Resistant Pest Management Newsletter

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Resistance Management from Around the Globe

Arthropod Resistance

Joint action of ready to use insecticide mixtures against rice leaffolder, *Cnaphalocrosis medinalis* Guenee

ABSTRACT Joint action analysis of four ready to use insecticide mixtures against rice leaffolder, *Cnaphalocrosis medinalis* Guenee indicated that all the four, Nurelle D. Bulldock star Upacy and Roket exhibited synergistic action *i.e*., Co-Toxicity Co-efficient (CTC>100) against rice leaf folder.

KEY WORDS *Cnaphalocrosis medinalis*, ready to use insecticide mixtures, joint actions, Co-Toxicity Co-efficient

INTRODUCTION Rice leaffolder, *C. medinalis* generally considered once as a pest of minor importance has increased in abundance at international level. The loss due to leaffolder was as high as 75 per cent in some areas (Choudhary and Bindra, 1970; Balasubramanian *et al*., 1973). In Tamil Nadu, the leaf folder complex assuming a major pest status in the recent years (Bentur *et al*. (1989; Saikia 1996 and Pandi 1997). Though the integrated pest management practices like use of resistant varieties, judicious fertilizer application, use of light traps *etc*., have substantial effect on management of this pest. But, insecticides still remain the major control measures against leaffolder. Carbofuran and fenthion (Chandramohan and Jayaraj, 1976), bendiocarb, acephate and carbosulfan (Saroja and Raju, 1982), quinolphos, monocrotophos and phosphamidon (Raju *et al*., 1990) and fenvalerate (Ramaraju and Natarajan, 1997) were the common insecticides used against the control of rice leaffolder. The frequent use of certain insecticides leads to development of insecticide resistance (Endo *et al*., 1987; Endo and Kozano, 1988; Endo *et al*., 1993; Xiao *et al*., 1994).

Farmers nowadays resort to use tank mixture of insecticides though not recommended to manage the resistant rice leaffolder, *C. medinalis*. Insecticide mixtures were reported to delay the development of resistance in *Helicoverpa armigera* Hubner (Yanachao *et al*., 1985; Vaissayre and Alaux, 1996). However, no other scientific report is available in India with respect to joint action of insecticides against *C. medinalis*. The cotoxicity coefficient analysis is not only useful for the estimation of joint toxicity in the laboratory, but also to decide the dosage of each toxicant in the mixture for preliminary field tests. Hence, a study was carried out to assess the joint action of insecticides in the ready to use mixtures by Co-Toxicity Co-efficient analysis (CTC).

MATERIALS AND METHODS Mass culturing of uniform and continuous culture of leaffolder population was maintained on 60 days old TN1 seedlings. The isogenic culture was initiated from the moths collected from the field in Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore. The collected moths were released in a oviposition cage (50 x 65 x 90 cm) containing 5 - 6 potted TN1 seedlings. Sugar solution (5%) was provided as adult food to improve fecundity. After oviposition, the eggs were hatched out 10 - 12 days after oviposition. The folded leaves along with larvae were clipped off and placed over healthy TN1 seedlings. Third instar larvae were used for experiment. The larvae selected for assay were placed in glass vials and anaesthetised by keeping in a freezer for 3 - 5 min. The anaesthetized larvae were transferred to Petridish with a moistured filter paper at the bottom to maintain turgidity of the leaves. The larvae were arranged on the filter paper in its natural position for dosing.

Predetermined concentration of formulated ready to use insecticide mixtures (Table 1) and their individual compounds were dispensed on the thoracic dorsum of larvae @ 1 µl using Hamilton repeating dispenser fitted with 50 µl syringe. The petridishes were tied with rubber bunds to avoid escape of larvae. The mortality of larvae was observed at 24 and 48 h after treatment. There were three replications and 15 larvae per replication. The experiments were carried out at Toxicology laboratory, Tamil Nadu Agricultural

University, Tamil Nadu, India. The dosed larvae were maintained at 27 ± 2°C and 80 ± 5 per cent relative humidity.

Table 1. Chemicals tested

Chemicals	Composition	Dose /ha	
		g a.i.	ml (or) g
Nurelle D 505	Chlorpyriphos 50% + Cypermethrin 5%	334	625 ml
Bulldock Star 262.5 EC	Betacyfluthrin 12.5% + Chlorpyriphos 250 g	393	1500 ml
Upacy 50DF	Acephate 45% + Cypermethrin5%	500	1000 g
Rocket 44EC	Profenofos 40% + Cypermethrin5%	440	1000 ml

The joint action of insecticides individually in the ready to use insecticide mixtures was decided on the basis of LC50 value of each insecticides and was decided on the basis of co-toxicity co-efficient (CTC) of mixtures. CTC was determined by the method described by Sun and Johnson (1960), using non-pyrethroid insecticides as standard insecticide.

Co-toxicity co-efficient of Mixture (M)

$$(M) = \frac{\text{Actual toxicity index of a mixture}}{\text{Theoretical toxicity index of a mixture}} \times 100$$

Where, Actual Toxicity Index (TI) of mixture

$$(TI) = \frac{\text{LC50 of A}}{\text{LC5 of mixture}} \times 100$$

M (using A as standard), Theroetical toxicity of mixture (M)
(M) = (TI of A) x (% of A in M) + (TI of B) x (% of B in M)

where, A and B individual insecticides in mixture (M)
Toxicity Index (TI)

$$(TI) = \frac{\text{LC50 of standard insecticide}}{\text{LC50 of sample insecticide}} \times 100$$

RESULTS AND DISCUSSION The results from the (Table 2) revealed that all the four ready to use insecticide mixtures *viz*., Nurelle D 505, Bulldock Star 262.5 DF, Upacy 50 DF and Roket 44 EC recorded co-toxicity co-efficient of more than 100, indicating that the chemicals in the mixture have synergistic action. Among these chemicals Bulldock Star recorded the highest CTC of 318.46 followed by Nurelle D 505 (222.90), Roket (173.49) and Upacy 50 DF (113.32).

The synergistic mechanism of profenofos, chlorpyriphos and acephate with synthetic pyrethroids is probably due to competitive inhibition of mixed function oxidase (MFO) for their activation and leaving less MFO available for detoxification of pyrethroids. Both protenofos and chlorpyriphos come under the group of phosphorothionates, the latent phosphorothionates, may be activated to become more toxic phosphates. This conversition is mediated through oxidation by MFO. Carboxyl esterase also plays a considerable role in detoxification of synthetic pyrethroids. Profenofos being a specific inhibitor of carboxyl esterase generally enhances the toxicity of synthetic pyrethroids in Roket 44 EC. Since, acephate is come under phosphoramidothioate, it also behave like protenofos.

The resistance monitoring studies conducted by Anbalagan (2001) indicated that the level of resistance to chlorpyriphos was 7.80 to 10.30 per cent. The LC50 value of chlorpyriphos against *C. medinalis* was 0.2638 ppm, but in the present study it was 2.328 ppm *i.e*. LC50 value increased approximately 10 fold, it indicated that the resistance may be increased to 10

S. No.	Chemicals	LC50	Fiducial limit	Regression equation	χ^2	CTC
I	Nurelle D 505	0.279	0.516-0.545	Y= -4.1451+1.6939X	0.18	222.9
a	Chlorpyriphos 50%	2.327	1.536-8.253	Y= -5.0361+1.4958X	1.436	-
b	Cypermethrin 5%	0.036	0.082-0.065	Y= -1.9949+1.2799X	1.713	-
II	Bulldock Star262.5EC	1.433	1.052-1.958	Y= -0.7562+2.3959X	1.877	318.46
a	Betacyfluthrin 12.5%	0.112	0.059-0.244	Y= -0.7123+0.8490X	1.325	-
b	Chlorpyriphos 250 g	2.327	1.536-8.253	Y= -5.0361+1.4958X	1.436	-
III	Upacy 50DF	0.574	0.453-0.695	Y= -1.4649+0.0026X	3.495	113.32
a	Acephate 45%	2.886	1.943-3.897	Y= -7.5602+2.0848X	5.928	-
b	Cypermethrin5%	0.036	0.082-0.065	Y= -1.9949+1.2799X	1.713	-
IV	Rocket 44EC	0.156	0.092-0.262	Y= -3.0482+1.3904X	3.158	173.49
a	Profenofos 40%	0.154	0.126-0.182	Y= -1.6692+0.0108X	2.253	-
b	Cypermethrin5%	0.036	0.082-0.065	Y= -1.9949+1.2799X	1.713	-

Table 2. Co-Toxicity co-efficient of ready to use insecticide mixtures against rice leaffolder *C. medinalis*

fold level. The similar results were also reported by Ramasubramanian and Regupathy (2004) when synthetic pyrithroids were mixed with organophosphates against *H. armigera*.

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Status of pyrethroid resistance in the cotton bollworm, *Helicoverpa armigera*, in Cameroon

ABSTRACT Over the last two years, from 2002 up to 2004, survey of *H. armigera* susceptibility to pyrethroid insecticides was carried out in order to diagnose control failure in the cotton growing area, in Nothern Cameroon. Vial tests on field-collected larvae and topical applications on wild strains confirmed the loss of pyrethroid susceptibility in *H. armigera*, both within and between cotton growing seasons. Most control failures reported by farmers in 2004 could be allotted to pyrethroid resistance.

KEY WORDS *Helicoverpa armigera*, insecticide resistance, pyrethroid, cotton.

