range was observed to be 0.0 to 30.0 percent at the Chhindwara location with a mean value of 16.33 percent. Armes *et al*., (1996) reported a 30 fold increase in resistance to methomyl in field strains of *Helicoverpa armigera*. Kranthi *et al*. (2000) found 31-40 percent resistance during September, October and December to 1.2µg/µl of methomyl.

Thus the development of resistance in H. armigera larvae against the insecticides under studies are in the order of: cypermethrin > fenvalerate > quinalphos > Endosulfan > methomyl.

The level of resistance against cypermethrin and fenvalerate, a pyrethroids group of insecticides, was found to be highest against metomyl and endosulfan; it was the lowest in *H. armigera* larvae collected from two location of Madhya Pradesh.

Acknowledgement:

The authors gratefully acknowledge Dr. K.R. Kranthi Central Institute for Cotton Research, Nagpur and Ministry of Agriculture, India for financial support for this work.

References:

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

Armes, N. J., Jadhavm D. R., and De Souza, K. R; 1996. A survey of insecticide resistance in *Helicoverpa armigera* in the Indian sub-continent. *Bull. entl. Res*. 86: 499-514.

Choudhary, R.K., Yadav, A.S., Shrivastava, V.K., Tomar, S.P.S. and Patel, Yogesh 2004. Studies on insecticide resistance in *Helicoverpa armigera* in Madhya Pradesh. Proceeding International Symposium on strategies for sustainable cotton production- A Global vision Crop protection 3:210-212.

Dhingra, S., Phokela, A. and Mehrotra, K.N. 1988. Cypermethrin resistance in the populations *Heliothis armigera*. National Academy of Science, India. *Science letter*11:123-25.

Hanumanth Rao, V., Rao, NHP; Nagesh, M. and Rao C. Raghundha 2000. Insecticide resistance frequencies in *Helicoverpa armigera* (Hubner) population on cotton. *Pestology* 7:21.24.

Kranthi, K. R., Banarjee, S. K. and Russell, D. 2000. IRM strategies for sustainable cotton pest management in India. *Pestology*. 14(12): 58-67.

Kranthi, K. R., Jadhav, D.R., Wangari, R.R., Kranthi, S., and Russel, D2001. Pyrethroid resistance and mechanisms in field strain of *Helicoverpa armigera* Hubner(Lepidoptera: Noctuidae) in India, *J. econ. Ent*. 94:253-63.

Kranthi, K. R., Jadhav, D.R., Wangari, R.R., Sakir Ali, S. and Russel, D. 2001. Carbamate and organophosphate resistance in

cotton pests in India.1995-1999.*Bull. ent. Res*.91:37-46.

Kranthi, K. R., Russel, D., Wangari, R., Kherde, M., Munje, S. K., Lavhe, N. K., and Armes, N. 2002. In Season Changes in Resistance to Insecticides in *Helicoverpa armigera* (Lepidoptera: Noctuidae) in India, *J. econ. Ent*. 95 (1): 134-42.

Mc Caffery. A. R., King. A. B. S., Walker, A. J. and Nayar, K. L. 1989. Resistance to synthetic pyrethroid in the bollworm, *Helicoverpa armigera* from Andhra Pradesh, India. *Pestic. Sci*. 27: 65-76.

Mehrotra, K.N. and Phokela, A.1992. Pyrethroid resistance in *Helicoverpa armigera* (Hubner). V. Response of populations in Punjab cotton. *Pestic. Res. J*. 4:59-61.

Pawar, C. S., 1998. *Helicoverpa armigera* a natural problem which needs a national policy and commitment for its management. *Pestlogy* 22(7):51-59.

Puri, S. N. 1995. Present status of IPM in India. National Seminar on Integrated Pest Management in Agriculture. December 29-30, 1995. Nagpur, Maharashtra.

Roush, T.R; Miller, G.L., 1986. Consideration for design of insecticide resistance monitoring programme. J. Econ.. Ent.

Vadodaria, M.P., Patel, R.B., Maisuria, I.M., Patel, U.G. and Patel R.H. 1998. Bioefficacy of Dipel EC as biopesticides for the management of bollworms of cotton. *Pestology* 23(12):13-15.

Table-1: Insecticide resistance in terms of percent larval survival of *H. armigera* observed weekly over different chemical insecticides at two location of Madhya Pradesh, India

S. No.	Insecticides	Discriminating Dose (µg/µl larva)	Range (% larval survival)		Mean (% larval survival)	
			Khandwa	Chhindwara	Khandwa	Chhindwara
1	Cypermethrin	0.1	100-100	44.50-65.22	100	56.0
2	Fenvalerate	0.2	100-100	44.45-63.34	100	53.63
3	Endosulfan	10.0	28.03-45.87	0.00-14.29	36.95	6.34
4	Quinalphos	0.75	35.50-56.50	0.00-29.50	46.0	12.51
5	Methomyl	1.2	26.97-44.0	0.00-30.0	35.48	16.33
6	Control	1.0	94.32-100	94.45-100	97.16	94.80

V.K. Shrivastavsa[1], A.S. Yadav[2], R.K. Choudhary[3], S.P.S. Tomar[4] and S.K. Parsai[5]

J.N. Krishi Vishwa Vidyalaya, ReGION Research Station, B.M. College of Agriculture, Khandwa (M.P.)- 460001 India

[1] Head Dept. of Entomology College of Agriculture, Gwalior (M.P.) India

[2] Corresponding Author: Krishi Vigyan Kendra, Harda (M.P.)-461331, India

Email- asyadav_75@ rediffmail.com

[3] College of Agriculture, Indore (M.P.) India

[4] Krishi Vigyan Kendra, Chhindwara (M.P.) India

[5] B.M. College of Agriculture, Khandwa (M.P.) India

Research in Resistance Management

Insecticidal activity of native *Bacillus thuringiensis* isolates against *Spodoptera litura* and *Helicoverpa armigera*

Abstract

Bacillus thuringiensis, a gram-positive bacterium, is most widely used in microbial control of insect pests on agricultural crops. The crystal protein produced by this bacterium is toxic to different orders of insects, such as lepidoptera, coleoptera and diptera. Experiments were carried out to test the efficacy of native *Bacillus thuringiensis* isolates, isolated from various ecological zones against, *Spodoptera litura* and *Helicoverpa armigera*. Native isolates P-1, D-1, PP-10, SI-3 and T-2 recorded 89.32, 90.66, 84.88, 70.66 and 80.00 percent mortality against *Helicoverpa armigera* respectively. There was no significant difference between HD-1 and D-1 in their ability to cause mortality in *H. armigera*. The cry toxin, the toxic component in *B. thuringiensis*, is reported to be more potent than synthetic pyrethroids and organophosphates**.** Among the 12 isolates bioassayed against *Spodoptera litura*, D1 recorded the lowest LC_{50} value (1124.73ppm) followed by PP9 (1283.76ppm). However, the reference strain HD-1 recorded a higher LC_{50} value (2410.54ppm). The highest LC_{50} was recorded for D21 (5262.22) followed by PP7 (4831.58ppm) and Bt-42(3737.2ppm).

Key words: *Bacillus thuringiensis*, *Helicoverpa armigera, Spodoptera litura*, Bioassay

INTRODUCTION

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

Of the many bio-control agents available, *Bacillus thuringiensis* insecticides have has proven to be effective and selective enough to eliminate outbreaks of Lepidopteran pests. *B. thuringiensis* is a

fascinating bacterium producing a number of insecticidal toxins which have spawned a whole industry. For more than 60 years, *B. thuringiensis* formulations have been sprayed as bio-insecticides against more than 300 insect species. The main advantage of *B. thuringiensis* is its high specificity (Carlton and Gonzalez, 1985), which makes it safer for non target organisms including humans and natural enemies. Furthermore, *B. thuringiensis*, being a bacterial fermentation product, is potentially less expensive to make than inorganic/organic chemical pesticides. In short, the high efficacy and specificity of Cry proteins (Gill, 1992) is largely responsible for the popularity of *B. thuringiensis.* However, *B. thuringiensis* formulations also have shortcomings. They are prone to early photo degradation, require repeated application and lack systemic action. These disadvantages have severely constrained the widespread utilization of *B. thuringiensis* in pest management (Carlton and Gonzalez, 1985). Some of the strategies developed to solve these problems include cloning of the toxin gene into endophytic bacteria and plants themselves.

The mechanism of action of *B. thuringiensis* crystal proteins involve solubilization of the crystal in the insect midgut and proteolytic processing of the protoxin by midgut proteases under alkaline condition, which then binds to the midgut receptors. The insertion of the toxin into the apical membrane creates pores and results in loss of transmembrane potential that leads to osmotic cell lysis (Knowles, 1994). Thus, sublethal concentration of toxin leads to change in larval behavior including avoidance of feed, ultimately resulting in death due to starvation (Aronson and Shai, 2001). In this article we report the efficacy and inceticidal activity of native isolates, isolated from various ecological zones, against *Spodoptera litura* and *Helicoverpa armigera*

MATERIALS AND METHODS

Different isolates were obtained from the repository in the Department of Biotechnology, University of Agricultural Sciences Dharwad. Bt-42 was a gift from S.P.S. khanuja, Director, CIMAP, Lunknow. P1, SI3, D1, D21, PP9, PP7, PP6, T-2, and PP-10 were obtained from culture collection of Department of Biotechnoogy UAS Dharwad. These isolates were isolated from various ecological zones like soil, phylloplane, rizhosphere etc. HD-1 is a reference strain. Bioassay studies were carried out to investigate the possibilities of using the native isolates as biocontrol agents against *Spodoptera litura* and *Helicoverpa armigera*. For preparation of crude protein from *B. thuringiensis*, the lactose acetone method of Dulmage *et al*. (1970*)* was followed. The crude protein was used for bioassay studies after estimating the protein content as per Lowry's method.

BIOASSAY OF *HELICOVERPA ARMIGERA AND SPODOPTERA LITURA*

Bioassay studies were conducted to assess the toxicity of *Bt* toxins to *Helicoverpa armigera.* The acetone powder made from spores was used for bioassay. The cultures were grown in MGM media for 72 hours and extraction of crude protein was done by the lactose acetone method. The cotton leaf dip bioassay was conducted on 2nd instar neonate larvae of *H. armigera*. For the bioassay of *spodoptera litura*, succulent leaves of the castor plants were washed with water containing 0.2% triton-X to remove the waxy coating. Leaf discs of a diameter of 5.0cm were smeared uniformly with the crude protein on both surfaces, allowed to dry and then placed in petriplate. Ten second instar larvae were placed per leaf disc and allowed to feed. The LC_{50} for each isolate was analyzed using the Maximum Likelihood Programme(MLPO.38)

RESULTS AND DISCUSSION

Bioassays of certain native isolates along with HD-1 and Bt-42 were carried out using the crude protein. The leaf dip bioassay method was used to assess the potency. Under experimental conditions, HD-1 gave a mortality of 94.6 percent at 72 hours on *H. armigera* larvae. Native isolates P-1, D-1, PP-10, SI-3 and T-2 recorded 89.32, 90.66, 84.88, 70.66 and 80.00 percent mortality, respectively (Table1). There was no significant difference between HD-1 and D-1 in their ability to cause mortality in *H. armigera*. The toxic component of *B. thuringiensis*, the cry toxin, is reported to be more potent than synthetic pyrethroids and organophosphates (Feitelson *et al.,* 1992).

In earlier studies, isolate D-1 was reported to be toxic to *Plutella xylostella*, the lepidopteran pest of cabbage (Bhat, 2000). Another native strain, P-1, which showed 89.32 percent mortality against *H. armigera*, showed 100 percent mortality to *Plutella xylostella* in an earlier study (Bhat, 2000) and 90.00 percent mortality to *H. armigera* (Prashanth Kumar, 2002). Although high variability of cry proteins have been described, it is necessary to search for more toxin genes because a large number of pests are not controlled with available cry toxins.

The leaf dip bioassay of *spodoptera litura* on castor leaves revealed that the extent of leaf damage is very high in the control, but the leaves treated with the crude protein PP9 and PP6 recorded less damage than other isolates. Among the 12 isolates, D1 recorded the lowest LC_{50} value (1124.73ppm) followed by PP9 (1283.76ppm). However, the reference strain, HD-1, recorded a higher LC_{50} value (2410.54ppm). The LC_{50} is highest in D21 (5262.22) followed by PP7 (4831.58ppm) and bt-42(3737.2ppm). The higher toxicity of native strains over positive control HD1 was observed. The result were similar to the results

Table 1: Efficacy of crude protein from different *B. thuringiensis* isolates on *Helicoverpa armigera*

Isolates	Mean number of dead larvae after 48 hours		Mean number of dead larvae after 72 hours	
D-1	20.667^{A}	(82.667)*	22.667^{AB}	(90.667)
P-1	20.33^{AB}	(81.32)	22.333^{AB}	(89.32)
T-2	17.33^{C}	(69.32)	20.000^{C}	(80.00)
Bt-42	18.667^{BA}	(74.664)	20.667^{BC}	(82.667)
SI-3	15.00^{D}	(60.00)	17.667^{D}	(70.667)
PP-10	20.00^{AB}	(80.00)	21.22^{C}	(84.88)
HD-1	21.33^{A}	(85.32)	23.667^{A}	(94.667)
Control	2.667^{E}	(10.64)	3.00^{E}	(12.00)
SEm±	0.408		0.486	
CD at 1%	2.648		3.154	

* Figures in parentheses indicate percent mortality

**The figures followed by different letters are significantly different according to DMRT at P=0.01

Table 2: LC_{50} value of different B. *thuringiensis* isolates

Strain	LC_{50}(ppm)	Feducial limits(95%)		Slope
		Lower	Upper	
D1	1124.37	941.75	1294.50	3.86 ±1.08
HD1	2410.54	2198.46	2666.24	6.72 ±2.18
SI3	2100.27	1817.62	3242.43	4.09 ±1.78
P1	1541.22	1356.51	1983.38	1.28± 0.11
Bt-42	3737.53	1462.15	2265.62	4.013± 1.20
PP6	3274.06	3013.42	3473.08	4.58± 1.70
PP9	1283.76	1007.00	1348.48	10.46 ±3.12
PP10	3304.01	2976.58	3511.02	0.86± 0.11
PP7	4831.58	4614.11	6483.07	23.43± 9.16
P21	5262.22	4899.68	512.73	19.28± 8.70

obtained by Bravo et al.(1998) wherein native Mexican strain IB126 was more toxic for *Spodoptera frugiperda* and *S. exigua* larvae(LC_{50} 22ng/cm^2) compared to HD1 (LC_{50} for *S. frugiperda* was 969ng/cm2 and LC_{50} for *S. exuigua* was 1330ng/cm^2).