INTRODUCTION In Nothern Cameroon, the noctuid *Helicoverpa armigera* (Hübner) is a major pest of cotton and vegetable crops, which directly affects production by attacking fruit-bearing organs. In Africa, pyrethroid insecticides have been used to control larvae on cotton crops for approximately 20 years. As a result, pyrethroid resistance first appeared in the 1996-97 in the southern (Van Jaarsveld *et al*., 1998) and western regions (Vassal *et al*., 1997; Martin *et al*., 2000). In

Northern Cameroon, central Africa, the national cotton company Sodecoton, with the help of the Institute of agricultural research (Irad-Prasac), implemented in 1999 a monitoring network for the early detection of resistance of target insects to the main recommended insecticides. In the 2001 cropping season, bioassays indicated the presence of resistant genes within *H. armigera* populations (Brévault *et al*., 2002). Over the last two years, from 2002 up to 2004, survey of *H. armigera* susceptibility to pyrethroid insecticides was carried out in order to diagnose control failure in the field.

MATERIAL AND METHODS

Vial tests - Assessment of *H. armigera* susceptibility to pyrethroid insecticides was carried out through vial tests, from 2002 to 2004. This method is based on the use of glass vials whose internal wall was previously coated with insecticide. For the experiment, cypermethrin was chosen as the active ingredient, because it is the most commonly used pyrethroid insecticide in Cameroon. Two treatments were tested in the laboratory in 30 ml vials: 10 non treated vials (control) and 30 vials, each treated with 30 µg of cypermethrin, dosage that was reported to have killed all the individuals of a susceptible strain BK77 and 60 to 80% in a resistant population collected in Benin in 1997 (Vaissayre et al., 2002). Larvae from 10 to 15 mm long (4th instar) were collected from cotton fields, not less than five days after the last insecticide application. They were immediately placed in individual vials with no food and maintained at ambient temperature. The number of dead larvae was recorded 24 hours later. Each season, the susceptibility survey was conducted in 13 sites of the cotton-growing area (Fig. 1), at the beginning and the end of the *H. armigera* infestation of cotton, August and October respectively. For each sampling site, two replicates were carried out in different plots.

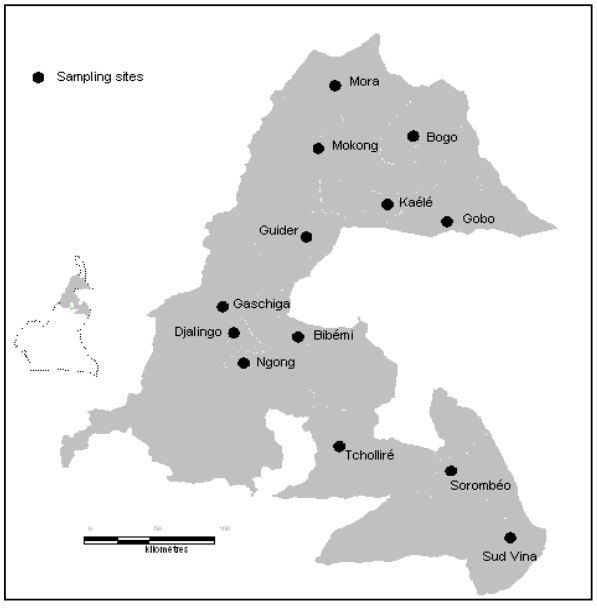

Figure 1. Sampling sites in the cotton growing area, Northern Cameroon

Topical bioassay procedure - A minimum of 300 larvae was collected for sampling sites with low mortality after vial tests. These were used to establish laboratory cultures. Additional samples were equally collected from tomato, an important host during the dry season. First generation larvae were used for susceptibility tests, using the BK77 strain as a reference (Martin *et al*., 2000). The insecticide solution for application was prepared from technical grade insecticide diluted with acetone. Once allocated in weight classes from 16 to 40 mg, fourth instar larvae were individually held in 6 cell-boxes containing artificial diet. A volume of insecticide solution, expressed in µg per g of larvae, was applied on the thorax with an Arnold micro-applicator (Burkard, UK). A minimum of 5 dosages were tested to build a regression line representative of dosage-mortality relation. For each test, a control population was treated with pure acetone solution. Mortality was observed 48 hours later. LD50 results were expressed as µg of active ingredient per gram of larvae. For each strain, the resistance coefficient (RC) was calculated as the proportion between LD50 of the strain and the susceptible reference strain.

Data analysis - Survival rates obtained from vial tests were corrected considering mortality in controls (Abbott, 1925). The LD50 WINDL software (Cirad, France) was used to analyze the data with the Finney's log-probit method (Finney, 1971).

RESULTS

Vial tests - Pyrethroid susceptibility in *H. armigera* regularly decreased both within and between cotton growing seasons (Fig. 2). As a result, mean survival rate reached the highest level (50) at the end of the 2004 cotton season. In the same way, it was noticed that the mean survival rate at the beginning of one cotton season (August, year n) was very near the mean survival rate at the end of the previous season (October, year n-1). The loss of susceptibility was

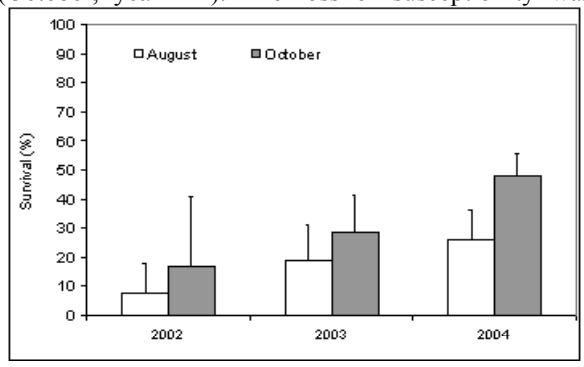

Figure 2. Mean and maximum survival of *H. armigera* field-collected larvae to 30 µg cypermethrin vial tests.

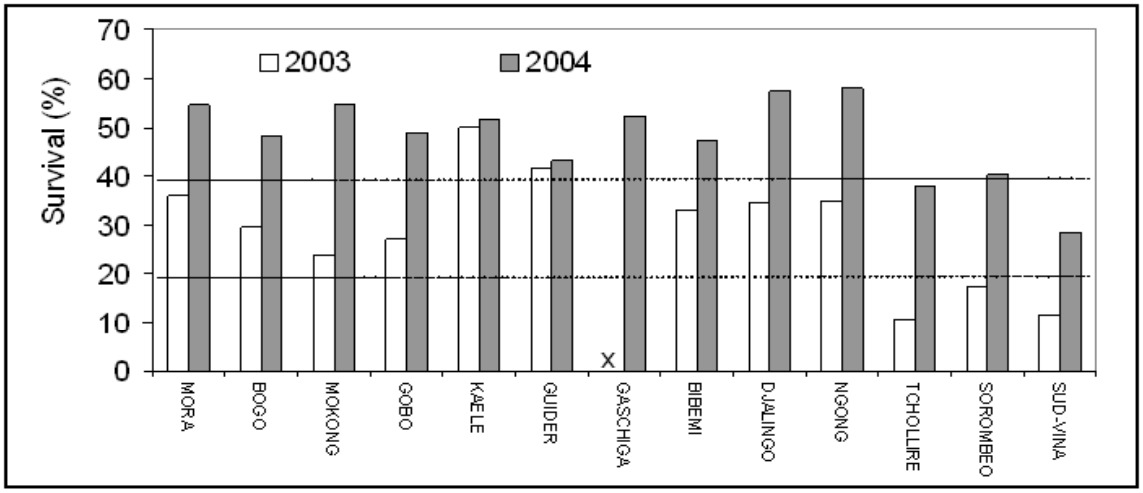

Figure 3. Mean survival of *H. armigera* field-collected larvae to 30 µg cypermethrin at the end of the infestation period on cotton (October 2003-2004). From left to right: North-South geographical range.

confirmed at a local scale from 2003 to 2004 (*Fig. 3*). If we consider that the survival range of a resistant population is about 20 to 40% (Vaissayre *et al*., 2002), then the majority of samples should be considered as pyrethroid resistant. Surprisingly, spatial heterogeneity in susceptibility was observed amongst the different sampling sites, with different resistance pattern in the south cotton growing area (Tcholliré, Sorombéo and Sud-Vina).

Topical bioassays - To complete this field survey, larval susceptibility was assessed in the laboratory by topical application on 6 and 2 strains collected on cotton and tomato crops respectively. LD50 measures confirmed the significant loss of susceptibility in wild populations of *H. armigera* from Northern Cameroon, with resistance coefficient around 60 (Tab. I). It was observed that populations from both tomato and cotton attained the same resistance level.

Year	Sampling site	Host plant	Month	LD_{50} (µg)	RC
2003	Kaélé	coton	10	11.3	28
	Djalingo	coton	10	10.5	26
	Ngong	coton	10	12.1	30
2004	Gaschiga	tomate	2	24.8	62
	Hamakoussou	coton	9	24.1	60
	Djalingo	coton	9	28.8	72
	Mayo Dadi	coton	9	16.6	42
	Gaschiga	tomate	12	24.3	61

Table I. LD50 and resistance factor to cypermethrin in *H. armigera* larvae collected from cotton and tomato plots in Nothern Cameroon (2003-2004).

DISCUSSION In Nothern Cameroon, the assessment of pyrethroid resistance in the cotton bollworm, *H. armigera*, revealed the presence of resistant individuals in local populations whose proportion have increased with time and pyrethroid applications. Resistance to pyrethroids in H. armigera was confirmed on several strains in West Africa in 1998-2002, where vial test with 30 µg cypermethrin performed on field-collected larvae at the end of the cotton season gave survival from 1 to 35% in Ivory Coast (Martin *et al*., 2003), whereas values superior to 20% were observed in Mali, Burkina and Benin within the same period (Vaissayre *et al*., 2002). In Cameroon, the situation has become very worrying as related by up to 40 survival rates in most sampling sites. In many cases, survey followed by laboratory bioassays on field-sampled populations showed that most control failures reported by farmers in 2004 could be allotted to pyrethroid resistance.