The efficacy of crude protein from different *B. thuringiensis* isolates on *Helicoverpa armigera* was shown in Table 1. The data pertaining to bioassay of spodoptera litura are presented in Table 2.

The isolates exhibited wide variation in the presence of *cry*IA. While isolates D-1, PP-6, and PP-9 possessed *cry*IA(a), *cry*IA(b) and *cry*IA(c), isolate P-1 had *cry*IA(a) and *cry*IA(c), and isolates PP-7, SI-3 and D-21 did not produce amplicon with any of the three *cry*IA(a), *cry*IA(b) and *cry*IA(c) primer. PP-10, PP-11, PP-12 and R-5 possessed both *cry*IA(b) and *cry*IA(c), but was not tested for *cry*IA(a). Although PP-7, SI-3 and D-21 did not amplify either *cry*IA(a), *cry*IA(b) or *cry*IA(c), they may posses another lepidopteran specific gene in them. D-1, the most potent of native isolates tested in this study, possessed *cry*IA(a), *cry*IA(b) and *cry*IA(c) genes(Mangala *et al*., 2007). It is possible that the presence of many *cry* genes can cause the strain to be more efficient. Thus, by screening isolates for diversity of *cry* genes and comparing them in bioassays we can select the novel insecticidal bacteria with an extended target spectrum for direct application.

Biological control of lepidopteran insect pests affecting crop plants has been possible using *B. thuringiensis* species. Other than using the bacterial spores in a spray formulation, the gene for crystal protein or δ-endotoxin has been engineered into plant systems, so that the transgenic plants are protected from the specific insect. Plants expressing toxin genes throughout the whole plant have several advantages over classical means used in plant protection including controlling borers that are difficult to reach by conventional means once they have penetrated plant tissues. In this contest searching for variability of strain for cry protein, identifying the most potent strain gains the important.

References:

ARONSON, A. I. AND SHAI, Y., 2001, Why *Bacillus thuringiensis* insecticidal toxins are so effective unique features of their mode of action. *FEMS Microbiology Letters*, **195:** 1-8.

BHAT, D., 2000, Molecular characterization of native *B. thuringiensis cry* genes. *M. Sc. (Agri.) Thesis*, University of Agricultural Sciences, Dharwad. biology of *B. thuriengiensis*. *The Molecular Biology of the bacilli*, **2**:211-249.

BRAVO, A., SARABIO, S., LOPEZ, L., VEROS, H., ABARCA, C., ORTIZ, A., ORTZ, M., LINA, L., VILLALOBOS, F. J., PENA, G., NUNEZ-VALDEZ, M., SOBERON, M. AND QUINTERO, R., 1998, Characterization of cry gees in a Mexican *Bacillus thuringiensis* strain collection. *Applied and Environmental Microbiology*, **64** : 4965-4972.

CARLTON, B.C. AND GONZALEZ, J.M., 1985, The genetics and molecular biology of *B. thuriengiensis*. *The Molecular Biology of the bacilli*, **2**:211-249.

DULMAGE, H. T., CORREA, J. A. AND MARTINEZ, A. J., 1970, Coprecipitation with lactose as a means of recording the spore crystal complex of *B. thuringiensis*. *Journal of Invertebrate Pathology,* **15** : 15-20.

FEITELSON, J., ZEIGLER, D. R. AND DEAN, D. H., 1992, *B. thuringiensis* and its pesticides crystal proteins. *Microbiology and Molecular Biology Reviews,* **62** : 775-806.

GILL, S.S., COWLES, E.A. AND PIETRANTONIO, P.V., 1992, The mode of action of *Bacillus thuriengiensis* endotoxin. *Annual review of Entomology*, **37:**615-636

KNOWLES, B. H., 1994, Mechanism of action of *Bacillus thuringiensis* insecticidal δ-endotoxins. *Advances in Insect Physiology*, **24:** 275-308.

MANGALA S.B., KRISHNARAJ P.U. AND KURUVINASHETTI M.S., 2007 Characterization of *cry*I gene profile in native *bacillus thuringiensis* isolates.*Crop Research*, **34:**263-267.

PRASHANTHKUMAR, N. G., 2002, Evaluation of molecular characterization of native *B. thuringiensis* isolates. *M. Sc. (Agri.) Thesis*, University of Agricultural Sciences, Dharwad.

Mangala S.Biradar, Shanker Patwari, Krishnaraj P.U. and Kuruvinashetti M.S.
Department of Biotechnology, University of Agricultural sciences, Dharwad-580005, Karnataka, India.

Comparative responses of malathion-susceptible and resistant *Musca domestica* L. strains to oxidative stress

Introduction

The extensive use of insecticides to control insect pests has provided a dramatic illustration of evolution in natural populations. It is not surprising that resistant and susceptible strains differ in properties other than their adaptation to insecticides, such as development time, fecundity and fertility.

Although the majority of fitness studies have shown that there are fitness costs associated with insecticide resistance (Ferrari and Georghiou 1981, Parello and Trumble 1989, White and Bell 1990), in many cases, in the absence of treatment, there is no fitness difference between resistance and susceptible strains or the resistant strain has a fitness advantage (Roush and Hoy 1981, Arnaud and Haubruge 2002).

Oxidative stress results from an imbalance of oxidants and antioxidants-either a surfeit of oxidants and/or a deficit of antioxidants (Gray and Clinton 1995). The antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) are the main enzymatic defences and act in concert with a panoply of non-enzymatic antioxidants (Halliwell and Gutteridge 1999).

Various classes of pesticides induce production of reactive oxygen species and oxidative tissue damage which may contribute to the toxicity of these xenobiotics (Bagchi et al. 1995). Enhanced activities of superoxide dismutase and catalase, two antioxidant enzymes that detoxify superoxide radical and hydrogen peroxide, respectively, were observed in Wister rats, after acute and sub-chronic malathion exposure (Possamai et al. 2007). Diazinon also increased a tissue specific catalase activity in the fish *Oreochromis niloticus* (Durmaz et al. 2006). Exposure of reference *R. dominica* to pure oxygen for two days resulted in 20% increase in catalase and reduced mortality of the treated population to 5% when post-treated with a discriminating dose of phosphine (Price et al. 1982).

In the present study, we evaluated fitness under oxidative stress, and the role of a major antioxidant enzyme, catalase, in malathion and fenitrothion resistant strains of the housefly *M. domesitca* L.

Materials and methods

Insect strains

The two housefly strains, maintained at the School of Biology, University of Newcastle upon Tyne, were used in this study. The 571 strain was reported (Ahmed et al. 1998) as malathion and fenitrothion resistant and the Cooper strain has been used as a susceptible reference.

Application of oxidative stress to the insects

Pure oxygen atmosphere conditions were maintained in a 30x30x30 cm clear plastic chamber kept within an incubator by passing a continuous gentle stream of humidified filtered 100% oxygen from a gas cylinder through the chamber. 24±2°C temperature and 50±5% relative humidity was maintained in the chamber. In the control, normal air (supplied from a compressed air cylinder) was used instead of pure oxygen. Responses of the two strains against stressors (malathion, potassium persulphate ($K_2S_2O_8$) and oxygen) were investigated, and the antioxidant effect of trolox (water-soluble Vitamin E analogue) was noted against them. Newly emerged adult flies (<1d old) were fed under normal rearing conditions on 50 ppm concentration of trolox in a sugar solution for 48 hours before being subjected to a single and then combination of stressors. Fifty flies were topically treated with a malathion solution of LD_{50} concentration (or were controls without malathion) and then kept in 100% oxygen in 300 ml glass jars with their openings

covered with mesh, or in normal air. The flies were anaesthetized with CO_2 for dosing. Other malathion-treated or control flies were then fed on a 200 ppm concentration of $K_2S_2O_8$ in a sugar solution from cotton buds dipped in 5ml glass vials. The mortality/survival was noted at 24 hours. The survivors of the four replicates were killed and kept at -20°C to determine the catalase activity.

Homogenisation of insects

Crude enzyme extracts were prepared by homogenising 100 mg of adult flies in 4 ml of ice cold 0.05M phosphate buffer with a pH of 7. The homogenates were centrifuged and the supernatants were used as an enzyme source.

Enzyme assay

Catalase activity was determined by using a modification of the spectrophotometric method of Durusoy et. al.(1995). Reactions, which are carried out at 30°C, were initiated by the addition of 50 µl of the supernatant to 3 ml of a substrate solution (pH 7) containing a 0.05M phosphate buffer and H_2O_2 at a final concentration of 20mM. Catalase activity was measured as a decrease in H_2O_2 concentration at 230 nM. Protein concentration of the homogenates was determined by the method of Bradford (1976).

Results and discussion

The adult survival rate, both with and without trolox treatment, in the strains of *M. domestica* L., after treatment with different stressors is shown in Figure 1. The results showed that the resistant strain survived significantly better against various prooxidants such as malathion, $K_2S_2O_8$ and oxygen than the susceptible strain of *Musca domestica*. Pre-feeding of synthetic antioxidant trolox increased the survival rate in both strains, against all stressors, but this increase was higher in the susceptible than the resistant strain of *Musca domestica*.

Catalase activities in the strains of *M. domestica*, following treatment with either individual or combined treatment of stressors in the trolox pre-treated and non-treated adult flies are presented in Figure 2. The results indicate that the catalase activity was significantly higher in the malathion resistant strain than the susceptible one and the enzyme activity was induced in response to the stress conditions; however, the induction was significantly higher in the resistant adults than in the susceptible. The flies pre-treated with trolox exhibited lower enzyme activity in both strains, but this decrease was significantly higher in the susceptible strain. The general response of the resistant strain was the induction of the catalase by the oxidative stressor; this response was variable, and the combined effects of multiple stressors was not additive. Treatment with the antioxidant trolox did not consistently reduce activity, perhaps because of the unreliable uptake into the insect. However, in the susceptible strain there was very little response to single or multiple stressors. However, trolox pre-treatment consistently reduced catalase activity across all stress combinations. This difference to the resistant strain may be a result of structural or enzymic changes to the gut (affecting uptake) in the resistant strain (Bughio and Wilkins 2004).

Our results suggest that pure oxygen and malathion exposure provoked oxidative stress and modulated catalase activity in the organophosphate (OP) resistant strain of *M. domestica*, and an induction of major antioxidant enzymes could have helped the strain to withstand the both insecticidal and oxidative stresses. The results would seem to suggest that adaptation to toxic stress such as exposure to insecticides implies some changes other than the usual mechanisms (reduced uptake, modified target, enhanced metabolism) and these can increase the fitness of the adapted generation in the absence of the selection.

References

Ahmed, S., R. M. Wilkins, and D. Mantle. 1998. Comparison of proteolytic enzyme activities in adults of insecticide resistant and susceptible strains of the house fly *M. domestica* L. Insect Biochemistry and Molecular Biology 28: 629-639.

Arnaud, L., and E. Haubruge. 2002. Insecticide resistance enhances male reproductive success in a beetle. Evolution 56: 2435-2444.

Bagchi, D., M. Bagchi, E. A. Hassoun, and S. J. Stohs. 1995. In vitro and in vivo generation of reactive oxygen species, DNA damage and lactate dehydrogenase leackage by selected pesiticides. Toxicology 104: 129-140.

Bradford, M. M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72: 248.

Bughio, F. M., and R. M. Wilkins. 2004. Influence of malathion resistance status on survival and growth of *Tribolium castaneum* (Coleoptera: Tenebrionidae), when fed on flour from insect-resistant and susceptible grain rice cultivars. Journal of Stored Products Research 40: 65-75.

Durmaz, H., Y. Sevgiler, and N. Uner. 2006. Tissue-specific antioxidative and neurotixic responses to diazenon in *Oreochromis niloticus*. Pesticide Biochemistry and Physiology 84: 215-226.

Durusoy, M., N. Diril, and A. N. Bozcuk. 1995. Age-related activity of catalase in different genotypes of *Drosophila melanogaster*. Experimental Gerontology 30: 77-86.

Ferrari, J. A., and G. P. Georghiou. 1981. Effects on insecticidal selection and treatment on reproductive potential of resistant, susceptible, and heterozygous strains of the southern house moquitoes. Journal of Economic Entomology 74: 323-327.

Gray, W. F., and B. S. Clinton. 1995. Antioxidant systems in insects. Archives of Insect Biochemistry and Physiolgoy 29: 187-197.

Halliwell, B., and J. M. C. Gutteridge. 1999. Free radicals in biology and medicine. Oxford Science Publications, Oxford.

Parello, M. P., and J. T. Trumble. 1989. Decline in resistance in *Liriomyza trifolii* (Diptera: Agromyzidae) in the absence of insecticide selection pressure. Journal of Economic Entomology 82: 365-368.

Possamai, F. P., J. J. Fortunato, G. Feier, F. R. Agostinho, J. Quevedo, D. W. Fillho, and F. Dal-Pizzol. 2007. Oxidative

stress after acute and sub-chronic malathion intoxication in wistar rats Environmental Toxicology and Pharmacology 23: 198-204.

Price, N. R., K. A. Mills, and L. A. Humphries. 1982. Phosphine toxicity and catalase activity in susceptible and resistant strains of the lesser grain borer (*Rhyzopertha dominica*). Comparative Biochemistry and Physiology 73C: 411-413.

Roush, R. T., and M. A. Hoy. 1981. Laboratory, glasshouse, and field studies of artificially selected carbaryl resistance in *Metaseiulus occidentalis*. Journal of Economic Entomology 74: 142-147.

White, N. D. G., and R. J. Bell. 1990. Relative fitness of malathion-resistant strain of Cryptolestes ferrugineus (Coleoptera: Cucujidae) when development and oviposition occur in malathion-treated and untreated wheat kernels. Journal of Stored Product Research 26: 23-37.

Rauf, A. and Richard M Wilkins
E-mail: abdur.rauf@ncl.ac.uk ; richard.wilkins@ncl.ac.uk
Phone: 0044 191 2226945 ; 0044 7805067957
School of Biology, King George VI Building, University of Newcaslte upon Tyne, NE1 7RU, United Kingdom

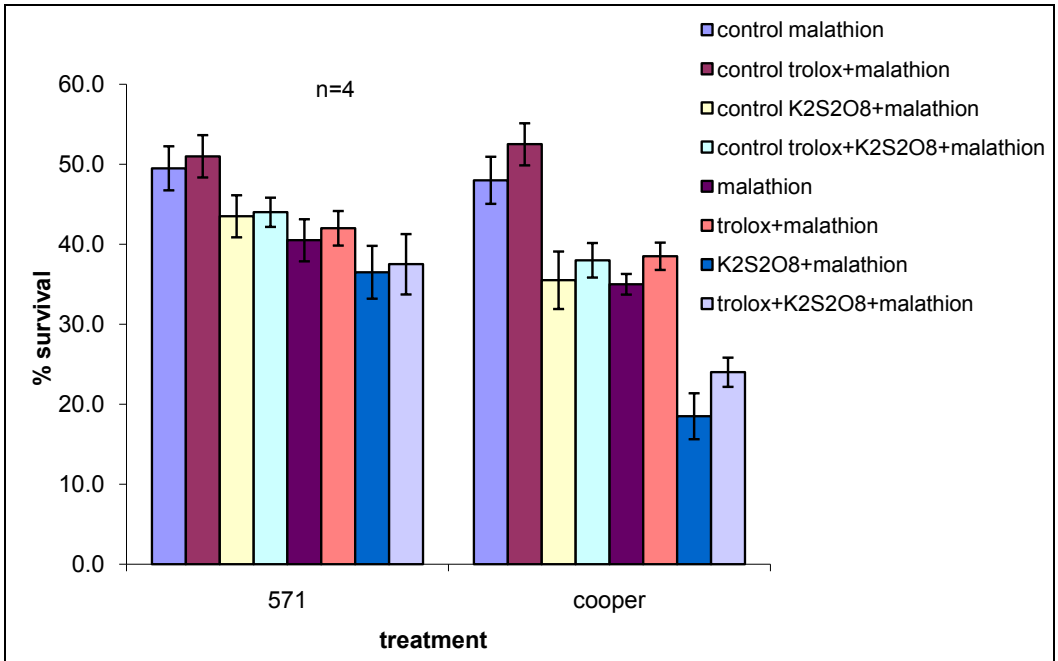


Figure. 1 Adult survival rate of the resistant and susceptible strains of *M. Domestica* against stressor treatment

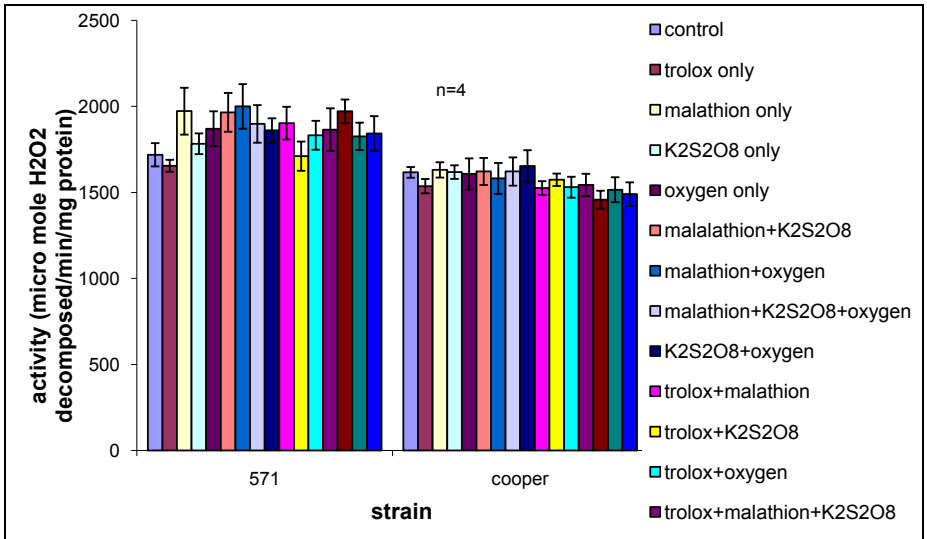


Figure. 2 Catalase activity in the strains of *M. domestica* after treatment with various stressors, pre-treated with trolox

Status of new insecticides vis-a-vis conventional insecticides against the American bollworm, *Helicoverpa armigera*

ABSTRACT

The American bollworm, *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae), is the most destructive pest of cotton. In the present experiment, the status of new insecticides vis-à-vis conventional insecticides in Kurnool population of *H. amigera* was estimated. The LC_{50} (expressed in µg per larva) values of emamectinbenzoate, indoxacarb, spinosad, profenophos, methomyl, quinalphos, endosulfan, deltamethrin, acephate, thiodicarb, chlorpyriphos, triazophos, monocrotophos, fenvalrate and cypermethrin were 0.0061, 0.22, 0.11, 0.38, 0.45, 0.53, 0.81, 1.07, 1.09, 1.86, 1.86, 1.86, 3.25, 4.16, and 8.70 respectively. Of all the insecticides tested emamectinbenzoate is the most toxic and cypermethrin was the least toxic. The LC_{90} (µg per larva) values were also calculated. This kind of study would serve as ready-reckoner of the selection of insecticides for the management of field strains and also helpful in development of resistant management strategies for this important polyphagous insect pest.

Key words: American bollworm, *Helicoverpa armigera*, new insecticides, conventional insecticides, Kurnool population

Introduction

The American bollworm, *Helicoverpa armigera* Hubner, is a polyphagous pest and causes severe economic damage to several economically important crops such as cotton, pulses and vegetables (Manjunath *et al*., 1987). During the 1980's, *H. armigera* emerged as a major pest of cotton besides other crops in India. Its outbreak often led to crop failures. Among other factors, its heavy infestations were attributed to the development of resistance to almost all conventional insecticides. High levels of resistance to synthetic pyrethroids in different strains of *H. armigera* were reported by several workers (Kranthi *et al*., 2002, Ramasubramanian 2004 and Prasada Rao *et al*., 2005)

Because of its ability to inflict serious economic damage *H.armigera* has been subjected to heavy doses of insecticide treatments. Typical control of this pest includes several synthetic pyrethroids, organophosphates and carbamates. Amongst different groups of insecticides synthetic pyrethroids constitute 8-75% and are widely used all over India to control this pest. Cypermethrin is most commonly used followed by fenvalrate. Since *H.armigera* has a strong propensity to develop resistance against different group insecticides, there is a need to study the status of new molecules vis-à-vis with old groups of insecticides especially synthetic pyrethroids. Such kind of studies will be helpful in using different group of molecules with care and preserving the usefulness of insecticides in pest management programme, and in further delaying the development of resistance in the meantime. Keeping these things in view, the present studies were contemplated to determine the relative toxicity of synthetic molecules with diversified modes of action against this pest in laboratory to provide organized guidance for the selection of insecticides that can be incorporated in pest management programmes. The LC_{50} values obtained would serve as ready reckoner for the selection of insecticides for field strains. Also, such base line data could be used as critical inputs in the deployment of new insecticides and insecticide resistance management programmes.

Material and Methods

Insect rearing

The eggs of *Helicoverpa armigera* along with the leaves, bracts and flowers of cotton were collected and brought to the laboratory. The neonates were allowed to hatch from the eggs and were then transferred to a chickpea flour-based artificial diet. The culture was maintained at ambient temperatures of 27 ± 1°C and 65 ± 5% relative humidity at Resistance Monitoring Laboratory, Regional Agricultural Research Station, Nandyal, Andhra Pradesh.

Insecticides

The insecticides spinosad, indoxacarb, emamectinbenzoate, endosulfan, quinalphos, chlorpyriphos, acephate, monocrotophos, triazophos, profenophos, nuvan, methomyl, thiodicarb, novaluron, lufenuron, cypermethrin, fenvalrate and deltamethrin were obtained from respective manufacturers and used for the present study.

Estimation of resistance levels and relative toxicity

The log-dose-probit-mortality (LDPM) assay of synthetic pyrethroids, organo phosphate compounds carbamates, spinosyns, and avermectins were used with concentrations of each test insecticide by diluting in analytical grade acetone. The third instar test insect larvae were topically exposed initially to concentrations of a wide range, and on the basis of mortality recorded a series of concentrations of a narrow range were selected to which the test insect is again exposed. The same procedure was repeated until mortality data in the range of 10-90% was recorded. Mortality was recorded until 144 hours after treatment. The moribund insects were counted as dead. The data thus obtained were subjected to probit analysis for calculating regression equation and lethal concentration by Finney's method of probit analysis. The values of relative toxicity of different insecticides were calculated by taking values of median lethal concentration of cypermethrin as unity.

Table. 1 Comparative toxicity of different insecticides against *H. armigera*							
Insecticide	**Chi^2**	**Regression equation**	**LC50**	**Fiducial limit**	**Relative toxicity**	**LC90**	**Fiducial limit**
E benzoate	11.49	Y=2.850+2.392 X	0.0061	0.0031-0.011	1426.2	0.06	0.030-0.13
Indoxacarb	11.84	Y=2.837+1.951 X	0.022	0.013-0.035	395	0.06	0.025-0.16
Spinosad	12.80	Y=3.443+1.482 X	0.11	0.07-0.13	79.0	0.489	0.269-0.891
Profenophos	0.60	Y=1.29+4.116 X	0.38	0.33-0.42	22	0.61	0.53-0.72
Methomyl	8.02	Y=3.171+1.307 X	0.45	0.29-0.70	19.3	1.09	0.54-2.16
Quinolphos	10.18	Y=3.278+1.035 X	0.53	0.34-0.85	16.4	7.76	2.51-23.44
Endosulfan	5.50	Y=2.513+1.130 X	0.81	0.34-1.86	10.7	10.71	6.61-18.6
Deltamethrin	5.64	Y=2.25+1.394 X	1.07	0.70-1.62	8.13	7.76	3.71-15.8
Acephate	6.05	Y=2.605+1.105 X	1.09	0.60-1.71	7.98	10.13	4.06-25.11
Thiodicarb	8.59	Y=3.043+0.801 X	1.86	1.022-3.38	4.6	12.30	4.26-34.67
Chlorpyriphos	0.44	Y=1.97+3.101 X	1.86	1.54-2.18	4.6	6.0	4.89-7.41
Triazophos	0.61	Y=2.08+3.15 X	1.86	0.58-2.18	4.6	3.63	3.09-4.31
Monocrotophos	3.24	Y=1.843+1.384 X	3.25	2.123-4.89	2.67	13.87	7.22-24.95
Fenvalrate	7.22	Y=2.501+0.967 X	4.16	2.81-6.16	2.09	30.19	16.59-54.95
Cypermethrin	1.02	Y=1.326+1.208 X	8.70	6.45-11.74	1.00	48.97	32.35-74.13

Results and discussion

The LC_{50} (expressed in µg per larva) values of emamectinbenzoate, indoxacarb, spinosad, profenophos, methomyl, quinalphos, endosulfan, deltamethrin, acephate, thiodicarb, chlorpyriphos, triazophos, monocrotophos, fenvalrate and cypermethrin were 0.0061, 0.22, 0.11, 0.38, 0.45, 0.53, 0.81, 1.07, 1.09, 1.86, 1.86, 1.86, 3.25, 4.16, and 8.70 respectively (Table 1). The dose mortality responses to the third-instar larvae of *Helcoverpa armigera* indicated that, at LC_{50} (sensitive point for relative evaluation of insecticides), out of 14 insecticides tested against *H. armigera*, all the insecticides viz., emamectinbenzoate, indoxacarb, spinosad, profenophos, methomyl, quinalphos, endosulfan, deltamethrin, acephate, thiodicarb, chlorpyriphos, triazophos, monocrotophos and fenvalrate were more toxic than cypermethrin being 1462.2, 395, 79.0, 22, 19.3, 16.4, 10.7, 8.13, 7.98, 4.60, 4.60, 4.60, 2.67 and 2.09 times more toxic respectively.

Based on the LC_{90} values, emamectinbenzoate was most toxic and cypermethrin was least toxic among the commonly available insecticides against *Helicoverpa armigera* (a point which indicates the concentration required for field recommendation to manage the pest population); the order of relative resistance remained the same being 816.16, 816.16, 100.14, 80.27, 44.9, 13.4, 8.16, 6.3, 6.34.8, 4.5, 3.98, 3.5 and 1.62 times respectively. Among the organophosphate insecticides profenophos was more toxic to the test insect and monocrotophos is the least toxic. Among different chemicals tested, the organochlorine compound endosulfan, carbamate compound methomyl and op compounds profenophos and quinalphos showed LC_{50} values <1 µg per larva and all other chemicals showed LC_{50} values >1 µg per larva.

In the present study emamectin benzoate demonstrated higher toxicity than other chemistries. It belongs to the Avermectins; these are a group of macro cyclic lactones isolated from fermentation of the soil microorganism *Streptomyces avermitilis*. These compounds act as agonists for gamma-amino butyric acid (GABA)-gated chloride channel. It is the most potent insecticide compound registered for agricultural use. It acts specifically on lepidopteran species. Bioefficacy of these avermectins mainly due to their unique mode of action and lack of cross-resistance with available insecticides. These compounds are considered best for IPM programmes because of their strong physiological and ecological selectivity (Ishaya and horowitz, 1988). Indoxacarb and spinosad were other two chemicals, which were found more toxic after emamectin benzoate. The indoxacab was found more toxic in the present study which may be because enhanced level of carboxyl esterase in pyrethroid resistant population was reported to activate indoxacarb into more potent metabolite DCJW (decarbo methoxylate JW-64) (Gunning *et al*., 2002). The negative cross-resistance reported in the *H. armigera* strain used in the present study strengthens view. It is a member of the new oxadiazine class of insecticide that acts by inhibiting sodium ion entry in to nerve cells (Wing *et al*., 1988).