Pyrethroid selection pressure during the cotton-growing season seems to be sufficient to significantly decrease the mean susceptibility between first invading populations until last generation. However, more surprising is the relative stability of resistance from one cotton season to the next, despite a long dry season allowing large decrease in the selection pressure. This stability could be explained by different non exclusive factors that it could be necessary to explore: maintenance of the selection pressure by pyrethroid use in intermediate cultivated host plants like tomato, relative weight of diapausing and migrating individuals in the reconstitution of populations after the dry season, remaining of resistance alleles originating from enhanced fitness, and the disappearance of refuges with the permanent reduction of natural ecosystems.

In conclusion, priority actions to be developed must focus on the rational and concerted management of pesticide use and the implementation of a regional monitoring network for the early detection of resistant populations. At the same time, more fundamental research should be undertaken on the mechanisms and gene flow in the epidemiological

profile of pyrethroid resistance in order to define practical ways of reducing the selection pressure and enhancing host plant use as potential refuges in a resistance management program for cotton production.

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Influence of Trap Crops and Application of Neem Seed Kernel Extract on the Occurrence of Natural Enemies in Cotton Ecosystem

ABSTRACT The present investigation on the influence of trap crops and NSKE on the occurrence of natural enemies in cotton (MCU 7) was made at Agricultural Research Station (ARS), Vaigaidam and ARS, Bhavanisagar, Tamil Nadu during winter 2003 -2004. Results revealed that the occurrence ratio of coccinellids towards cotton: bhendi and cotton: redgram varied from 1: 0.59 - 1: 0.60 and 1: 0.88 - 1: 1.08 respectively. Spiders registered 1: 0.45 - 1: 0.91 and 1: 0.94 - 1: 1.31 as occurrence ratio on cotton: bhendi and cotton: redgram respectively. But, when the NSKE applied on cotton, the populations moved from cotton to trap crops (bhendi and redgram) and the occurrence ratio was altered towards trap crops. The increased occurrence ratio of coccinellids towards cotton: bhendi and cotton: redgram varied from 1: 0.76 - 1: 0.78 and 1: 1.09 - 1: 1.26 respectively. The occurrence ratio of spiders was altered to 1: 0.86 - 1.33 and 1: 1.10 - 1: 1.98 on cotton: bhendi and cotton: redgram respectively.

KEY WORDS Cotton, Trap crops, Neem, Natural enemies, Occurrence ratio

INTRODUCTION Cotton crop is known to be damaged by 1326 species of pests and in India 162 species of pests attack this crop (Hargreaves, 1948 and Puri, 1998). Frequent crop failures were reported due to very high incidence of sucking pests and bollworms. The important reason ascribed for it was the development of insecticide resistance by sucking pests and bollworms. In this search, a new impetus with emphasis to cropping system, plant products and biocontrol agents is given to Integrated Pest Management in cotton. The basic principle in pest management through cropping system is that any change in the vegetation will influence the population of pests and natural enemies. Diversified cropping system may lead to low resource concentration and also enhance the activity of natural enemies which may result in lower pest build up. In diversified cropping system there will be exchange of natural enemies between crops and increased activity of natural enemies, on main crop might have resulted decreased bollworms egg load in cotton (Stern, 1967). Natural enemies play an important role in suppressing pest populations in the crop whenever suitable conditions prevail for their survival, development, conservation

and multiplication in any agro ecosystem. Keeping this in background, a field experiment was conducted to study the influence of trap cropping system and application of Neem Seed Kernel Extract (NSKE) on the occurrence of natural enemies in cotton.

MATERIALS AND METHODS Two field experiments were conducted on cotton at Agricultural Research Station (ARS), Vaigaidam and ARS, Bhavanisagar, Tamil Nadu during Winter 2003 -2004 with trap crops bhendi (Arka Anamika) and redgram (APK1). A cotton variety MCU7 was sown (60 x 30cm) on one side of the ridge. In each plot having 10 rows of cotton, fifth row was substituted with trap crops, which was sown simultaneously on the other side of the ridge. The trap cropping systems were compared with the cotton sole crop with 10 rows of cotton in each plot. A11 the plots received recommended agronomic practices of the region except the treatment operations. NSKE 5% was applied on cotton leaving trap crops to diversity the pests to trap crops commencing from 46 DAS at weekly interval upto maturity phase. The treatment combinations were cotton (NSKE treated) + bhendi, cotton (NSKE treated) + redgram, cotton (NSKE untreated) + bhendi, cotton (NSKE untreated) + redgram, cotton sole crop (NSKE treated) and cotton sole crop (NSKE untreated). A Randomized Block Design with four replications was used for the experiment with an individual plot size of 8m x 8m.

To know the influence of trap crops and restricted application of NSKE on cotton leaving trap crops on the occurrence of coccinellids and spiders were counted at ten randomly selected plants per replication. The population of predators was recorded based on number of eggs, grubs, pupae and adults in coccinellids and adults in spiders. In case of trap crops, observations on the occurrence of natural enemies were taken from the randomly selected plants as detailed above. The occurrence ratio (OR) of natural enemies as calculated by Balakrishnan (2002) were worked out by using the following formula

$$OR = \frac{\text{Occurrence of natural enemies on trap crops}}{\text{Occurrence of natural enemies on cotton}}$$

RESULTS AND DISCUSSION Effect of trap crops and NSKE spray on cotton on the occurrence of natural enemies such as coccinellids and spiders were recorded and the pooled data are furnished in Table 1.

Coccinellids species such as *Menochilus sexmaculatus* Fabr., *Coccinella transversalis* and *Alesia discolor* Muls. were observed during the study. *M. sexmaculatus* was predominant in all the crops. *C. transversalis* and *A. discolor* were predominant in redgram. The occurrence ratio of coccinellids towards cotton: bhendi and cotton: redgram varied from 1: 0.59 - 1: 0.60 and 1: 0.88 - 1: 1.08 respectively. But, when the NSKE applied on cotton, the populations moved from cotton to trap crops (bhendi and redgram) and the occurrence ratio was altered towards trap crops. The increased occurrence ratio of coccinellids towards cotton: bhendi and cotton: redgram varied from 1: 0.76 - 1: 0.78 and 1: 1.09 - 1: 1.26 respectively (Table 1).

The spider species such as *Oxyopes* sp., *Argiope* sp., *Araneus* sp., *Neoscona* sp., *Plexippus* sp., were observed in all the three crops. *Plexippus* sp., *Argiope* sp and *Neoscona* sp., were predominant in redgram. Spiders registered 1: 0.45 - 1: 0.91 and 1: 0.94 - 1: 1.31 as occurrence ratio at two different locations on cotton: bhendi and cotton: redgram respectively. Further, application of NSKE on cotton due to repellent action of the neem the population moved to bhendi and redgram and the occurrence ratio was altered to 1: 0.86 - 1.33 and 1: 1.10 - 1: 1.98 as occurrence ratio of spiders on cotton: bhendi and cotton: redgram respectively (Table 1).

The occurrences of natural enemies on trap crops were comparatively low than on cotton. Among the trap crops, redgram in cotton recorded more number of coccinellids and spiders than bhendi. Application of NSKE on cotton diverted the population of coccinellids and spiders to trap crops. This was due to repellent action of NSKE the populations moved to

Table 1. Influence of trap crops and restricted application of NSKE on cotton on the occurrence of natural enemies

Cropping system	NSKE 5% spray on cotton	Crops	Vaigaidam, Winter 2003-04				Bhavanisagar, Winter 2003-04			
			Coccinellids		Spiders		Coccinellids		Spiders	
			P	OR	P	OR	P	OR	P	OR
Cotton + Bhendi	Cotton untreated with NSKE	Cotton	20.86	-	7.53	-	17.35	-	4.28	-
		Bhendi	12.33	01:00.6	3.4	01:00.4	10.36	01:00.6	3.9	01:00.9
	Cotton treated with NSKE	Cotton	19.06	-	5.69	-	14.71	-	3.21	-
		Bhendi	14.5	01:00.8	4.9	01:00.9	11.5	01:00.8	4.27	01:01.3
Cotton + Redgram	Cotton untreated with NSKE	Cotton	34.73	-	8.76	-	22.28	-	4.78	-
		Redgram	30.67	01:00.9	8.3	01:00.9	24.07	01:01.1	6.28	01:01.3
	Cotton treated with NSKE	Cotton	30.33	-	7.69	-	20.78	-	3.92	-
		Redgram	33.2	01:01.1	8.53	01:01.1	26.28	01:01.3	7.78	01:02.0
Cotton sole crop	Cotton untreated with NSKE	Cotton	22.6	-	7.61	-	21.07	-	4.28	-
	Cotton treated with NSKE	Cotton	18.06	-	6.38	-	18.42	-	3.71	-

P – Mean population per ten plants OR – Occurrence ratio

untreated trap crops which is in confirmation with the findings of Saminathan (2000). Increasing crop diversity normally favours the population of natural enemies of pests (Edwards *et al*., 1992). Cotton trap cropped with redgram recorded higher population of coccinellids and spiders compared to cotton trap cropped with bhendi and cotton sole crop. The studies gave a clue that trap cropping of cotton with redgram may form an important basic step towards habitat manipulation for the management of insect pests of cotton.

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Humoral immune reactions participation in resistance formation of Colorado beetle (*Leptinotarsa decemlineata* Say) larvae and imago to a biopreparation for potato

INTRODUCTION *L. decemlineata* resistance to the pathogen microorganism action to considerable extent depends on the level of defence systems activity and reactivity. At the same time there are not numerous evidences of the raise of insect immune response reactivity through immunization (Bartnikayte, Babonas, 1984; Dunn, 1990; Saltykova *et al*., 2005). The aim of this work was a study of the humoral immune responses during initial stage of infectious process in *L. decemlineata* larvae and imago infected per os with bitoxibacillin (BTB) consistently increasing concentrations. This investigation was made through determination of agglutinating, phenoloxidase, catalase and peroxidase activities in L. decemlineata hemolymph under the single and twofold BTB action. Choice of these parameters was not casual because they are inalienable components of the insect humoral immune response and demonstrate close intercommunication. Phenoloxidase system (POS) takes up one of the central place in insect immunity (Glupov, Bachvalov, 1998). In result of the prophenoloxidase activation many interacting proteins acquire the biological activity and take place in cellular and humoral defence reactions against pathogens. So important step of the insect immune response as recognition is bound up with hemagglutinins and POS functions (Yu, Kanost, 2003). Lastly, the antioxidant system (AOS) included such enzymes as catalase and peroxidase reduces toxic action of reactive oxygen metabolites accumulated during POS functioning (Whitten *et al*., 2001).