Spinosad is the first member of Dow agro sciences naturalyte class of insecticides. Spinosad is comprised primarily two macrocyclic lactones, spinosyn A and D, secondary metabolites produced by the actinomycete, *Saccharopolyspora spinosa*, under natural fermentation conditions. The mode of action of spinosad is two fold; the primary target site is the nicotinic acetylcholine receptor, but the GABA receptor is also affected to some degree. Toxicity of spinosad may be attributed to their novel mode of action.

Gupta *et al*. (2005) reported that new insecticides like emamectinbenzoate, indoxacarb and spinosad were more toxic than conventional

insecticides, mainly synthetic pyrethroids, against Delhi population of *H. armigera*. He also opined that this might be mainly due to their unique modes of action and translaminar movement and lack of cross-resistance with other commercially used pesticides.

Owing to their high specificity, these novel insecticides are superior in safety for beneficial insects, humans and animals. These findings do not reflect field efficacy since these are based on a linear response to a variety of dosages under laboratory conditions, where as field efficacy is influenced by several other factors including coverage and environmental conditions. Coupled with their efficacy against resistant pests and selectivity towards natural enemies, these new molecules can greatly reduce the number of sprays applied per season. Sine these new chemicals are mostly contact and stomach poisons, they are reported to be highly efficient in the field. Since growers have a wide range of alternatives in the form of old and new chemicals, the best strategy would be to use effective compounds as one of the components of pest management strategy.

References

Gunning, R.V. and Devonshire, AL. 2000. Negative cross-resistance between indoxacab and pyrethroids in Australia Helicoverpa armigera: a tool for resistance management. *Resistant Pest Management* **11**:52.

Gupta, G. P., Bariah, A., Rani. S. and Raghuraman, M. 2005. Relative toxicity of novel insecticides to American bollworm (Helicoverpa armigera). *Indian Journal Agricultural Sciences* **75** (4): 235-237.

Ishaaya, I and Horowitz, A. R. 1988. Insecticides with novel modes of action. An over view. In. Ishaaya, I. And Degheele, D (eds) Insect ides with novel modes of action. Narosa Publishing House, New Delhi, pp 1-25.

Kranthi, K.R., Jhadav, D.R., Wanjari, R.R., Shaker Ali, S. and Russel. D. 2002. Insecticide resistance in five major insect pests of cotton in India. *Crop Protection* **21**:449-60

Manjunath, T. M., Bhatnagar, V. S., Pawar, C.S. and S. Sithanandam, 1987. Economic importance of *Heliothis* spp. In India and assessment of their natural enemies and host plants. *In Proceedings of the International Workshop on Biological Control of Heliothis*, 11-15, November 1985, New Delhi, India.

Prasada Rao, G.M.V., Hariprasada Rao, N. and Raju, K. 2005. Insecticide Resistance in field population of American Bollworm, Helicoverpa armigera Hub. (Lepidoptera: Noctuidae). *Resistant Pest Management News Letter* **15** (1) 15-17

Ramasubramanian, T. 2004. Magnitude, Mechanism and Management of Pyrethroid resistance in Helicoverpa armigera (Hubner) in India. *Journal of Entomology* **1** (1): 6-11.

Wing, K. D., Schnee, M. E., Sacher, M and Connair, M. 1988. A novel oxadiazine insecticide is bioactivated in lepidopteran larvae. *Archives of Insect Biochemistry and Physiology* **37**: 91-103.

G. M. V. Prasada Rao* and Grace A. D. G

*Sr. Scientist (Ento.) Regional Agril. Research Station, Lam, Guntur-34

Sensitivity of the entomopathogenic fungus, *Beauveria bassiana* (Bals.) Vuill. to certain fungicides

ABSTRACT: The fungal entomopathogen *Beauveria bassiana* (Bals.) Vuill. has been recognized as a potent biocontrol agent against several insect-pests in diverse agro-ecosystem. The effect of 10 fungicides on mycelial growth of *B. bassiana* isolate: BbCm KKL 1100 was evaluated *in vitro* each at three concentrations: the field recommended rate (1.0X), a 10 fold lower rate (0.1X) and a 10 fold higher rate (10.0X). The fungicides tested, in general, were antagonistic to *B. bassiana* at varying intensities. Benomyl, carbendazim, hexaconazole, propiconazole and tricyclazole caused total inhibition of mycelial growth even at the lowest concentration (0.1X). Edifenphos, iprobenphos and mancozeb appeared to be fungistatic at normal field dose (1.0X). At sub-normal concentrations, chlorothalonil, copper oxychloride and edifenphos were moderately toxic to *B. bassiana* exhibiting 63.0, 72.6 and 76.3 per cent mycelial inhibition respectively. Our study suggests that *B. bassiana* is very sensitive to the fungicides tested, particularly at normal as well as higher field recommended dosage. Consequently, it would be expected that negative effects of fungicides on *B. bassiana* would occur during epizootics. Extensive field studies complemented by parallel laboratory experiments should consider assessing the interaction between selective fungicides and *B. bassiana* isolates to evaluate their ecological impact in cropped environments.

Key words: Entomopathogenic fungus, *Beauveria bassiana*, fungicides, sensitivity.

INTRODUCTION

Fungicides are routinely used crop protection chemicals, but the broad spectra of activity of some may exert adverse effects on non-target organisms, including several forms of entomopathogens that occur in the natural environment. Prominent among the entomopathogens involved in crop protection are fungi. Entomopathogenic fungi have been proposed as biocontrol agents against diverse insect pests in agriculture. In particular, *Beauveria bassiana* (Bals.) Vuill. has long been tested against a wide range of insects in different geographical regions. In India, natural epizootics of *B. bassiana* have been often observed on a range of insect pests in the rice crop (Hazarika and Puzari, 1990; Ambethgar, 1991). The fungus may be applied in the form of conidia or mycelium, which sporulates after application. Field application of *B. bassiana* conidia has provided significant reduction in the insect pest populations in the rice ecosystem (Ramamohan Rao, 1989). The exploitation and effective use of this fungus as a biocontrol agent of insect pests is partially dependent upon survival of infective propagules in the field environment (Clark *et al.,* 1982). Laboratory assessment of growth inhibition of *B. bassiana* is a useful criterion for testing sensitivity (Aguda *et al*., 1988). The knowledge of interactions between introduced entomofungus and all commonly used pesticides would facilitate the choice of chemicals compatible with or less harmful to naturally occurring

or artificially inoculated biocontrol fungi. The aim of this study was to determine the influence of selected fungicides intended for plant disease control, on the mycelial growth *in vitro* of a virulent *Beauveria bassiana* isolate (BbCm KKL 1100) to predict their biological impact on natural epizootics.

MATERIALS AND METHODS

The laboratory experiment was conducted in the Department of Entomology, Tamil Nadu Agricultural University, Coimbatore, India. The fungus isolate: BbCm KKL 1100 used in this study was originally derived from larvae of rice leaffolder, *Cnaphalocrocis medinalis* Guenee from rice leaffolder endemic area of Karaikal, Pondicherry Union Territory, India (Ambethgar, 1997). The mycelial growth of *B. bassiana* was examined on Sabouraud dextrose agar fortified with 1% yeast extract (SDAY) medium adopting the poisoned food technique (Moorhouse *et al.,* 1992).

The fungicides selected for the compatibility test against the entomofungus were among those tested for control of foliar diseases of rice. Ten fungicides (benomyl, carbendazim, chlorothalonil, copperoxychloride, edifenphos, hexaconazole, iprobenphos, mancozeb, propiconazole and tricyclazole) at three concentrations (0.1X, 1.0X and 10.0X times field recommended rates) were fixed for evaluation *in vitro* at 500L spray fluid ha^{-1} (Crop Production Guide, 2000). The fungicides were added to the sterilized SDAY medium at the luke-warm temperature of approximately 55°C, thoroughly mixed, and poured into 9 cm diameter sterile Petri dishes. The agar plates were allowed to set overnight under a laminar flow cabinet. Mycelial plugs cored out from the periphery of a 7-day-old colony were transferred-inverted to plates of SDAY amended with respective fungicides. The inoculated plates were incubated at 26±2°C in complete darkness for 14 days, and colony diameter/linear growth in excess of the plugs in each Petri dish was measured. The degree of sensitivity was expressed as proportion of mycelial colony diameter remaining/extent of mycelial inhibition compared to corresponding control using the formula described by Hokkanen and Kotiluoto (1992).

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

Where: X, Y, Z stand for percent growth inhibition, radial mycelial growth of fungus in control and radial mycelial growth of fungus in poisoned medium, respectively. The toxicity of the fungicides was graded based on a 1-4 evaluation score, where 1 = harmless (< 50% reduction in beneficial capacity), 2 = slightly harmful (50-79 %), 3 = moderately harmful (80-90%), and 4 = harmful (>90 %) in toxicity tests *in vitro* according to Hassan's classification scheme (Hassan, 1989). This study was performed twice on different days and each fungicide/concentration was replicated three times. The data was analyzed statistically.

RESULTS AND DISCUSSION

The effects of fungicides on *B. bassiana* have been studied because of their potential for interference in natural epizootics. The results on the sensitivity of *B. bassiana* isolate BbCm KKL 1100 for ten fungicides is shown in Table 1. The fungicides tested were harmful to growth and development of *B. bassiana* either partially or completely at the three concentrations tested. At the highest (10X) concentration, all the ten fungicides tested caused total inhibition of mycelial growth, indicating their co-application at higher than normal concentration is not desirable due to their fungitoxic nature. Benomyl, carbendazim and hexaconazole entirely inhibited the mycelial growth in all the three concentration indicating very high sensitivity of the fungus. At normal field dose (1.0X), benomyl, carbendazim, hexaconazole, propiconazole and tricyclazole caused complete inhibition of mycelial growth, while rest of the fungicides suppressed as much as 81.7-88.2 percent mycelial growth.

Table 1. Effect of certain fungicides on the growth of *B. bassiana* in SDAY at 14 DAT

Fungicides	Mean mycelial colony diameter in mm (Inhibition (%) over control)			
	Lower dose (0.1X)	Field dose (1X)	Higher dose (10X)	Scoring at (0.1X)
Benomyl	0.0 (100.0)	0.0 (100.0)	0.0 (100.0)	4
Carbendazim	0.0 (100.0)	0.0 (100.0)	0.0 (100.0)	4
Chlorothalonil	33.3 (63.0)	16.5 (81.7)	0.0 (100.0)	2
Copperoxychloride	24.7 (72.6)	16.0 (82.2)	0.0 (100.0)	2
Edifenphos	21.3 (76.3)	15.6 (82.6)	0.0 (100.0)	2
Hexaconazole	0.0 (100.0)	0.0 (100.0)	0.0 (100.0)	4
Iprobenphos	16.3 (81.8)	10.7 (88.2)	0.0 (100.0)	3
Mancozeb	17.3 (80.7)	13.7 (84.8)	0.0 (100.0)	3
Propiconazole	17.6 (80.4)	0.0 (100.0)	0.0 (100.0)	3
Tricyclazole	10.0 (88.9)	0.0 (100.0)	0.0 (100.0)	3

Value in parenthesis is per cent inhibition of mycelial growth over control

1 = Harmless (<50% inhibition): 3 = Moderately harmful (80-90% inhibition)

2 = Slightly harmful (50-79% inhibition): 4 = Harmful (>90% inhibition)

The graphic results of Figure 1 indicate mean radial growth status of *B. bassiana* at three different concentrations of fungicides. In general, *B. bassiana* was highly sensitive to all the ten fungicides tested. At sub-normal concentrations, chlorothalonil, copper oxychloride and edifenphos were moderately toxic to *B. bassiana* with mean mycelial radial growth of 33.3, 24.6 and 21.3 mm respectively, as against 90 mm normal growth in poison free agar. The sensitivity of *B. bassiana to various pesticides* has been studied

primarily in the laboratory (Olmert and Kenneth 1974: Aguda *et al*., 1988; Vanninen and Hokkanen, 1988; Majchrowiez and Poprawski, 1993; Todorova *et al.,* 1998). Several fungicides including maneb, benomyl, mancozeb, copperoxychloride, chlorothalonil and carbendazim were reported to be either toxic or have fungistatic action on *B. bassiana* (Srivastava *et al.,* 1991; Gardner *et al.* 1979; Gupta *et al.,* 2002). Similarly, mancozeb recommended for control of foliar diseases in potato crop was detrimental to the survival of conidia of *B. bassiana* and impaired natural mycosis on the Colorado potato beetle, while foliar application of chlorothalonil and metalaxyl did not hamper natural mycosis in the same field environment (Clark *et al.,* 1982; Loria *et al.,* 1983). Natural infections of *B. bassiana* on soybean semilooper, *Rivula* sp., were strongly inhibited by foliar spraying of carbendazim and mancozeb intended for field control of soybean foliar diseases (Srivastava *et al.,* 1991). Fungal sporulation is less affected at low pesticide concentrations (Todorova *et al.,* 1998). Contradictory results and variation among fungal isolates of the same species in reaction to the same pesticide suggest that it is important to evaluate the compatibility of different strains of entomofungi with more commonly used selective fungicides.

Our results showed pronounced inhibition of *B. bassiana* by all the fungicides tested at all three concentrations. Of the fungicides, benomyl, carbendazim and hexaconazole exhibited very strong inhibition on the mycelial growth of *B. bassiana* even at lower concentrations. However, despite these adverse effects, a study by Vanninen and Hokkanen (1988) concluded that benomyl did not significantly inhibit the growth of *B. bassiana* in their trials. In favour of their results, Sandhu *et al.* (2001) experimented on the development of benomyl resistant *B. bassiana* strains and described their infectivity against the gram pod borer, *Helicoverpa armigera*. In our study, copper oxychloride, chlorothalonil, edifenphos, iprobenphos and mancozeb were found to be moderately harmful. In previous studies, the toxic effects of copper oxychloride, mancozeb, zineb (Tedders, 1981), and carbendazim (Machowicz-Stefaniak, 1985) have been reported on *B. bassiana.* Tedders (1981) found that copper oxychloride was innocuous to *B. bassiana.* Clark *et al* (1982) demonstrated that chlorothalonil strongly inhibited mycelial growth of *B. bassiana* in laboratory tests. In contrast, Nanne and Radcliffe (1971) found that the extent of mycosed aphids in fields treated with mancozeb was merely 4.0%; however in untreated control fields, the disease prevalence was 22.4%, which was four times as much as in fungicidal treatments. In another field study, chlorothalonil was not detrimental to the survival of conidia of *B. bassiana,* when used for the control of foliar diseases in potato ecosystems (Clark *et al.,* 1982). Similarly, Loria *et al* (1983) demonstrated that chlorothalonil had a neutral or stimulatory effect on *B. bassiana* sporulation. However, Rama Mohan Rao (1989) opined that co-application of fungicides and entomopathogenic fungi may not be a desirable practice due to high detrimental nature of fungicides.