MATERIALS AND METHODS

Insects - *L. decemlineata* larvae and imago were collected from potato field and reared on potato foliage in glasses with volume 0.5 dm3. Artificial infection of insects was conducted through feeding of potato foliage moistened with BTB solution (*Bacillus thuringiensis var. thuringiensis* product). Primary treatment of *L. decemlineata* was accomplished with most unletal BTB concentration which didn't cause mortality significantly distinguished from control variant, and secondary treatment - with BTB LC50 preliminary determined for larvae and imago. Larvae was primary treated at third instar with 0.01% BTB solution and secondary - at fourth instar with 0.1% BTB. Imago was primary treated with 0.1% BTB and repeatedly - a ten days later with 0.5% BTB. Change dynamics of the enumerated indices was determined during first 24 hours after the single and two-fold treatment of L. decemlineata. Insect mortality was valued by quantity calculation of died larvae and imago seven days later treatment with BTB LC50.

Hemagglutination assays - Agglutination activity in hemolymph and hemagglutinins saccharine specificity was determined by D. Stynen *et al.* (1982) method. D-galactose, D(-)-fructose, D(+)-ramnose,

D(-)-mannose, L(+)-arabinose, N-acetil-D-glucosamine (NAGA), lactose, maltose, saccharose, chitooligosaccharides (COS) (oligomers included fifteen monomers), heparine and gyaluronic acid (GUA) were used for the hemagglutination inhibition test.

Enzyme activity in hemolymph -Phenoloxidase activity was determined in trys-HCl buffer (pH 7.5). DOPAoxidase activity with respect to substratum L-digydroxiphenilalanin was measured on spectrophotometer SP-46 at 475 nm by optical density change during 5 min. Tyrosinoxidase activity was evaluated by velocity of L-tyrosine oxidation on spectrophotometer SP-46 at 475 nm by optical density change during 30 min. Peroxides activity was determined at incubation in acetate buffer (pH 5.6) by velocity of benzidine oxidation on spectrophotometer SP-46 at 540 nm by optical density change during 1 min. Catalase activity was determined by manganometric method. Activity of these enzymes evaluated in protein concentration, which was measured by Bredford method (Skopes, 1985). Values of activity were normalized by control.

Statistical analysis received data was carried out with using arithmetical mean, arithmetical mean error and Student t-criterion.

RESULTS AND DISCUSSION The experiment results demonstrated valuable decrease of the larvae and imago mortality after the treatment with BTB LC50 when insects were preliminary treated with unletal BTB concentration (Tab. 1). Analogous results were obtained early with bee moth larvae (Bartnikayte, Babonas, 1984) and honeybee workers (Saltykova *et al*., 2005) under immunization and subsequent infection with *B. thuringiensis*.

Table 1. Mortality (%) of *L. decemlineata* under the single and two-fold treatment with BTB

ontogenetic stage	single treatment	two-fold treatment
larvae	55.56±5.56	8.33±4.81
imago	48.43±6.64	7.24±1.44

Hemagglutination assays - Agglutinin titre doubled in hemolymph of the *L. decemlineata* larvae and imago in 24 and 2 hours respectively after the treatment with BTB LC50 (Tab. 2). In result of the preliminary treatment with BTB unletal concentration agglutinin titre in larval and imaginal hemolymph twice exceeded the control values at the moment of repeated treatment. After repeated infection agglutinin titre in the hemolymph of immunized larvae and imago reached the value of 4096 in 2 hours, and agglutinating activity of the imago hemolymph quadrupled in comparison with initial point 24 hours later. Hemagglutinins of *L. decemlineata* larvae and imago had a similar saccharine specificity and bound with only compound oligo- and polymeric carbohydrates namely with heparin, COS and GUA at minimal inhibitory concentration 0.125, 0.125 and 0.250% respectively.

Table 2. Hemagglutinin titre in *L. decemlineata* lunder the single and two-fold treatment with BTB

ontogenetic stage	Time after treatment with BTB LC_{50}, hour			
	0	2	4	24
larvae				
0.1% BTB	512	512	512	1024
0.01+0.1% BTB	1024	4096	4096	4096
imago				
0.5% BTB	1024	2048	4096	4096
0.1+0.5% BTB	2048	4096	4096	8192

Induction of hemagglutination activity under infection development is shown to be stipulated by intensification of agglutinins synthesis or (and) activation of nonactive glycoprotein forms as was shown for the lectin Sarcophaga (Komano *et al*., 1981). Hemocytes are supposed to produce agglutinins only after the direct contact with antigen (Glupov, Bachvalov, 1998). Therefor the increase of agglutinin titre in the first hours after the treatment of insects with BTB can be stipulated by intensity rise of the hormone regulated lectin synthesis in fat body cells. So these reasons suggest us the agglutinins induced during initial stage of infection development to be plasmatic lectins realized opsonic function. It is remarkable that in our results COS - oligomers consisted of NAGA - inhibit the agglutination of mouse erythrocytes whereas NAGA is not inhibitory. Obviously carbohydrate must to have sufficiently large molecular mass or (and) additional bonds for binding with hemagglutinins. During infection development in imago minimal inhibitory concentration of GUA didn't change whereas minimal inhibitory concentration of heparin and COS doubled. This fact indicates inclusion of the specific with respect to BTB defence mechanisms because toxins produced by B. thuringiensis include similar with COS glycosid fragments (Dulmage, Rhodes, 1971).

Activity of POS and AOS enzymes. According to received data immunization of larvae in third instar causes decrease of the phenoloxidase activity in hemolymph during initial stage of the repeated infection in fourth instar (Fig. 1). Most likely the action

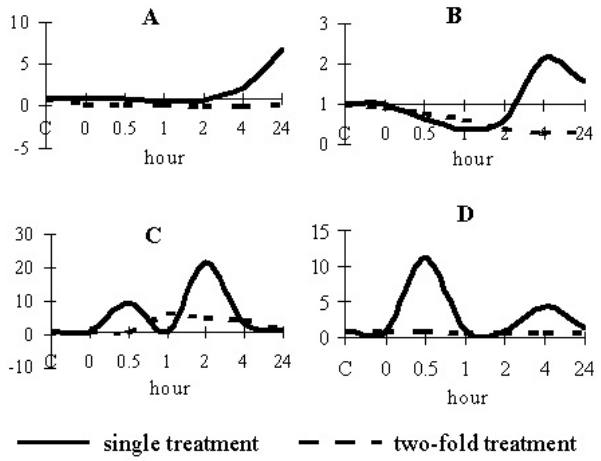

Fig. 1. Dinamics of POS and AOS enzymes activity in the hemolymph of *L.decemlineata* larvae under the single and two-fold treatment with BTB. Data is normolized by control. A - DOPAoxidase activity, B - tyrosinase activity, C - peroxidase activity, catalase activity.

of pathogen low doses preceded to moult stimulates activity of the phenoloxidases inhibitors including inhibitors of serine proteinases. On the contrary in imago the action of BTB unletal concentration provokes the phenoloxidases activation within the first hours after repeated infection in comparison with the single treated with BTB individuals (Fig. 2). DOPAoxidase functions are largely connected with nonspecifical defence mechanisms namely with its participation through quinone oxidation in regulation of the titre of biogenous amines, which are one of the central link of insect stress reaction (Wright, 1987). Early activation of tyrosinase provides insects with done in good time increase of the metabolites pool necessary for proceeding of defence reaction during the infection acute phase. Moreover, tyrosinase is supposed to participate in agglutination and recognition processes intensified during infection initial stage (Glupov, Bachvalov, 1998). This supposition is corroborated by the facts that tyrosinase is a strongly glycosylated protein and participates in the internalization of insect cells and pathogen through formation of the linking bridges consisted of tyrosine quinone derivatives (Marmaras et al., 1996). Thus, increase of the agglutinating and tyrosinase activity in the hemolymph of *L. decemlineata* imago after the two-fold treatment with BTB can be an evidence of increase of the recognition process efficiency by the preliminary action of BTB unletal concentration. In the over hand preliminary treatment of *L. decemlineata* larvae and imago with BTB unletal concentration causes decrease of the level and stabilization of the catalase and peroxidase activity during initial stage of repeated infectious process (Fig.1, Fig. 2). The increase of phenoloxidase activity together with the decrease of AOS enzymes activity in imago once again demonstrates interaction of the phenoloxidase and oxidative ways. The mechanism founded on this interaction is supposed to be accompanied by the temporary inhibition of own AOS and production of reactive oxygen intermediates playing important role in insect antimicrobial immunity (Whitten *et al*., 2001). According this point of view immunization of *L. decemlineata* with BTB unletal concentration can be supposed to stimulates expression of the factors activating phenoloxidases and inhibiting AOS enzymes activity for the accumulation of the cytotoxic arsenal high level at the moment of the infectious process transition from initial stage to acute phase.

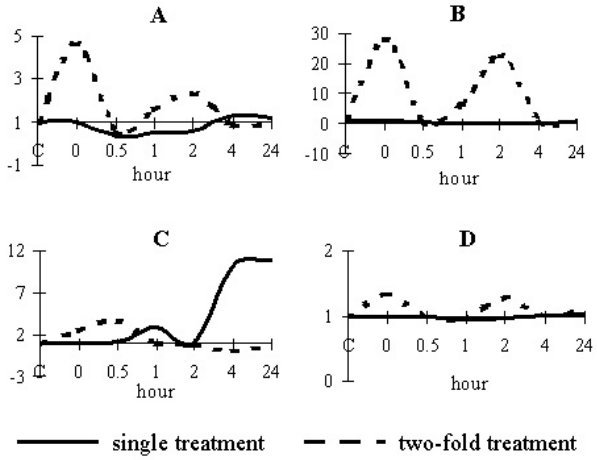

Fig. 2. Dinamics of POS and AOS enzymes activity in the hemolymph of *L.decemlineata* imago under the single and two-fold treatment with BTB. Data is normolized by control. A - DOPAoxidase activity, B - tyrosinase activity, C - peroxidase activity, catalase activity.

CONCLUSIONS Thus, received results demonstrate possibility of *L. decemlineata* immunization with bacterial preparation unletal concentration don't causing serious pathological changes in insect organism and mortality distinguished from control. Preliminary treatment of *L. decemlineata* larvae and imago with BTB unletal concentration significantly increases insect's survival and stimulates development of the humoral defence reaction during repeated infection. Realizing of *L. decemlineata* defence mechanisms during the initial stage of repeated infection is characterized by the ontogenetic peculiarities. In four instar larvae it displays in the stabilization of AOS enzymes activity and increase of hemagglutinin titre, whereas in imago - in the stabilization of AOS enzymes activity and increase of POS enzymes and hemagglutination activity. Observed changes of humoral immunity components at the physical isolation of gut pathogen during the first hours after the treatment of beetles with BTB suggest the existence of mediators signalizing about the penetration of infectious agent into the gut and in short

time activating defence mechanisms. As a whole heightened activity of *L. decemlineata* humoral defence systems under the repeated infection and conservation of immunization effect after the change of larval instar indicates the insects ability for development of immune memory. Ability for the increase of resistance to disease through immunization demonstrated also in some other *Insecta* species may be one of the causes of whole resistance formation in insect populations supplementing the natural selection action that is necessary to be taken into account during production and application of microbiological preparations as well as of transgenic plants.

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Suppression of mites *Tetranychus macfarlanei* (Baker and Pritchard) and *Amblyseius indicus* (Narayanan and Kaur) with certain acaricides

ABSTRACT Phytophagous mites are becoming more aggressive in nature as pests on vegetable crops due to environmental changes and cropping pattern. Pumpkins are the most important vegetable crop among the cucurbits throughout the India. Among the vegetable crops the cucurbites suffer the worst due to phytophagous mites. The pumpkin mite, *Tetranychus macfarleni* (Baker and Pritchard) has been identified as a serious mite pest on pumpkins and a predatory mite, *Amblyseius indicus* (Narayanan and Kaur) was observed to be associated with phytophagous mite during summer months. Experiments were conducted to screen out the safer, and economical effective acaricides *viz*. dicofol 18.5% EC, phosalone 35% EC, sulphur 80% WP, N.S.K.E. 5%, azadirachtin 0.03% EC, and eucalyptus oil 2% against pumpkin mite and its predatory mite under laboratory condition and field condition during 2002 and 2003. The results of laboratory and field condition revealed that dicofol and phosalone showed maximum mortality (95.66% & 89.77%) and (90.55% & 72.22%) whereas the lowest mortality was shown with eucalyptus oil and N.S.K.E, (22.49% & 36.99%) and (24.99% & 46.22%) respectively, during 2002. The same trend was observed *i.e*. dicofol and phosalone responded maximum mortality (92.77% & 88.96%) and (90.44% & 74.62%) whereas lowest mortality by eucalyptus oil and N.S.K.E, (23.55% & 34.44%) and (28.77% & 45.77%) respectively, against pumpkin mite during 2003. The maximum mortality under laboratory and field conditions showed against predatory mite by dicofol and phosalone (98.88% & 75.99%) and (87.77% & 72.88%), whereas lowest mortality by eucalyptus oil and N.S.K.E., (19.33% & 20.44%) and (34.22% & 25.88%) respectively, during 2002. Same trend was shown *i.e*. dicofol and phosalone responded

maximum mortality (99.66% & 77.88%) and (88.66% & 71.66%) whereas lowest mortality were responded by eucalyptus oil and N.S.K.E, (18.55% & 23.50%) and (36.11% & 26.55%) respectively against predatory mites during 2003.

KEY WORDS Acaricidal, response, pumpkin mite, predatory mite.

INTRODUCTION The spider mite has been identified as a detrimental mite pest of several vegetable crops like okra, cowpea, brinjal, cucurbits, cucumber and melons in India. This mite pest found very injurious through out tropical and sub- tropical parts of the world (Jeppson *et.al* 1975). In recent past years the outbreaks of pumpkin mite, *Tetranychus macfarlanei* (Baker and Pritchard) on pumpkin, *Cucurbita moschata* (Dutch), a common vegetable of summer months have attracted attention to the growers and archaeologists in eastern part of Uttar Pradesh. Heavy populations of mites built profuse webs on plants, which cover them. This mite pest problem has gained momentum because of rapid changes that have occurred in cultural practices to grow pumpkin crop, *Cucurbita moschata* (Dutch). The spider mite causes direct damage like loss of chlorophyll, stunting of growth defoliation, reduction in size and quality of fruits, appearance of various types of plant deformities etc. and all these severely affect the yield. Besides causing direct damage few phytophagous mites inject toxic substances in to their hosts, which cause increased localized growth and disruption of tissue (Jeppson *et al*., 1975). The monoculture and indiscriminate use of insecticides/acaricides on pumpkin, *Cucurbita moschata* (Dutch) has been enhancing the resurgence of this mite. The utilization of natural enemies of mite pest is eco-friendly approach in the management of injurious spider mite, *Tetranychus macfarlanei* (Baker and Pritchard) on pumpkin, *Cucurbita moschata* (Dutch) used predatory mite, *Amblyseius tetranychivorus* (Gupta) that belongs to family Phytoseiidae. The predatory mite, *Amblyseius tetranychivorus* (Gupta) is sensitive to commonly used pesticides (Jagdish and ChannaBasavanna, 1983). The population of spider mite can be suppressed effectively without affecting *A. tetranychivorus* using some indigenous plant extracts. The seed extracts of neem, pongamia, mahua; leaf extracts of *Clerodendron inerme* and *Vitex negundo* recorded 72 to 100% mortality of *T. urticae* (Anon., 1994-96; Chandrashekhera, 1997). Overmeer (1985) reported that phytoseiids are best-known predators among the acari and may be mass reared easily and shipped. Some phytoseiids are currently reared and sold for the biological control of spider mite, *Teteranychus urticae* Koch (McMurtry, 1982). Chemical management is an important component in Integrated Mite Management. Once the grower manages them then he gets good quality and quantity of produce. The lack of proper management against insect-pest and mite-pest are one of the major constraints for production. Therefore, keeping this in mind the present investigation has been envisaged in this direction to asses the response of some commonly available acaricides at their recommended doses on populations of pumpkin mite and its predatory mite, *Amblyseius indicus* (Narayanan and Kaur).

Our aim is to manage the pumpkin mite population and evaluate the response of acaricides against, *Amblyseius indicus* (Narayanan and Kaur) on pumpkin, *Cucurbita moschata* (Dutch). These are the good components for integrated mite management.

MATERIAL AND METHODS The experiments were carried out under laboratory and field conditions in the department of entomology and agricultural zoology, institute of agricultural sciences, B.H.U. and vegetable grower's field respectively during the summer months of 2002 and 2003. The response of acaricides *viz*. dicofol, phosalone, sulphure, azadirachtin, N.S.K.E. (Neem Seed Karnal Extract) and eucalyptus oil against pumpkin mite, *Tetranychus macfarlanei* (Baker and Pritchard) and its predatory mite, *Amblyseius indicus* (Narayanan and Kaur) on pumpkin under laboratory and field conditions.

LABORATOY CONDITION The experiments were conducted to find out the responses of acaricides at their recommended concentration against pumpkin mite, *Tetranychus macfarlane*i (Baker and Pritchard) and its predatory mite, *Amblyseius indicus* (Narayanan and Kaur) on pumpkin leaves in Completely Randomized Design with three replications under the laboratory. The available acaicides *viz*. dicofol, phosalone, sulphure, azadirachtin, N.S.K.E. (Neem Seed Karnal Extract) and eucalyptus oil were used through Leaf Dipped Method (FAO method No 10a, Busvine, 1980) at their recommended concentration. The chemical solution was prepared just before the application, water treated leaves (control) were also maintained in each experiment with each acaricides at its respective concentration and the pumpkin leaves were dipped in that solution up to 5 second to ensure the complete wetting then allowed them to dry. The dried leaves were placed on the moist cotton bed of the petri - dishes, the petiole of the treated leaves were covered with moist cotton buds to avoid dryness. Twenty mites were transferred to each treated leaf from mother culture of pumpkin mite and predatory mite separately. The percent mortality data recorded after 12, 24, 48, 60 and 72 hrs of each acaricides. The observe mortality of pumpkin mite and predatory mite were converted into percentage mortality.

FIELD CONDITION The experiments were conducted to find out the responses of acaricides viz. dicofol,

phosalone, sulphure, azadirachtin, N.S.K.E. (Neem Seed Karnal Extract) and eucalyptus oil at their recommended concentration against pumpkin mite, *Tetranychus macfarlanei* (Baker and Pritchard) and its predatory mite, *Amblyseius indicus* (Narayanan and Kaur) on pumpkin leaves in Randomized Block Design (R.B.D.) with three replications at vegetable growers field. The field size was 41 x 24.5m, plot size was 6 x 4 m, row-to-row 1m plant-to-plant 50 cm and bund size 30-cm. spacing was maintained. The chemical solutions were prepared just before the application; a water treated plot (control) was also maintained in each experiment with each acaricides at its respective concentration. In this experiment cloth screen was used for avoiding drifting from plot to plot. The observations were taken from five randomly selected tagged and numbered plants from each plot. Five leaves plucked from upper, middle and lower portion of the each plant and a total number of twenty-five leaves were collected from each plot for taking observation. The mite population was counted on the basis of 2-cm2 leaf areas at four spots per leaf under stereoscopic-binocular microscope. The mortality of pumpkin mite and predatory mite were observed in different intervals at pre spray, 1, 3, 7, and 14th days after. The percent mortality data of pumpkin mite and its predatory mite was calculated.