From our data, all the fungicides tested though showed inhibition on the fungus growth, their rate of inhibition varied. It is clear from the studies of Hall and Dunn (1959) that any pesticides which are innocuous under *in vitro* conditions need not be safe in the open field conditions and vice-versa. Therefore, fungicides harmful in sensitivity tests on *in vitro* culture should be treated *in vivo* as well with the pathogen on the insect host to predict their interference in epizootics. Spore application early in the season, coupled with judicious use of selective fungicides could fit into a long-lasting pest management programme.

CONCLUSION AND RESEARCH NEEDS

The impact of fungicides on fungal entomopathogens has been extensively studied employing the poisoned food technique by incorporation of chemicals into agar, although some of the results are conflicting. Numerous previous investigations and the results of our experiment have clearly demonstrated high sensitivity of *B. bassiana* to commonly used crop protection fungicides. However, *B. bassiana* is one of the predominant fungal entomopathogens prevailing elsewhere in different cropped ecosystems. Consequently, it would be expected that the negative effects of fungicides on *B. bassiana* would occur during epizootics, either by killing or limiting fungal colonization. Selective fungicides that can be used to control plant diseases without any adverse effects on the biological properties of entomopathogenic fungi need to be screened. Systematic field studies, complemented by parallel laboratory experiments are essential for clear understanding of the ecological impact of fungicides on the introduced fungal entomopathogens. Continued research should also be concentrated on the characterization and improvement of biocontrol fungi for spotting fungicides resistant strains of *Beauveria* to be exploited in the integrated crop protection programme.

ACKNOWLEDGEMENTS

The study was conducted as partial fulfillment of the Doctor of Philosophy degree by the senior author at Tamil Nadu Agricultural University, Coimbatore, India. The study was supported in part by a grant from the Indian Council of Agricultural Research, New Delhi (India) in the form of Senior Research Fellowship.

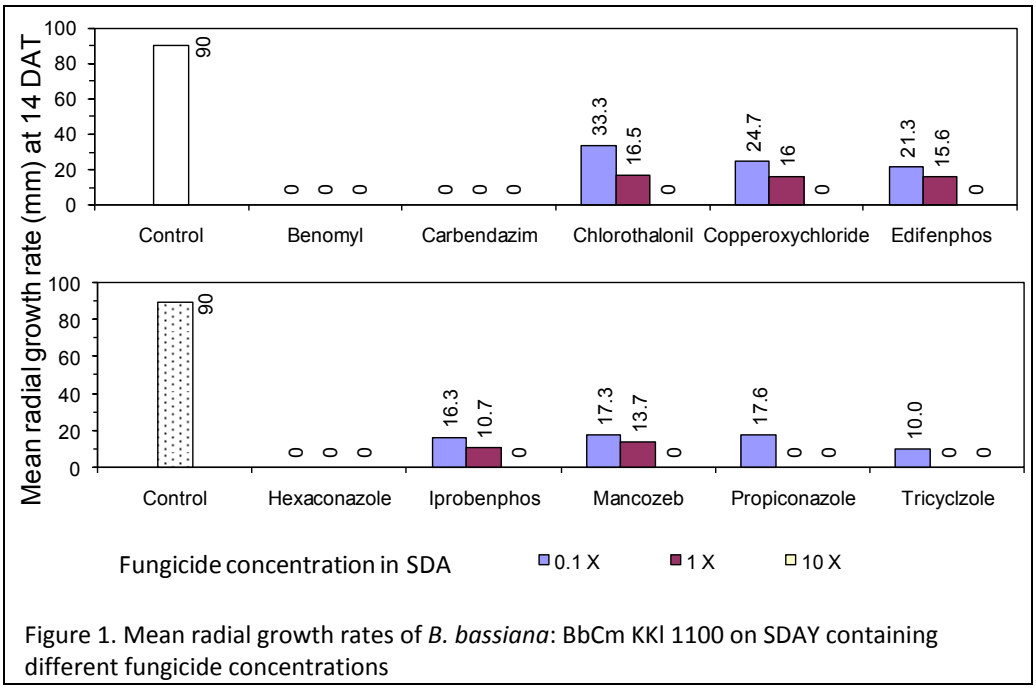


Figure 1. Mean radial growth rates of *B. bassiana*: BbCm KKl 1100 on SDAY containing different fungicide concentrations

REFERENCES

Aguda, R.M., Rombach, M.C. and Roberts, D.W. (1988). Effect of pesticides on germination and growth of three fungi of rice insects. *International Rice Research Newsletter,* 13(6): 39- 40.

Ambethgar, V. (1991). Biochemistry of epizootics of the fly fungus, *Pandora delphacis* (Hori.) Humber in the rice brown planthopper, *Nilaparvata lugens* (Stal), M.Sc. (Ag) Thesis, Annamalai University, Annmalainagar, India.

Ambethgar, V. (1997). Record of white muscardine fungus, *Beauveria bassiana* (Bals.) Vuill. on rice leaf folder complex from Karaikal, Pondicherry Union Territory (India). *Journal of Entomological Research,* 21 (2): 197-199.

Clark, R.A., Casagrande, R.A. and Wallace, D.B. (1982). Influence of pesticides on *Beauveria bassiana* a pathogen of the Colorado potato beetle. *Environmental Entomology,* 11: 67 - 70.

Crop Production Guide. (2000). Rice, Tamil Nadu Agricultural University, Coimbatore and Directorate of Agriculture, Chennai, Tamil Nadu, India.

Gardner, W.A., Sutton, R.M. and Noblet, R. (1979). Evaluation of the effects of six selected pesticides on the growth of *Nomuraea rileyi* and *Beauveria bassiana* in broth cultures. *Journal of Canadian Entomological Society,* 14: 106 -113.

Gupta, R.B.L., Sharma, S. and Yadava, C.P.S. (2002). Compatibility of two entomofungi *Metarhizium anisopliae* and *Beauveria bassiana* with certain fungicides, insecticides and organic manures, *Indian Journal of Entomology,* 64: 48-52.

Hall, I.M. and Dunn, P.H. (1959). The effect of certain insecticides and fungicides on fungi pathogenic to the spotted alfalfa aphid. *Journal of Economic Entomology*, 52: 28-29.

Hassan, S.A. (1989). Testing methodology and the concept of the IOBC/WPRS Working Group. In: *'Pesticides and Non-Target Invertebrates'* (Ed. Jepson, P.C.), Intercept, Wimborne, Dorset, pp.1-8.

Hazarika, L.K. and K.C. Puzari. (1990). *Beauveria bassiana* (Bals.) Vuill. for biological control of rice hispa (RH) in Assam, India. *International Rice Research Newsletter,*15: 31.

Hokkanen, H.M.T and Kotiluoto, R. (1992). Bioassay of the side effects of pesticides on *Beauveria bassiana* and *Metarhizium anisopliae*: standardized sequential testing procedure. *IOBC/WPRS Bull., XI (3)*: 148-151.

Loria, R., Galaini, S. and Roberts, W. (1983). Survival of inoculum of the entomopathogenic fungus, *Beauveria bassiana* as influenced by fungicides. *Environmental Entomology,* 12: 1724-1726.

Machowicz-Stefaniak, Z. (1985). The effect of systemic fungicides used in orchard protection on the growth of entomogenous fungi (Hyphomycetales: Mycophyta). *Roczniki Nauk Rolniczych,* 11: 63-75.

Majchrowiez, I. and Poprawski, T.J. (1993). Effects *in vitro* of nine fungicides on growth of entomopathogenic fungi. *Biocontrol Science and Technology,* 3: 321-336.

Moorhouse, E.R., Gillespie, A.T. Sellers, E.K. and Charnley, A.K. (1992). Influence of fungicides and insecticides on the entomogenous fungus, *Metarhizium anisopliae*, a pathogen of the vine weevil, *Otiorhynchus sulcatus*. *Biocontrol Science and Technology,* 2: 49-58.

Nanne, H.W. and Radcliffe, E.B. (1971). Green peach aphid population on potatoes enhanced by fungicides. *Journal of Economic Entomology*, 64: 1569-1570.

Olmert, I. and Kenneth, R.G. (1974). Sensitivity of entomopathogenic fungi, *Beauveria bassiana, Verticillium lecanii* and *Verticillium* sp. to fungicides and insecticides. *Environmental Entomology,* 3: 33-39.

Rama Mohan Rao, P. (1989). Studies on culture techniques, safety and control potential of certain entomopathogenic fungi of rice pests. Ph.D Thesis, *Tamil Nadu Agricultural University, Coimbatore, India*, 212 pp.

Sandhu, S.S., Unkles, S.E., Rajak, R.C. and Kinghorn, J.R. (2001). Generation of benomyl resistant *Beauveria bassiana* strains and their infectivity against *Helicoverpa armigera. Biocontrol Science and Technology*, 11: 245-250.

Srivastava, S.K., Singh, K.J. and Singh, O.P. (1991). Effect of fungicides on the infection of grey semilooper larvae (*Rivula* sp.) by *Beauveria bassiana* (Balsamo) Vuill. in soybean. *Journal of Biological Control,* 5 (1): 57-58.

Tedders, W.L. (1981). *In vitro* inhibition of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* by six fungicides used in peach culture. *Environmental Entomology,* 10: 346-349.

Todorova, S.I., Coderre, D., Duchesne, R.M. and Cote, J.C. (1998). Compatibility of *Beauveria bassiana* with selected fungicides and herbicides. *Biological Control,* 27: 427-433.

Vanninen, I. and Hokkanen, H. (1988). Effect of pesticides on four species of entomopathogenic fungi *in vitro*. *Annales Agriculturae Fenniae*. 27: 159-169.

V. AMBETHGAR[1] M. SWAMIAPPAN[2], R.J. RABINDRA[3] AND R. RABINDRAN[2]

[1]Regional Research Station TNAU, Vridhachalam-606 001, Tamil Nadu, India
[2.]Centre for Plant Protection Studies, TNAU, Coimbatore-641 003, Tamil Nadu, India
[3.]Project Directorate of Biological Control, ICAR, Bangalore-560 024, Karnataka, India
Corresponding Author E-mail: drva_1965@yahoo.co.in

Acute and Selective Toxicity of Diafenthiuron and Profenofos against *Sciothrips cardamomi* Ramk. and *Conogethes punctiferalis* Guenee of Small Cardamom

ABSTRACT: The acute toxicity of diafenthiuron (Polo®) and profenofos (Curacron®) was assessed against target pests (small cardamom thrips *Sciothrips cardamomi* Ramk., shoot and capsule borer C*onogethes punctiferalis* Guenee) and non-target pollinators of four species of honey bees (*Apis cerana indica* F, *Apis florea F*, *Apis mellifera* Capensis and *Trigona iridipennis* Smith) in terms of LD_{50}/LC_{50} and LD_{95}/LC_{95}. The LD_{50} values of diafenthiuron, profenofos and endosulfan to *C. punctiferalis* by topical application was 67.932, 0.116 and 0.254 µg respectively and to *S.cardamomi* by capsule dip bioassay was 23.492, 3.249 and 26.092 µg respectively. The LC_{50} value of diafenthiuron, profenofos and endosulfan by capsule dip bioassay was 156.410, 7.548 and 10.905 ppm to *A.mellifera*; 112.203, 6.381 and 9.420ppm to *A.c.indica*; 74.896, 5.835, and 8.909 ppm to *A. florea* and 49.520, 4.912 and 7.217ppm to *T.iridipennis*. The safety index was higher for diafenthiuron (6.86 to 14.34) and lower for profenofos (0.65 to 0.69) when compared to the standard endosulfan.

Introduction

The shoot and capsule borer *Conogethes punctiferalis* Guenee and thrips *Sciothrips cardamomi* Ramk. are two major pests in small cardamom of Western Ghats in South India. Both pests not only cause direct crop loss in terms of quality and quantity but also result in a decline in the total area of the plants over period of years. For management of these pests, planters mainly depend on chemical insecticides. An array of chemicals (quinalphos, monocrotophos, methyl parathion, fenthion, dimethoate, phosalone, phenthoate, carbosulfan, permethrin, fenvalerate and fluvalinate) against *S. cardamomi* (Kumaresan, 1983; Chandrasekaran, 1984; Kumaresan *et al*, 1988; Krishnamurthy *et al.,* 1989; Kumaresan and Gopakumar, 1993; Valarmathi,1997) and (fenthion, monocrotophos, chlorpyrifos, malathion, triazophos, dimethoate, quinalphos, endosulfan and lambda cyhalothrin) against *C. punctiferalis* on cardamom (Valarmathi, 1997; Spices Board, 2001; Sureshkumar *et al*.,2004) have been evaluated and found effective. Even though ecofriendly neem has been evaluated (Rajabaskar and Regupathy, 2005, 2008) and suggested the major focus falls on chemical control with insecticides among planters. In view of earlier experiences in terms of insect resistance to insecticides, repeated use of the same insecticide is to be avoided. For this, newer molecules are needed. Diafenthiuron (1-tert-butyl-3-(2, 6-di-isopropyl-4-phenoxyphenyl thiourea) is an effective novel insecticide cum acaricide (Soo *et al*., 2002) with a mode of action of inhibiting mitochondrial ATPase (Ruder *et a*l, 1991). It was found effective against *S.cardamomi* and *C.punctiferalis* on cardamom (Rajabaskar, 2003). Profenofos 50 EC is one of the insecticides registered in India found to be effective against *S.cardamomi* and *C.punctiferalis* on cardamom (Renuka, *et al*., 2002; Rajabaskar, 2003). The effect of pesticides on bees assumes a special significance in cardamom where pollination by bees is of tremendous importance. It is therefore necessary to generate the baseline toxicity of diafenthiuron and profenofos for future monitoring of the development of resistance and safety to honey bees.

MATERIALS AND METHODS

Insecticides: The insecticide dilutions required for assays were prepared from technical grade diafenthiuron of 93.5 percent purity, profenofos of 90.5 percent purity (M/S Syngenta India Ltd., Mumbai) and endosulfan of 98.75 percent purity (Aventis Crop Science India Ltd., Mumbai) by diluting with analytical grade acetone.

Bio assay

a) Thrips: The capsule dip bioassay method was followed. Uniform size capsules were dipped in different insecticide dilutions for five seconds, and then dried on filter paper in open air for 20 minutes. The capsules were placed in a 12 well plastic tray. Thrips nymphs collected from the field using an aspirator were placed on a black cloth. Using a fine zero point camel hair brush, 20 thrips were then transferred on to the treated capsules and the tray was covered with a lid. Capsules immersed in water alone served as control. The test was replicated thrice. Mortality was recorded 48 hours after exposure to the insecticides.

b) Shoot and capsule borer: The insects were mass cultured as per the methodology developed by Rajabaskar (2003). Field collected *C. punctiferalis* larvae were reared on castor and ginger hosts using a plastic tray kept under a mosquito net. The daily emerged adults were sexed and caged separately for observation. Five pairs were allowed to lay eggs on a ovipositional substrate in the cage (60 x 30 x45 cm) and a cotton pad soaked with 10 percent sugar solution was provided as adult food. The oviposition substrate contained two plastic tea stainers put in a juxta position (8cm diameter) containing host material as an odour

source and was wrapped with khada cloth. This was then hung from the top of the cage for oviposition. The eggs obtained were utilized for further culturing of the test insect. The topical application method was followed considering the internal feeding habits of the pest and their avoidance of treated surfaces. The larvae weighing 18 - 22 mg (length 1.2 cm) were used for bioassays. An aliquot of 1 µl of a known dilution of an insecticide was placed on the thoracic dorsum of each larva using 1µl repeating dispenser (PB 600 -01, Hamilton Co. Ltd.,) fitted with a 50 µl syringe and Rheodyne needle. The control was treated with acetone alone. No less than 30 larvae per dose were used per treatment. Mortality counts were taken 24 hours after treatment.

c) Honeybees: The dry film contact toxicity method used by Rajathi *et. al*. (2006) was followed. One to three day old honeybee workers of each species (*Apis cerana indica* F, *Apis florea F*, *Apis mellifera* Capensis and *Trigona iridipennis* Smith) were obtained from the apiary of Tamil Nadu Agricultural University, Coimbatore, India and conditioned for 20 hours at the standard experimental conditions. Filter paper of 7 cm diameter was impregnated with 0.5 ml of insecticide test solution and dried. The filter paper was then placed in a plastic container with sufficient aeration for the bees. Worker bees were anaesthetized by keeping them in a refrigerator for a few minutes. Approximately 30 bees were quickly transferred with the help of paper used like a spoon into each of the containers. They were then covered with black cloth in order to reduce their flying or "balling" so as to bring about maximum contact with the treated surface of filter paper. The bees were kept in contact with the treated surface of filter paper for one hour, after which the bees were transferred into cages (40x40x40 cm) made of muslin cloth over an iron frame and provided with cotton soaked in 40 percent sucrose solution as a source of food. The bees were observed for mortality 20 hours later. Moribund honey bees were counted as dead. Acetone was used as a control. Endosulfan was included as a standard check for comparison. The median lethal dose/concentration (LD_{50}/LC_{50}) of insecticides used was determined (Regupathy and Dhamu, 2001). The safety index was worked by the following formula.

$$\text{Safety index} = \frac{LC_{50} \text{ of test compound}}{LC_{50} \text{ of standard}}$$

RESULT AND DISCUSSION

Profenofos was found to be more toxic to nymphs of *S.cardamomi* than diafenthiuron as revealed by the LC_{50} values of 3.249 and 23.492 ppm respectively (Table 1), whereas LC_{50} of endosulfan was 26.092 ppm. The LC_{50} of profenofos by capsule dip method to nymphs of *S.cardamomi* was 3.25ppm and was 8.03 times toxic than that of endosulfan. The LD_{50} of diafenthiuron, profenofos and endosulfan estimated by topical application to third instar larvae of *C. punctiferalis* was 67.932, 0.116 and 0.254 µg/larva respectively (Table 2). Profenofos was found to be 2.19 times more toxic than endosulfan. Renuka and Regupathy (2008) reported the LD_{50} value of profenofos and endosulfan as 0.082 and 0.105 ppm to *C.punctiferalis* respectively. Bishara and Shakeel (1985) reported the LD_{50} value of profenofos by topical application to larvae of *P. gossypiella* as 12.49. The lowest LD_{50} value was recorded by Kosugi (1999) against smaller tea tortrix *Adoxophyes honmai*.

The LC_{50} values of diafenthiuron, profenofos and endosulfan was 156.410, 7.548 and 10.905 ppm to *A.mellifera*; 112.203, 6.381 and 9.420ppm to *A.c.indica*; 74.896, 5.835, and 8.909 ppm to *A. florea* and 49.520, 4.912 and 7.217 to *T.iridipennis* (Tables 3-6). The safety index was higher for diafenthiuron (6.86 to 14.34) and lower for profenofos (0.65 to 0.69) when compared to the standard endosulfan. Profenofos was more toxic to bees than endosulfan, while diafenthiuron was less toxic. The relative toxicity of profenofos was 1.44, 1.48, 1.53 and 1.47 fold, and that of diafenthiuron was 0.07, 0.08, 0.12 and 0.15 fold to *A. mellifera, A.c.indica, A. florea* and *T. iridipennis* respectively in comparison with endosulfan. The toxicity of profenofos is relatively low when compared to other insecticides used in cardamom. Phosalone and chlorpyrifos were 1.96 and 38.25 times more toxic than that of endosulfan, respectively, by topical application (Mishra and Verma, 1982), while quinalphos was 13.25 times more toxic by the dry film method (Deshmukh, 1991). Profenofos was found to be moderately toxic to honey bees as earlier reported by El-Banby and Kansouh (1981) and Hameed and Singh (1998), and it was also found to be toxic in nectar only on the day of application while pollen was the least toxic material tested compared to leaves and petals in cotton. Cardamom, being a highly cross pollinated crop where honey bee is the principal pollinating agent, increases fruit set considerably when compared to flowers prevented from bee visits (Chandran *et al.,* 1983). Cardamom flowers remain open for 15 - 18 hours, and stigma receptivity to pollen is at its peak for just 3 - 4 hours (between 8.00 a.m. to 12.00 noon) (Krishnamurthy *et al.,* 1989). This is the most critical period for pollination. If pollination does not occur, by late evening the flower withers away and dies. Maximum foraging activity of bees has been found during 08.00 - 11.00 and 04 – 16.00 hours of the day. Therefore, emphasis should be given to control the pests effectively with minimal hazard to honeybees. It is desirable that profenofos 50 EC should be applied by avoiding peak activity of honeybees. Profenofos is one of the insecticides with low residual toxicity (Hameed

and Singh, 1998) and profenofos 50 EC being an emulsion will be less hazardous when compared to dusts and wettable powders, as bees are less likely to contact with emulsions, because they are readily absorbed in plant tissues (Mishra and Sharma, 1988). Diafenthiuron, a new molecule of insecticides, inhibits growth of the insect and also is found to be less toxic to honeybees when compared to profenofos and endosulfan. The effectiveness of diafenthiuron has been reported against sap feeders like *S.cardamomi* on cardamom (Rajabaskar,2003), *B. tabaci* on cotton (Chinnabbai, *et al*., 2000; Cai, 1998) and brinjal (Saradha and Nachiappan, 2003), *S. dorsalis* on hot pepper (Vos *et al.,*1991) and lepidopteron pests, *P. xylostella* (Zuhua and Shusheng, 1998; Ellis *et al*., 1992) and *S .litura* (Cheng *et al*.,1999). Instead of using repeated application of conventional insecticides, a new insect growth regulator, like diafenthiuron, could be effectively used in a rotation with profenofos for IRM programme. This will minimize risk to honeybees and other non target organism by exploiting ecological selectivity. Moreover the IPM modules developed by Rajabaskar (2003) which comprises sequential application of neem, profenofos, diafenthiuron, neem and profenofos was found to be effective against *C. punctiferalis* and another module comprising sequential application of neem , diafenthiuron, diafenthiuron , profenofos and profenofos was found to be effective against *S.cardamomi*. These IPM modules may be included for minimizing the resistance problem while managing *S.cardamomi* and *C.punctiferalis* in the cardamom plantation.

Table 1. LC_{50} values of diafenthiuron, profenofos and dimethoate to *S.cardamomi*-Capsule dip bio assay

S1. No	Chemical	LC_{50} (ppm)	95% fiducial limits		Y= a + bx	x^2 at P= 0.05	Relative Toxicity
			UL	LL			
1.	Diafenthiuron	23.492	43.046	12.821	0.7799+0.9655x	1.562^{NS}	1.110
2.	Profenofos	3.249	4.318	2.445	1.6793+1.9020x	1.002^{NS}	8.03
3	Endosulfan	26.092	39.046	17.435	0.4043+1.2241x	0.203^{NS}	1.00

n= number of thrips used (20)
NS= Not significant

Table 2. LD_{50} values of profenofos ,diafenthiuron and endosulfan to third instar larvae of *C. punctiferalis* - Topical method (µg/larva)

S1. No	Chemical	LD_{50} (µg)	95% fiducial limits		Y= a + bx	x^2 at P= 0.05	Relative Toxicity
			UL	LL			
1.	Diafenthiuron	67.932	90.898	50.768	4.0993+1.8831x	0.589^{NS}	0.004
2.	Profenofos	0.116	0.160	0.084	1.2238+1.8287x	0.658^{NS}	2.190
3	Endosulfan	0.254	0.371	0.174	1.3725+1.5088x	2.190^{NS}	1.00

n= number of larvae used (30)
NS= Not significant

Table 3. LC_{50} values of diafenthiuron , profenofos and endosulfan to honey bee, *A. mellifera*

S1. No	Chemical	LC_{50} (ppm)	95% fiducial limits		Y= a + bx	x^2 at P= 0.05	Safety Index	Relative Toxicity
			UL	LL				
1.	Diafenthiuron	156.410	218.897	111.298	3.0761+1.5548	1.873^{NS}	14.34	0.07
2.	Profenofos	7.548	10.332	5.514	1.8128+1.7569	5.954^{NS}	0.69	1.44
3	Endosulfan	10.905	18.220	6.527	1.1435+0.9552	1.752^{NS}	1.00	1.00

n= number of larvae used (30)
NS= Not significant

Table 4. LC_{50} values of diafenthiuron , profenofos and endosulfan to *A.c. indica*

S1. No	Chemical	LC_{50} (ppm)	95% fiducial limits		Y= a + bx	x^2 at P= 0.05	Safety Index	Relative Toxicity
			UL	LL				
1.	Diafenthiuron	112.203	154.479	81.497	4.0617+1.7944	0.896 ^{NS}	11.91	0.08
2.	Profenofos	5.835	8.565	4.751	1.1220+1.0298	3.614 ^{NS}	0.68	1.61
3	Endosulfan	9.420	16.443	5.397	1.5405+0.8705	1.661 ^{NS}	1.00	1.00

n= number of bees used (30)
NS= Not significant

Table 5. LC_{50} values of diafenthiuron , profenofos and endosulfan to *A. florea*

S1. No	Chemical	LC_{50} (ppm)	95% fiducial limits		Y= a + bx	x^2 at P= 0.05	Safety Index	Relative Toxicity
			UL	LL				
1.	Diafenthiuron	74.896	94.504	59.357	6.5142+2.3671x	1.873 ^{NS}	8.41	0.12
2.	Profenofos	6.381	9.594	3.548	2.5763+1.9912x	5.954 ^{NS}	0.65	1.40
3	Endosulfan	8.909	14.973	5.301	0.9124+1.0499x	2.136 ^{NS}	1.00	1.00

n= number of bees used (30)
NS= Not significant

Table 6. LC_{50} values of diafenthiuron, profenofos and endosulfan to *T. iridipennis*

S1. No	Chemical	LC_{50} (ppm)	95% fiducial limits		Y= a + bx	x^2 at P= 0.05	Safety Index	Relative Toxicity
			UL	LL				
1.	Diafenthiuron	49.520	69.000	35.542	3.4565+1.8013x	0.242 ^{NS}	6.86	0.15
2.	Profenofos	4.912	6.493	3.716	2.9148+2.1442x	4.131 ^{NS}	0.68	1.47
3	Endosulfan	7.217	11.877	4.386	1.0578+1.0217x	2.522 ^{NS}	1.00	1.00

n= number of bees used (30)
NS= Not significant

REFERENCES

Bishara, S.I.and M.A. Shakeel. 1985. Toxicity levels of some insecticides to the pink bollworm in district Multan, Pakistan. *Proc. Entomol. Soc. Karachi*, **14/15**:107-112.

Cai, L.W. 1998. Efficacy of insecticides for controlling *Plutella xylostella* L. of cabbage: *Prog. Hort*., **31**(3): 176-180.

Chandran, K., P. Rajan, D. Joseph and M.C. Suryanarayana. 1983. Studies on the role of honey bees in the pollination of cardamom. *Proc. Second Int. Conf. Apic. Trop. Climates,* IARI, New Delhi. pp 497-504.

Chandrasekaran, R.1984. Field control of cardamom thrips. **In:** Venkataram, C.S. (Ed.) *Proc. PLACROSYM-V***,** Indian Society for Plantation Crops, Kasaragod, Kerala. pp 644-652

Cheng, X.F., C.Z. Liao and W.Y.Han.1999.Preliminary report on tests for preventing and controlling *Spodoptera exigua* with some new type insecticides. *China Vegetables.* **3**: 22-24.