RESULT AND DISCUSSION The results of laboratory and field condition revealed that dicofol and phosalone showed maximum mortality (95.66% & 89.77%) and (90.55% & 72.22%) whereas the lowest mortality was shown with eucalyptus oil and N.S.K.E, (22.49% & 36.99%) and (24.99% & 46.22%) respectively, during 2002 (Table 1.). The same trend was observed *i.e*. dicofol and phosalone responded maximum mortality (92.77% & 88.96%) and (90.44% & 74.62%) whereas lowest mortality by eucalyptus oil and N.S.K.E, (23.55% & 34.44%) and (28.77% & 45.77%) respectively, against pumpkin mite during 2003 (Table 1). Many workers, Sandhu *et al* (1983), Singh *et al* (1989), Singh and Singh 1992, Kumar *et al* (2001) and Kumar and Singh (2003) report similar results.

The maximum mortality under laboratory and field conditions showed against predatory mite by dicofol and phosalone (98.88% & 75.99%) and (87.77% & 72.88%), whereas lowest mortality by eucalyptus oil and N.S.K.E., (19.33% & 20.44%) and (34.22% & 25.88%) respectively, during 2002 (Table 2). Same trend was shown *i.e* dicofol and phosalone responded maximum mortality (99.66% & 77.88%)

Table: 1. Acaricidal response against pumpkin mite, *Tetranychus macfarlanei* Baker and Pritchard on pumpkin, *Cucurbita moschata* Dutch.

S.No	Treatments	Con.	Mean per cent mite mortality under laboratory condition.		Mean per cent mite mortality under field condition.	
		(%)	2002	2003	2002	2003
1	Dicofol (18.5% EC)	0.045	95.66 (10.28)*	92.77 (10.13)	89.77 (9.97)	88.96 (9.93)
2	Phosalone (35%EC)	0.07	90.55 (10.01)	90.44 (10.00)	72.22 (8.99)	74.62 (9.13)
3	Sulphure (80% WP)	0.25	43.77 (-7.11)	40.55 (6.86)	65.44 (8.58)	63.88 (8.49)
4	NSKE	5	24.99 (-5.49)	28.77 (5.86)	46.22 (7.29)	45.77 (7.26)
5	Azadirachtin 0.03% EC	0.5	38.6 (-6.71)	36.55 (6.54)	56.03 (7.98)	51.74 (7.69)
6	Eucalyptus oil	0.2	22.49 (-5.24)	23.55 (5.35)	36.99 (6.58)	34.44 (6.36)
7	Control	-	13.88 (-4.22)	11.55 (3.89)	12.72 (4.06)	12.46 (4.02)
	SEM ±	-	12.23	10.7	10.23	12.62
	CD (0.01%)	-	32.78	28.68	27.4	33.83

* Figures in parenthesis are the ? X+0.5 transformed value, where X= mean corrected percent mortality.

Table: 2. Acaricidal response against predatory mite, *Amblyseius indicus* (Narayanan and Kaur) on pumpkin, *Cucurbita moschata* Dutch.

S.No	Treatments	Con.	Mean per cent mite mortality under laboratory condition.		Mean per cent mite mortality under field condition.	
		(%)	2002	2003	2002	2003
1	Dicofol (18.5% EC)	0.045	98.88 (10.44)*	99.66 (10.48)	75.99 (9.21)	77.88 (9.32)
2	Phosalone (35%EC)	0.07	87.77 (-9.86)	88.66 (9.91)	72.88 (9.03)	71.66 (8.96)
3	Sulphure (80% WP)	0.25	60.94 (-8.3)	62.49 (8.40)	52.88 (7.77)	56.33 (8.00)
4	NSKE	5	34.22 (-6.34)	36.11 (6.50)	25.88 (5.58)	26.55 (5.65)
5	Azadirachtin 0.03% EC	0.5	53.88 (-7.84)	58.88 (8.17)	44.58 (7.17)	45.15 (7.21)
6	Eucalyptus oil	0.2	19.33 (-4.89)	18.55 (4.80)	20.44 (5.02)	23.50 (5.34)
7	Control		16.44 (-4.55)	20.44 (5.02)	11.45 (3.88)	11.17 (3.84)
	SEM ±		0.429	0.648	0.609	0.1919
	CD (0.05%)		0.89	1.337	1.264	0.398

* Figures in parenthesis are the ? X+0.5 transformed value, where X= mean corrected percent mortality.

and (88.66% & 71.66%) whereas lowest mortality were responded by eucalyptus oil and N.S.K.E, (18.55% & 23.50%) and (36.11% & 26.55%) respectively against predatory mite during 2003 (Table 2). Some workers, Moment *et al* (1997), Stark *et al* (1997), Kumar and Singh (1998), Chinnaiah (1999) and Kumar and Singh (2004) support the results.

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Insecticide Resistance in field population of American bollworm, Helicoverpa armigera Hub. (Lepidoptera: Noctuidae)

ABSTRACT Insecticide Resistance studies on *Helicoverpa armigera* (Hubner) have been carried out at Resistant Monitoring Centre (RMC), Regional Agricultural Research Station (RARS), Nandyal, Andhra Pradesh (India) to monitor the resistant frequencies in *Helicoverpa armigera* during peak cotton growth periods of 2003-04 and 2004-05 crop seasons to different groups of insecticides and resistance development during 2004-05 crop season. The resistance monitoring was carried out against cypermethrin 0.1µg/µl, fenvalrate 0.2 µg/µl, endosulfan 10µg/µl, quinalphos 0.75µg/µl, and methomyl 0.1µg/µl. Among these, synthetic pyrethroids have shown high resistance frequencies (>80%). Mean resistance frequencies were low to moderate against methomyl (27.5±2.48) and endosulfan (22.75± 2.5). Quinalphos showed moderate to above moderate level. Frequencies ranged from 26.35 ± 4.4 to 78.24± 8.24 and the mean was 52.41±2.78. The LD50 values of cypermethrin, fenvalrate, and quinalphos were 8.51 µg/Larva, 7.07 µg/Larva, 1.04 µg/Larva respectively. The *Helicoverpa armigera* has developed more resistance i.e., 946 folds resistance against cypermethrin, followed by 491 folds against fenvalrate. Whereas, only 13 folds resistance was observed against quinalphos.

KEYWORDS Insecticide resistance, *Helicoverpa armigera*, cypermethrin, fenvalrate, quinalphos, endosulfan, and methomyl

INTRODUCTION The cotton bollworm, *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae), is the major insect pest on cotton in India. Indiscriminate and excessive use of chemical insecticides during past few decades have led to the emergence of several problems of which development of resistance to insecticides by this pest created alarming situations in different states of India (Armes *et al*., 1996). Systematic monitoring attempts to know the extent of resistance development by *H. armigera* to different groups of insecticides were carried out by several workers in different cotton growing parts of India (Armes *et al*., 1994; Kranthi *et al*., 1997). However, there was no such study on the development of insecticide resistance in *Helicoverpa armigera* on cotton in Rayalaseema region of Andhra Pradesh. Hence, studies were carried out to at RARS, Nandyal, to monitor the resistant frequencies and extent of resistance development in *Helicoverpa armigera* to different groups of insecticides during peak cotton growth periods of 2003-04 and 2004-2005.This type of study would be of immense use for formulating location specific Insecticide Resistance Management strategies against this important polyphagous pest.

MATERIALS AND METHODS

Discriminating Dose Assay - *Helicoverpa armigera* eggs were collected from cotton plants and transferred in to multi cellular trays containing chickpea based semi synthetic diet. The larvae hatched from the eggs when reached to 3-4 instar stage were subjected to discriminating dose test. The five technical grades insecticides cypermethrin 0.1µg/µl, fenvelrate 0.2 µg/µl endosulfan 10 µg/µl, quinolphos 0.75 µg/µl and methomly 0.1µg/µl were used. At least 100 larvae were treated with each insecticide. Equal number of larvae was simultaneously dosed with 1.0 µg/µl of acetone alone as a check. Larval mortality was assessed after 24 hours up to six days. Per cent mortality was calculated after correcting the mortality by Abbots's (1925) formula. The Resistance level was expressed as the percentage of larvae surviving the discriminating dose. From the weekly observations, averages frequencies of resistance for peak crop growth period were worked out and pooled weekly standard errors (±) were calculated.

Log Dose Probit Mortality Assay - Log dose probit assay (LDP) of cypermethrin, fenvalrate, quinalphos, were tested. The data recorded were subjected to probit analysis (Finney 1952). After determining the LD50 value to test insecticides the resistance ratios were computed as suggested by FAO (1979). The mortality data was subjected to Abbot's (1925) correction before computing LC50 values.