Chinnabbai, C., G.V.Subbarathnam and T. Madhumathi.2000.Relative toxicity of some new insecticides to resistant population of *Bemisia tabaci* (Gennadius) infesting cotton in Andhra Pradesh. *Pest Mgmt. Econ. Zool.* **8**: 1-4.

Deshmukh, P.B. 1991. Toxicity of some insecticides to Indian honey bee *Apis indica* F. *Pestology*, **15**(9): 31-33.

El-Banby, M.A. and A.S.H. Kansouh.1981.The residual toxicity to honeybees of certain insecticides on different parts of cotton plants. *Proc. XXVII Int. Congr. Apiculture***,** *Acapulco,* 1981. pp.319-329.

Ellis, W. W.; Sribuddhachart, J.; Chaimongkol, I. and Hare, C. J. 1992. Diafenthiuron: field studies in *Plutella xylostella* (L) control in SE Asia, 1986-88.In: Ooi, P. A. C., G. S.Lim and P. S.Teng (Eds). *Proc. 3rd Int.Conf. Pl. Prot. Tropic., Genting Highlands*, Malaysia, 20-23. March 1990, 2, 66-70.

Hameed, S.F. and S.P. Singh. 1998. Pesticide bee poisoning in changing scenario of Indian Agriculture. **In**: Mishra, R.C. and Rajesh Garg (Eds.).*Perspectives in Indian Apiculture.* 1997-98. Agro Botanica, Bikaner. pp.334-362.

Kosugi, Y. 1999. Difference in efficacy of insecticides to oriental tea tortrix, *Homona magnanima* between either surface and both

surface treatments of tea leaves. *Annual Report of the Kanto-Tosan Pl. Prot. Soc.,* **46** : 129-131.

Krishnamurthy, K., M.M. Khan, K.K. Avadhani, J. Venkatesh, A.L. Siddaramaiah, A.K. Chakravarthy and S.R. Gurumurthy. 1989. *Three Decades of Cardamom Research at Regional Research Station, Mudigere (1958-1988).* Station Technical Bulletin No.2. Regional Research Station, Mudigere, Karnataka. 94p

Kumaresan, D. 1983. Field evaluation of insecticides for the control of cardamom thrips. *South Indian Hort.,* **31**: 151-152.

Kumaresan, D. and B. Gopakumar. 1993. Evaluation of fluvalinate and methamidophos against cardamom thrips *Sciothrips cardamomi* (Ramk.). *Pestology***, 17**(6): 34-36.

Kumaresan, D., A.Regupathy and P.Baskaran. 1988. *Pests of Spices*. Rajalakshmi Publications, Nagercoil, India. 241p.

Mishra, R.C. and A.K. Verma. 1982. Relative toxicity of some insecticides to *Apis cerana* F. workers. *Indian Bee J.,* **44**(3): 67-71.

Mishra, R.C. and S.K. Sharma. 1988. Save bees – Use Pesticides judiciously. *Bulletin No.1, AICP-Honeybee Research and Training,* Haryana Agric, University, Hisar. pp.12.

Rajabaskar, D. 2003. Studies on the evaluation of IPM modules against *Conogethes punctiferalis* Guenee and *Sciothrips cardamomi* Ramk. on cardamom. , Ph.D., Thesis, Tamil Nadu Agric University, Coimbatore.

Rajabaskar, D. and A. Regupathy 2005. Evaluation of neem formulations against cardamom shoot and capsule borer (*Conogethes punctiferalis* Guenee) and its effects on honey bee visits in cardamom plantation. In:Ignacimuthu, S. and S.jayaraj (eds) Proc. *National Symposium on Green pesticides for Insect Pest Management*, February 5-6, 2003, Loyala College, Chennai- p96-99. Narosa Publishing House, Delhi.ISBN 82-7319-686-9.

Rajabaskar,D. and A.Regupathy.2008.Deterrence of shoot and capsule bore, C*onogethes punctiferalis* Guenee and thrips, S*ciothrips cardamomi* Ramk. of small cardamom by certain neem formulations. *Res. Report. 67th Annual Pacific Northwest Insect Management Conference , January* 7 & 8, 2008, Portland, OR 97204, USA.p.19-22.

Rajathi, D.S., S.V. Krishnamoorthy and A. Regupathy. 2006. Selective toxicity and discriminating dose of lambda cyhalothrin 5 CS against *Earias vitella* Fab. Resistant Pest Management Newsletter 15 (2): 35-38.

Regupathy, A. and K.P. Dhamu. 2001. *Statistics Workbook for Insecticide Toxicology.* Softeck Computers, Coimbatore. 206p.

Renuka,S. and A.Regupathy.2008. Acute and selective toxicity of profenofos against the shoot and capsule borer C*onogethes punctiferalis* Guenee of small cardamom. Resistant Pest Management Newsletter 17(2): 33-35.

Renuka,S.,A. Regupathy and D. Rajabaskar**.** 2002. Bio-efficacy of profenofos (Curacron 50EC) against cardamom thrips *Sciothrips cardomomi* (Ramk). **In** :Rethinam, P., H. H. Khan, V. M. Reddy, P. K. Mandal and K. Suresh (Eds.). *Proc. PLACROSYM XIV*, 12-15 December 2000, Hyderabad, India 491-492.

Ruder, F.J., W. Guyer, J.A. Benson and H. Kayser. 1991. The thiourea insecticide / acaricide diafenthiuron has a novel mode of action: inhibition of mitochondrial respiration by its carbodiimide product. *Pestici. Biochem. Physiol.,* **41**: 207-219.

Saradha, C. and R.M. Nachiappan. 2003. Evaluation of diafenthiuron 50% WP (Polo) against whitefly,*Bemisia tabaci* Genn. (Aleyrodidae: Homoptera) on Brinjal (*Solanum mlongena*). *Pestology*, **27**(1): 12-14.

Soo, K.Y., K.J. Han, K.Y. Whan, K. Kyun and Q.X. Li. 2002. Photo degradation of diafenthiuron in water. *Pest Mgmt Sci*., **58**(5): 496-502.

Spices Board. 2001. *Cardamom Package of Practices. Spices Board,* Cochin-25. p 51.

Suresh Kumar, R.S., D. Rajabaskar, K. Chozhan and A. Regupathy. 2004. Evaluation of a new insecticide Lambda cyhalothrin (Kerate® 5EC) for management of shoot and capsule borer *Conogethes punctiferalis* (Guen.) on cardamom. *Pestology* Vol. XXVIII No. :40-433

Valarmathi, S. 1997. Bioefficacy and residues of quinalphos applied as ecofriendly formulations aquaflow (AF) and colloidal suspension (CS) on cardamom and cotton. Ph.D. Thesis, Tamil Nadu Agricultural University, Coimbatore.161p.

Valarmathi, S. and A. Regupathy. 2004. LD50 values of quinalphos to third instar larvae Conogethes punctiferalis (g/ larva). National symposium on "Recent trends in applied biology" January 28th & 29th, 2004. Avinashilingam Home Science University, Coimbatore. Abst.

Vos, J. G. M., S.Sastrosiswojo, T.S.Uhan and W.Setiawati, 1991. Thrips on hot peppers in Java, Indonesia. AVRDC Publication, (91-342): 18-28.

Zuhua, S. and L.Shusheng.1998. Toxicity of insecticides commenly used in vegetable fields to the diamond back moth, *Plutella xylostella* and its parasite, *Cotesia plutellae. Chinese J. Biol. Cont.* 14(2): 53-57.

D.Rajabaskar[1] and A.Regupathy
Department of Entomology, Tamil Nadu Agricultural University, Coimbatore- 641003, India
[1]Present address: Assistant Professor (Agricultural Entomology), Tapioca and Castor Research Station, Yethapur-636 119, Tamil Nadu, India.
email:rajabaskard@yahoo.co.in

CHANGE IN SUSCEPTIBILITY IN MALE HELOPELTIS *THEIVORA* WATERHOUSE (JORHAT POPULATION, ASSAM, INDIA) TO DIFFERENT CLASSES OF INSECTICIDES

(Susceptibility change in male *Helopeltis theivora* to different insecticides)

ABSTRACT

By using dry film bioassay method, relative toxicity of thirteen insecticides was evaluated against the male *Helopeltis theivora* (Jorhat population, Assam). On the basis of LC_{50} values, the order of toxicity was found to be cypermethrin > λ-cyhalothrin > fenpropathrin > thiomethoxam > acephate > profenophos > deltamethrin and dimethoate > oxydemeton methyl > imidacloprid > phosalone > quinalphos > endosulfan. The relative toxicity calculated on the basis of the LC_{50} value of endosulfan which proved that cypermethrin (26.75 times) followed by λ-cyhalothrin (24.76 times), and fenpropathrin (16.03 times) are more toxic than endosulfan. Effective field dosages of the thirteen insecticides were computed based on the LC_{50} values and compared with the recommended dosages. Pronounced shift in the level of susceptibility of male *H. theivora* to all the chosen insecticides (1.04 – 82.85 times increase) except acephate was noticed. The decrease in susceptibility of *H. theivora* to deltamethrin and imidacloprid was the largest. Monitoring of resistance development in field populations of *H. theivora* in North East India could be done by using these base-line values of different insecticides established in the present study.

Key words: Relative toxicity, Susceptibility change, *Helopeltis theivora*, Tea

INTRODUCTION

The tea mosquito bug, *Helopeltis theivora* (Miridae : Heteroptera), is one of the notorious pests of tea, infestation has been increasing day-by-day in an alarming proportion all over northeast India and a major problem for the tea planters in terms of its control. Due to its feeding on young succulent shoots, the quality of made tea is deteriorated and causes a crop loss to the tune of 10-15% (Rahman *et al*., 2005; Somnath Roy *et al*., 2008a). The insecticide consumption has been doubled from 4.47-7.67 l/ha to 7.35-16.75 l/ha due to its escalating population trend, and cost of control has increased by Rs.2500 to Rs.6000 per hectare (Chakravartee, 1994; Borbora and Biswas, 1996; Gurusubramanian and Bora, 2008; Gurusubramanian *et al*., 2008; Somnath Roy *et al*., 2008). Based on our recent survey it was found that male population outnumbered the female (1:2.12 female: male). The resistance developed by population of *H. theivora* collected from different regions of North East India ranged from 1.47 – 62.99 fold for males and 1.25 – 62.82 fold for females to different insecticides (Gurusubramanian and Bora, 2008), and further, three different colour variants were observed in both male and females of *H. theivora* (Gurusubramanian and Bora, 2007). Against this backdrop, a suspicion was raised as to whether this pest in Jorhat, Assam, India would develop a strategy of change in their susceptibility towards different classes of insecticides. In order to confirm the above fact, a study was conducted to assess the relative toxicity and expected effective concentration of some commonly used thirteen insecticides of organochlorine, organophosphates, synthetic pyrethroids and neonicotinoids against males of *H. theivora* collected from Jorhat, Assam, India.

MATERIALS AND METHODS

Collection of test insects and insecticides used

Helopeltis theivora males were collected from the plots of Tocklai experimental station, Jorhat. Field collected males were preconditioned for seven days in the laboratory (27± 2^{o} C, 70-80% RH and a 16:10 LD photoperiod). Thirteen insecticides were used (endosulfan-Thiodan; acephate-Asataf; dimethoate-Rogor; quinalphos-Flash; profenophos-Celcron; oxydemeton methyl-Metasystox; phosalone-Zolone; deltamethrin-Decis; cypermethrin-Challenger; λ-cyhalothrin-Karate, fenpropathrin-Meothrin); imidacloprid-Chemida; thiomethoxam-Actara) in this study and their formulation details are given in Table 1.

Median lethal dose and Probit analysis

Graded concentrations of insecticides were prepared in distilled water from commercial formulations of the insecticides. TV 1 clone two and a bud healthy shoots were collected from the Tocklai experimental garden plots and brought to the laboratory. The leaves were washed thoroughly with distilled water and air dried. Fifteen tea shoots for each treatment were sprayed with each insecticide separately at the respective dilutions by using a glass atomizer; then, they were kept in a glass tube containing water and wrapped with cotton. The sprayed tea shoots were kept under a ceiling fan for the evaporation of the emulsion for 15 minutes. Glass tubes containing tea shoots were placed in the glass chimneys. The muslin cloth was tied with the help of rubber bands on the top of the glass chimneys, and the tubes were kept at 27 ± 2^{o}C in culture room. Thirty field collected and preconditioned males of *H. theivora* were released separately in each glass chimney containing tea shoots. Observations for adult mortality were recorded in all the five replications of each concentration for 24 hours after the treatment. Moribund insects were counted as dead (Rahman *et al*., 2006). Five to seven concentrations of each insecticide were tested to obtain the concentration – probit mortality curve. The mortality data was converted to percent mortality and subjected to probit analysis (Finney, 1971; Busvine, 1971) to obtain LC_{50} and LC_{95} values and a regression equation, from which the relative toxicity values were calculated by taking LC_{50} and LC_{95} values of endosulfan and dimethoate for males, as unity.