Table. 1. Frequency of Insecticide Resistance in field strain of *Helicoverpa armigera* to different insecticides

Discriminating Dose	2003-2004	2004-2005	Mean
Cypermethrin (0 .1 µg/ µl)	94.25 (2. 2)*	85.1 (1. 96)	89.67 (2. 08)
Fenvalrate (0 .2 µg/ µl)	96.75 -1.65	67.2 (1. 83)	81.97 -1.74
Methomyl (1.2 µg/ µl)	21.25 -4.13	33.75 -0.84	27.5 -2.48
Quinalphos (0 .75 µg/ µl)	26.35 -4.4	78.24 -1.17	52.41 -2.78
Endosulfan (10 µg/ µl)	10.5 -4	28.35 -1	19.2 -2.5

* Standard error

RESULTS AND DISCUSSION Frequency of insecticide resistance in *Helicoverpa* against different insecticides for both the years, was given in (Table-1). Higher resistance levels were observed for both the pyrethroids (>80%). Resistance frequencies observed for cypermethrin for both consecutive years showed marginal differences and moderate differences were observed with respect to fenavalrate. Mean resistance frequency observed against cypermethrin and fenvalrate were 89.67±2.08 and 81.97±1.74 respectively. Resistance frequency for methomyl ranged from 21.25 ± 4.13 to 33.75 ± 0.84 and mean resistance was 27.5 ±2.48. Resistance frequency for endosulfan ranged from 17±4.0 to 28.35±1.0 and mean resistance was 22.75±2.5. However, quinalphos showed large variation in resistance levels, which ranged from 26.35 ± 4.4 to 78.24±78.24 and mean was 52.41±2.78. Insecticide resistance monitoring carried out by Armes *et al.*, 1994, Surilivelu *et al*., 2004 and Chavan and Nimbalkar, 2003 showed high level of resistance to synthetic pyrethroids, moderate to above moderate level and fluctuating trends to organophosphates and low to moderate levels to carbomates and cyclodiene compounds. These variations in resistance levels indicate that the phenomenon of resistance development due to insecticide selection pressure is time and area specific. Results of Log Dose Probit Mortality (LDPM) were presented in (Table-2). LDPM assays were conducted for different molecules indicated that LD50 of cypermethrin was 8.51 µg/larva, fenvalrate was 7.07 µg/larva and quinalphos was 1.04 µg/larva. Synthetic pyrethroids have recorded high LD50 values as compared to organophosphate compound indicating that OP compounds are more effective even at lower concentrations. The pest has developed 946 folds resistance against cypermethrin and 491 folds against fenvalrate. However, against quinolphos it was only 13

folds. Ramasubramanyam (2004) reported that pyrethroid resistance is ubiquitous through out India. The Raichur strain exhibited very high level of resistance to cypermethrin (2489 folds) followed by Guntur strain (1213 folds). In case of fenvalrate magnitude of resistance in *H. armigera* was 350 folds in Kannivadi strain of Tamila Nadu. Armes *et al*., 1992, reported low levels of resistance that i.e., 9 folds in *H. armigera* to quinalphos. Kranthi *et al*., 2001, also reported 1 to 15 folds (Guntur strain). In the present study magnitude of resistance recorded was 13 folds. In the present study the magnitude of resistance recorded was 13 folds. This is in conformity with the studies conducted by the above researchers. This clearly indicates the importance of OP compounds like quinalphos in resistance management schedule against the American bollworm *Helicoverpa armigera*.

Table.2 Log Dose probit response of field strain of *Helicoverpa armigera* to insecticides

Insecticide	LD_{50}	95% FL	Slope	X^2	df	Resistance Factor
Cypermethrin	8.51	6.26-10.75	0.8	9.04	7	946
Fenvalrate	7.07	3.86-10.27	0.61	9.22	5	441
Quinalphos	1.04	0.728-1.35	1.21	5.63	4	13

Note: LD_{50} expressed as µg / larva; FL. Fiduciary limits; X^2= Chi square calculated value; df = Degrees of freedom

RF= LD_{50} of field strain/LD_{50} of susceptible strain*

* Cypermethrin-Reading strain=0.009 µg/larva
* Fenvalrate-reading strain=0.016 µg/larva
* Quinalphos-Reading strain=0.08 µg/larva

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The Resistance Forming Dynamics to Different Stressors in Housefly Larvae

ABSTRACT The dynamics of resistance and cross-resistance forming in Musca domestica larval stage III to 4 stressors (malathion, bitoxybacilline, low and high temperature) during 20 generations was investigated. It was shown that the resistance to all selectants was forming slowly. The toxicants-selected larvae developed the cross-resistance to all stressors investigated. Low temperature-selected larvae developed the bitoxybacilline cross-resistance and thermostable larvae become more resistant to low temperature and bitoxybacilline.

INTRODUCTION The problem of the insect resistance exists already about 100 years, but it has not lost its actuality also at present. In literature questions of the insect resistance and cross-resistance are spared big attention to insecticides from different chemical classes (Ozaki, Kassai, 1984; Amirhanov, Arzhavitina, 1990; Peric, 1990; Denholm, Rowland,. 1992; Roslavceva, 2003). As a rule, the closer the insecticides structure or manner of disadvantage factors acting, the higher cross-resistance. But the big amount of data concerning cross-resistance to preparations with the different chemical nature and mechanism of action exists. The

DDT- resistant insects often show the cross-resistance to pyrethroids (Gunning et al., 1990) Treatments by organophosphorus pesticides lead to forming of the resistance to pyrethroids (Zilberminc, 1988) and chitin synthesis inhibitor diflubenzuron (Rupes et al., 1977;. Ivanova, 1980). The cross-resistance to juvenile hormone analogue methoprene was developed during selection of the Egyptian cotton leafworm by diflubenzuron (El-Guindy et al., 1980). Moreover, the facts of resistance forming during the selection by insecticides to such different factors as starvation, heating, drying and cooling are known (Tewari, Pandey, 1978). All these facts confirm the presence of nonspecific resistance, which can be formed both under the influence of insecticides possible as under the influence of other stressors. In our work it was taken an attempt of the comparative analysis of the processes of resistance and cross-resistance forming to number of such different by the manner of action factors, as short-term influence of high or low temperature and chronic influence of the bacterial preparation bitoxybacilline (BTB) and organophosphorus insecticide malathion (carbophos, 10% w.p.) in laboratory strains of the housefly.

MATERIALS AND METHODS In the investigations conducted the larvae of the housefly *Musca domestica* L. were used. For the research of the resistance forming the larvae in the sensitive (S) strain Cooper were divided into groups and each group was selected by the corresponding stress-factor. The larvae of III age beginning were treated. For forming of the resistance to high temperature (the strain RH) the glasses with substrate and larvae were placed for 30 minutes in thermostat with corresponding temperature, starting with the temperature 35ºC. In the following selections the temperature was increased on 5-1ºC depending on surviving of larvae. For forming of the resistance to low temperature (the strain RC) the glasses were placed in refrigerator with corresponding temperature (starting with 15ºC and then reducing it on 3-1ºC). In the course of level determination of the resistance to the non-favorable temperatures the glasses with larvae were placed for 30 minutes in thermostat at the temperature from 30º to 60º C (with a step in 5º) or in refrigerator at the temperature from 20º to 0ºC (with a step in 5º). The control glasses were kept at the temperature 25ºC, the account of mortality was taken after the eclosion of adults. In the course of level determination of the resistance to toxicants the substrate with addition of corresponding preparation was placed in the glasses with larvae. 6 concentrations of each preparation were used in experience with 20 larvae in three-fold repeats. For control group of larvae the dry substrate was moistened by water. The glasses were contained under 25 ºC, the mortality account was taken after the adults eclosion. The criterion of flies' larvae sensitivity to the preparations was the efficient concentration, led to 50% mortality of persons (EC50,%), which was defined by the method of probit-analysis (Prist, 1965). The degree of reached resistance in housefly larvae was characterized by the resistance index (RI), which presents itself the ratio of resistant line EC50 to EC50 of sensitive line (Methodical instructions, 1990).

RESULTS AND DISCUSSION The toxicological estimation results of the housefly sensitive strain and strains selected with malathion (RM) and BTB (RBTB), are presented in table 1.

Table 1.Changes of the sensitivity to toxicants in housefly larvae during 20 generations

Generation	Index	Strain Rm	Strain Rbtb
F0(S strain)	EC50(%)	0.0168±0.0017	0.0047 ± 0.0008
F5	EC50(%)	0.021 ± 0.004	0.0067 ± 0.0009
	RI	1.25	1.43
F10	EC50(%)	0.031 ± 0.002	0.017 ± 0.001
	RI	1.8	3.6
F15	EC50(%)	0.058±0.006	0.028±0.002
	RI	3.45	5.96
F20	EC50(%)	0.13 ±0.003	0.024±0.002
	RI	9.42	5.12

From this table it is seen that the resistance to malathion forms very slowly - in 5-th generation RI=1.25, and only to 20-th generation it reaches the significant value - 9.42. Much quicker the resistance to BTB was formed at the beginning of selection; then this process was retarded and in 15-th and 20-th generations the RI was almost equal (5.96 and 5.12 accordingly). Apparently, cause of this is that this preparation has a broad spectrum of the action and is not only neirotoxicant, but also renders antifidant, metatoxic and dereproductive action (Poskryakov et al., 1995). The last effect we had observed after 14 selections with BTB. The sensitivity to selectant changes in houseflies larvae selected with high (RH) and low (RC) temperatures is shown on figures 1 and 2. In the selection of high temperatures the curve of mortality has typical form of dependency "dose - effect" and allows to use probit-analysis for calculation of the index named ST50 - a temperature, which is lethal for 50% exposed persons under given method of exposure. For the houseflies' larvae, selected with high temperature, as in the event with toxicants, the tendency of resistance to selectant increasing is revealed (fig.1), but this trend has not a single meaning. On extent of 10 generations selected the resistance of larvae increased: ST50 for S strain was +43ºC, and in 10-th generation - +53º C, then resistance of larvae decreased, remained higher than in sensitive strain.