Calculation of expected effective concentration and expected effective dose

The expected effective concentration of each insecticide was calculated by doubling the LC_{50} value (%) to attain a LC_{100} value, and then the effective field dosages of the thirteen insecticides were computed based on the following formula and compared with recommended dosages as per the standard method of Misra (1989):

Expected effective concentration (LC_{100}) (%) = 2 X LC_{50} %

Expected effective dose (g a.i./ ha) = ED /100 X EC X 20 fold

ED = Percent Concentration / EC X 1000 X 400 litres of spray fluid/ha

RESULTS AND DISCUSSION

The lowest LC_{50} value was determined to be cypermethrin, which was followed by λ-cyhalothrin, fenpropathrin, thiomethoxam, acephate, profenophos, deltamethrin, dimethoate, oxydemeton methyl, imidacloprid, phosalone, quinalphos and finally endosulfan (Table 1). However, at the LC_{95} level, the toxicity of the insecticides differed and the toxicity in descending order was: cypermethrin > thiomethoxam > phosalone > acephate > fenpropathrin > quinalphos > endosulfan > deltamethrin > λ-cyhalothrin > imidacloprid > profenophos > dimethoate and

oxydemeton methyl. The data on the dosage-mortality response of the male population of *H. theivora* collected from Jorhat area revealed that chi-square values indicated a good fit of the probit responses in all the bioassays showing that there was no heterogeneity between observed and expected responses. The values of relative toxicity when calculated from the LC_{50} value of endosulfan showed that all the insecticides were found to be more toxic than endosulfan (Table 1) whereas at the LC_{95} level dimethoate and oxydemeton methyl were used together (Table 1). Based on relative toxicity derived on the basis of LC_{50} and LC_{95} values, the highly toxic and most effective insecticide against the male *H. theivora* was cypermethrin (Misra, 1989; Kapoor, 2002; Gurusubramanian and Bora, 2008) which was observed to be 26.75 times more toxic than endosulfan at the LC_{50} value and 10.47 times than dimethoate and oxydemeton methyl at the LC_{95} value (Table 1). Among synthetic pyrethroids, a higher LC_{50} value was observed in deltamethrin than cypermethrin, λ-cyhalothrin, and fenpropathrin wherein 6-10 times lesser LC_{50} values were recorded. Among the organophosphates, acephate and profenophos are highly toxic; dimethoate and oxydemeton methyl are moderately toxic; and phosalone and quinolphos are least toxic to male *H. theivora* (Table 1). Thiomethoxam and imidacloprid being the new generation molecules like neonicotinoids, falling under the category of highly and moderately toxic, respectively (Table 1).

Generally it is accepted that the field application rate of insecticides should at least be 20 folds or more of the LC_{50} value determined through the bioassay method for achieving satisfactory control of the pest in agriculture (Misra, 1989; Kapoor, 2002). By this simple logic, the expected effective dosages of the chosen thirteen insecticides were calculated and their results are summarized in Table 2. When these computed dosages were compared with the recommended dosages of the insecticides, it was observed that 1.04 – 82.85 times more of the recommended dosages of different insecticides might be required to achieve desirable control of the pest (Table 2). Higher dosage difference was noticed in deltamethrin and imidacloprid than in other insecticides (Table 2). However, there was no change in acephate, which may prove effective even at a lower dose than the recommended dose. A comparison of the expected effective doses of thirteen insecticides based on their LC_{50} values with recommended dose revealed a pronounced shift in the level of susceptibility of the male *H. theivora* to all of the chosen insecticides except acephate. One of the reasons for the change in susceptibility to insecticides was the over use of synthetic pyrethroids (8-16 rounds per year) against this pest (Sannigrahi and Talukdar, 2003; Gurusubramanian *et al*., 2005). Among the neurotoxins, pyrethroids were the most toxic compounds to the Jorhat population of male *H. theivora* followed by neonicotinoids, organophosphates and organochlorine. The same results were observed in male and female *H. theivora* collected from Darjeeling (Bora *et al*., 2007) and Jorhat (Gurusubramanian and Bora, 2008). Further, the susceptibility of male *H. theivora* to various organophosphates varied greatly as reported earlier in Jorhat, Dooars and Darjeeling populations of *H. theivora* (Rahman *et al*., 2006; Bora *et al*., 2007). The base-line values of different insecticides established in the present study can be used to monitor resistance development in field populations of *H. theivora* in North East India.

Table 1. Relative toxicity values of different insecticides against male *Helopeltis theivora* (Jorhat population)

Insecticide	χ^2	Regression equation	LC_{50} (ppm)	LC_{95} (ppm)	S.E.	Fiducial limits (95%)	Relative toxicity	
							LC_{50}	LC_{95}
Endosulfan 35 EC	4.051	Y=6.050x-6.500	79.983	295.12	3.89	86.69-72.44	1.000	2.817
Cypermethrin 10 EC	9.250	Y=3.800+2.780x	2.990	79.43	4.71	3.54 – 2.50	26.750	10.470
Deltamethrin 2.8 EC	1.343	Y=1.649+2.590x	29.992	363.08	4.71	48.75-18.40	2.667	2.290
Fenpropathrin 30 EC	4.850	Y=4.029+1.414x	4.988	257.03	3.50	6.88 – 3.61	16.035	3.236
λ-cyhalothrin 5 EC	0.441	Y=3.999+1.904x	3.230	501.18	3.60	3.95 – 2.52	24.762	1.659
Acephate 75 SP	1.640	Y=2.148+2.502x	14.790	251.88	4.66	18.13 – 12.06	5.407	3.302
Dimethoate 30 EC	1.443	Y=1.320+2.590x	29.992	831.76	4.71	36.64– 24.43	2.667	1.000
Oxydemeton methyl 25 EC	6.450	Y= 3.100x - 0.032	39.810	831.76	3.60	46.00-34.00	2.009	1.000
Phosalone 35 EC	1.220	Y=4.758x – 3.482	58.884	251.18	3.70	64.85 – 53.48	1.358	3.311
Profenophos 50EC	1.8500	Y=2.300+2.100x	19.950	794.38	3.58	24.50 – 16.20	4.009	1.047
Quinalphos 25 EC	6.800	Y= 5.400x – 5.200	79.430	269.15	3.50	87.00-72.10	1.006	3.090
Imidacloprid 17.8 SL	6.689	Y=1.628+2.120x	39.811	630.96	3.61	49.54– 31.98	2.009	1.320
Thiomethoxam 25 WG	0.942	Y=0.105+ 4.780x	10.000	280.92	4.70	11.14 – 8.97	7.998	3.981

Table 2. Comparison of effective field dosages with recommended dosages of different insecticides against male *Helopeltis theivora* (Jorhat population)

Insecticide	LC_{50} (%)	Expected effective concentration (%)	Expected effective dose g a.i./ha	Recommended dose g a.i./ha	Times increase
Endosulfan 35 EC	0.0079	0.0158	1264	350	3.61
Cypermethrin 10 EC	0.00029	0.00058	46.4	10	4.64
Deltamethrin 2.8 EC	0.0029	0.0058	464	5.6	82.85
Fenpropathrin 30 EC	0.00049	0.00098	78.4	75	1.04
λ-cyhalothrin 5 EC	0.0003	0.0006	48	10	4.80
Acephate 75 SP	0.0014	0.0028	224	750	0.29
Dimethoate 30 EC	0.0029	0.0058	464	300	1.54
Oxydemeton methyl 25 EC	0.0039	0.0078	624	250	2.49
Phosalone 35 EC	0.0058	0.0116	928	350	2.65
Profenophos 50EC	0.0019	0.0038	304	200	1.52
Quinalphos 25 EC	0.0079	0.0158	1264	250	5.04
Imidacloprid 17.8 SL	0.0039	0.0078	624	23.49	26.56
Thiomethoxam 25 WG	0.0010	0.0020	160	50	3.20

ACKNOWLEDGEMENT

The authors are grateful to the Director, Tocklai Experimental Station, Tea Research Association, Jorhat, Assam for providing necessary facilities, constant encouragement and valuable suggestions and, also, wish to thank In-charge, Statistics Department, TRA, Jorhat for statistical analysis.

REFERENCES

Bora S, Sarmah M, Rahman A and Gurusubramanian G (2007) Relative toxicity of pyrethroid and non-pyrethroid insecticides against male and female tea mosquito bug, *Helopeltis theivora* Waterhouse (Darjeeling strain). *Journal of Entomological Research* 31 (1): 37-41.

Borbora, B.C. and Biswas, A.K. 1996. Use pattern of pesticides in tea estates of North East India. *Two and Bud* 43 (2): 4-14.

Busvine, J.R., 1971. *A critical review of the techniques for testing insecticides*, C.A.B. Publication, London, pp. 271-276.

Chakravartee, J. 1994. Tea pest management: Present Status and Research Needs. *Two and Bud*, 43 (1): 23-32.

Finney, D.J. 1971. *Probit Analysis*, Cambridge University Press, Cambridge, pp. 333.

Gurusubramanian, G. and Bora, S. 2007. Relative toxicity of some commonly used insecticides against adults of *Helopeltis theivora* Waterhouse (Miridae: Hemiptera) collected from Jorhat area plantations, South Assam, India. *Resistant Pest Management Newsletter*, 17(1): 8-12.

Gurusubramanian, G. and Bora, S. 2008. Insecticidal resistance to tea mosquito bug, *Helopeltis theivora* Waterhouse (Miridae: Heteroptera) in NorthEast India. *Journal of Environmental Research Development*, 2(4):560-567.

Gurusubramanian G., Borthakur M., Sarmah M. and Rahman A., 2005. Pesticide selection, precautions, regulatory measures and usage. *In* Dutta, A., Gurusubramanian, G. and Barthakur, B.K. (Eds.), *Plant Protection in tea: Proc. Plant Protection Work shop*. Tocklai Experimental Station,TRA, Jorhat Assam Printing Works Private Limited, Jorhat, Assam, India, pp 81-91.

Gurusubramanian, G., Rahman, A., Sarmah, M., Bora, S. and Somnath Roy (2008) Pesticide usage pattern in tea ecosystem, their retrospects and alternative measures. *Journal of Environmental Biology,* 29(5/6) (*in press*).

Kapoor, S.K., Joginder Singh, Derek Russell, Balwinder Singh and Kalra, R.C., 2002. Susceptibility change of *Helicoverpa armigera* Hub. to different insecticides in Punjab. *Pesticide Research Journal*, 14: 177-180.

Misra, R.K., 1989. Toxicity of various insecticides against *Heliothis armigera* Hub. Guntur strain. *Pesticide Research Journal*, 1: 105-109.

Rahman, A., Sarmah, M., Phukan, A.K., Borthakur, M. and Gurusubramanian, G. (2005) A plant having insecticidal property for the management of tea pests. *In* Proc Int Symp on Innovation in Tea Science and Sustainable Development in Tea Industry,Tea Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou, China, 2005, p. 731-748.

Rahman, A., Sarmah, M., Phukan, A.K., Somnath Roy, Sannigrahi, S., Monorama Borthakur and Gurusubramanian, G., (2006) Approaches for the management of tea mosquito bug, *Helopeltis theivora* waterhouse (Miridae: Heteroptera). *In Strategies for Quality: Proc. 34th Tocklai Conference*. Tocklai Experimental Station, Tea Research association, Jorhat, Assam, India, pp 146-161.

Sannigrahi, S. and Talukdar, T. 2003. Pesticide use patterns in Dooars tea industry. *Two and Bud*, 50: 35-38.

Somnath Roy, Ananda Mukhopadhyay and Gurusubramanian, G. 2008. Use pattern of insecticides in tea estates of the Dooars in North Bengal, India. *North Bengal University Journal of Animal Science*, 2(1): 35-40.

Somnath Roy, Gurusubramanian, G. and Ananda Mukhopadhyay 2008a. Insecticide persistence and residual toxicity monitoring in tea mosquito bug, *Helopeltis theivora* Waterhouse (Hemiptera: Heteroptera: Miridae). *Resistant Pest Management Newsletter*, 17(2): 9-14.

G.Gurusubramanian[1], N. Senthilkumar[2], S. Bora[3], Somnath Roy[4], and Ananda Mukhopadhyay [4]

Department of Zoology[1] and Department of Biotechnology[2], Mizoram University, Tanhril, Aizawl – 796 009, Mizoram

E-mail: gurus64@yahoo.com; nskmzu@gmail.com; Fax No.0389-2330834/644

[3]Department of Entomology, Plant Protection Division, Tocklai Experimental Station, Tea Research Association, Jorhat- 785 008, Assam, India

[4]Entomology Research Unit, Department of Zoology, University of North Bengal, Darjeeling-734 430, West Bengal, India.

E-mail: entosomnath@yahoo.co.in; dr_amukherjee_nbu@rediffmail.com; Fax No.0353-2581546

Abstracts in Resistance Management

Record of sugarcane woolly aphid, *Ceratovacuna lanigera* Zehntner (Homoptera: Aphididae) and its natural enemies in Uttar Pradesh and Uttaranchal

The Sugarcane woolly aphid, *Ceratovacuna lanigera* Zehntner, is a new emerging pest of sugarcane, which is causing severe losses to the sugarcane crop (Figures 1 and 2). Both nymphs and adults suck the cell sap from the lower side of the leaves. Infestation of sugarcane woolly aphid was first noticed during the winter season of 2001-02 with low to severe intensity in large area of different sugar mills of the Muzaffarnagar, Meerut, and Saharanpur districts of Western Uttar Pradesh and the Haridwar and Dehradun districts of Uttarakhand. In the following years, infestation of sugarcane woolly aphid was reduced drastically to low or very low in these areas. During 2004 and 2007, low to severe infestation of the pest based on area and pest intensity was observed in and around Triveni Eng and Industries Ltd. (Sugar Unit), Khatauli, Muzaffarnagar district of Western Uttar Pradesh followed by Uttam Sugar Mills Ltd, Liberhedi, Haridwar district of Uttaranchal. Predators of the pest *viz., Dipha aphidivora* (Figure 3), *Micromus igorotus* (Figure 4) *Metasyrphus confrator* and coccinellids (*Cheilomenes sexmaculata* (Fabricius); *Coelophora saucia* Mulsant) were observed predating upon sugarcane woolly aphid. This is the first report of sugarcane woolly aphid and its predators from Uttar Pradesh and Uttaranchal.

Figure: 1. Infestation of C. lanigera

Figure: 3. Sugarcane woolly aphid predator, *Dipha aphidivora* with *C. lanigera* infestation.

Figure: 2. Sugarcane cultivated field affected by Woolly aphid

Figure 4. Larvae of *Micromus igorotus* feeding with C. lanigera.

Satyendra K. Singh* and G. M. Tripathi**
Indian Institute of Sugarcane Research, Lucknow-226002, Uttar Pradesh, India
*Present address- Ladybird Research Laboratory, Department of Zoology University of Lucknow, Lucknow-226 007, India
**Sr. Scientist & PI, Division of Plant Protection
* **To whom correspondence should be addressed at: E-mail:** satyendra_55@hotmail.com

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/rpm/submission.html for submission information).

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

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

The next two **submission deadlines** are:

Monday, March 16, 2009
Monday, September 21, 2009

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/rpm/index.html**