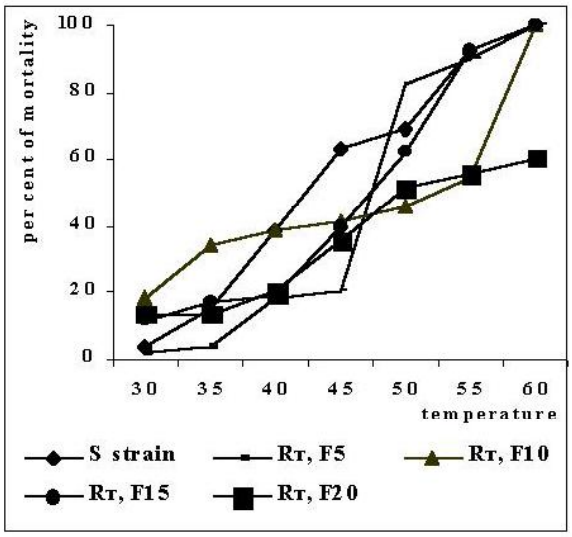

Fig. 1. The sensitivity of housefly larvae to high temperature

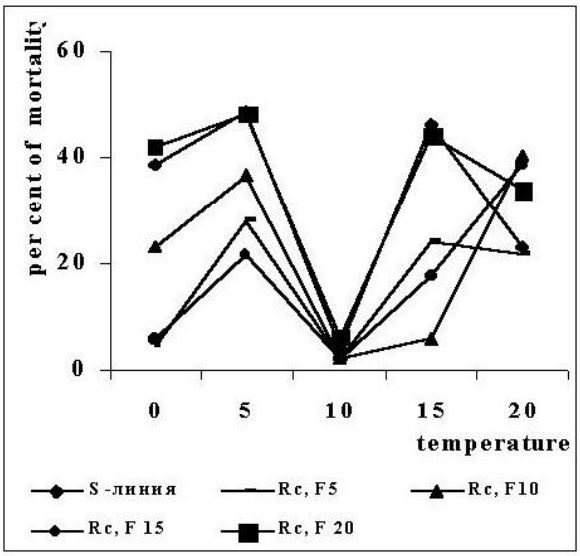

Fig. 2. The sensitivity of housefly larvae to low temperature

Very interesting appropriateness is revealed for larvae, selected with low temperatures (fig.2). The minimal mortality of larvae was observed under the influence +10º C. Moreover, the emerged flies of the sensitive strain. Copulated and postponed the eggs (without substrate and water). Thereby, it is possible to consider that 30-minute exposure under +10ºC is adaptogenic stress-factor for sensitive and selected strains. Direct applying of the probit-analysis method for temperature sensitivity estimation turned out to be difficult. If in course of insecticides treatment outside of class (pyrethroids, inhibitors of the chitin syntheses, organophosphorus or organochlorus combinations) it is revealed proportional dependency of the mortality percent from concentration substance then in the event of influence of the low temperature this curve looks like sinusoid. Apparently, the complex nature of surviving dependencies from the low temperature is explained that damaging influence in this case is due not by one primary reason, as under the heat stress, but by complex of factors. The form of dose-effect curve for low temperature indicates the presence of several critical temperature ranges that is probably caused by nonsimultaneously starting of several different compensatory mechanisms. However the certain critical range exists, within, of which all explored strains demonstrated proportional acute increase of mortality. Under the influence of the high temperatures in our experiment the most rectilineal increase of mortality is registered from +40ºC to +50ºC. In the variant with low temperatures using the similar increase of mortality is noted from +10º to +5ºC. We offer to use for comparing the different groups of insects, in our case - high and low temperature selected house fly strains, the coefficient of temperature sensitivity (CT), defined as ratio of mortality values differences in extreme points of the critical temperature range for selected strains to the same differences for sensitive, or "basic" strain:

$$Ct = \frac{|\ N(i1) - N(i2)\ |}{|\ N(c1) - N(c2)\ |}$$

Where N(i1) and N(i2) -mortality values in extreme points of the critical temperature range for basic (sensitive) strain; N(c1) and N(c2) - mortality values in the same points for selected (or compared) strain

For such index value 1.0 means absence of some difference in sensitivity to the changes of the temperature. If CT value exceeding 1.0, it evidences the decrease of sensitivity (increase of resistance) in comparing with basic group of insects; value CT smaller than 1.0 indicates the increase of sensitivity (the reduction of resistance). Thereby, it was possible to estimate and compare the results of selection in R-H and R-C strains their (tab. 2).

Table 2. Changes of the temperature sensitivity to in housefly larvae during 20 generations

Generation	Index	Strain R_C	Strain R_T
F_0 (S strain)	d	44.2	30.7
F_5	d	26.1	63.9
	C_T	1.69	0.5
F_{10}	D	37.7	6.8
	C_T	1.2	4.5
F_{15}	D	17.7	43.4
	C_T	2.5	0.7
F_{20}	D	42	31.1
	C_T	1.1	1

Comment: d – difference of mortality in extreme points of the critical temperature range, Ct – coefficient of temperature sensitivity.

Table 3.The cross–resistance to toxicants in 10-th and 20-th generations of selected strains.

Insecticide	Generation	Index	Strain S	Strain R_{BTB}	Strain R_M	Strain R_C	Strain R_T
BTB	F_{10}	EC50(%)	0.0047± 0.0007	0.0171± 0.0013	0.01± 0.001	0.014 ± 0.002	0.064± 0.007
		RI		3.64	3.2	2.98	13.62
	F_{20}	EC50(%)		0.024±0.002	0.029±0.003	0.028±0.002	0.044±0.004
		RI		5.12	6.17	5.96	9.36
Malathion	F_{10}	EC50(%)	0.0168± 0.0017	0.012± 0.001	0.031± 0.002	0.026 ±0.002	0.0041± 0.0016
		RI		0.71	1.85	1.55	0.24
	F_{20}	EC50(%)		0.079±0.009	0.13±0.003	0.023±0.002	0.018±0.002
		RI		4.7	7.65	1.35	1.08

In the strain, selected with low temperatures, value CT remains during 20 generations exceeding 1.0. In our opinion that is evidence of process continuing of the resistance forming to low temperatures though its rate changes. In the course of selection with high temperature the significant increasing of resistance in 10-th generation was changed by reversion of resistance level in 15-th generation and then returned to initial level in 20-th generation. It will be noted that this criterion, characterizing the direction of the sensitivity changing process must not be single. As the data (tab. 2) evidence, the selection with high temperature seemed to be unsuccessful, since in 20-th generation actual reversion of resistance is registered. In the same time, surviving data under maximal temperature (+60º) in the strain RH indicate the great qualitative advance of resistance to high temperature in this strain: in 20-th generation was registered 40% surviving of larvae after exposure against the value 0 in F5 -F15.

Results of the cross-resistance in 10-th and 20-th generations of selected strains to toxicants investigations are presented in table 3.

Practically in all selected strains of the housefly in 10-th generation the cross resistance to BTB was formed, but the strain selected with high temperature has more appreciable resistance (RI=13,6). At the same time cross-resistance to malathion is rather low (RI=1.55 in RC) or is absent (RI=0.71 in RBTB and RI=0.24 in RT). The cross- resistance to these preparations was increased in 20-th generation in BTB and malathion-selected strains cross- resistance to malathion increased. In the RT strain and in RC strain increased the cross-resistance to BTB while cross-resistance to malathion did not change.

Using the proposed coefficient of temperature sensitivity there is possible to present data on cross-resistance of selected strains to high and low temperatures in the manner of tables also (tab.4).

The strains selected with BTB and low temperature show cognate with sensitive strain relation to high temperature i.e. resistance does not observed. Cross-resistance of the malathion-selected strain to high temperature was registered only in 20-th generation. The curve of mortality in selected strains under influence of the low temperatures has, either as in the event of with sensitive strain sinusoid shape. The minimal mortality also was registered under the temperature +10º ?. Thereby the exposure by temperature +10º ? is an adaptogenic stress-factor for all selected strains. In 20-th generation of all selected strains was observed the lower low-temperature sensitivity than in S strain (CT>1) i.e. cross- resistance is formed to low temperature, but it is possible that low temperature make more active defensive powers of the insects' organism in consequence of which surviving of the larvae increases irrespective of selectant nature (tab.5).

Table 4. Cross-resistance level of housefly larvae to high and low temperatures

Parameter	Generation	Index	Strain R_{BTB}	Strain R_M	Strain R-c	Strain R_T
High temperature	F_{10}	d	29.6	32,0	37,3	6,8
		C_T	1.04	0.96	0.82	4.51
	F_{20}	D	44.7	5	34	31.1
		C_T	0.69	6.14	0.9	0.99
Low temperature	F_{10}	D	43	16	34.7	34.8
		C_T	1.03	2.76	1.27	1.27
	F_{20}	d	18.8	13	42	20
		C_T	2.35	3.4	1.05	2.21

Table 5. Surviving of the housefly larvae selected with different stressors under extreme temperatures (%)

Temperature	Generation	Strain R_{BTB}	Strain R_M	Strain R_C	Strain R_T	Strain S
60° C	F_{10}	0	2.1	0	0	0
	F_{20}	34	29.5	10	40	
0° C	F_{10}	34.9	58	76.9	22.4	61.8
	F_{20}	64.6	81.4	58	57.8	

CONCLUSION The results obtained indicate that as specific so and nonspecific defense mechanisms are used in the process of resistance forming to stress-factors of the different nature. Presence of such nonspecific component cause the cross-resistance forming to all selectants in the BTB-selected strains In the same time, results of the cross-resistance level estimation demonstrate the prevailing importance of nonspecific component in the resistance to temperature influences forming. In our opinion it is based on difficulty and multiplicity of the organism defensive systems, participating in the forming of so stable adaptation to temperature factor, as the temperature resistance in the course of selection acquired. Not all changes occurring during the selection it is possible to explain, using the result, on organism level obtained. So it is necessary to produce the biochemical studies, which will allow us to elaborate the picture of the defensive systems interaction in the processes of directional selection.

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Susceptibility to Insecticides in Representative Canadian Populations of Colorado potato beetle, *Leptinotarsa decemlineata* (Say)

ABSTRACT Second instar larvae from representative Canadian populations of Colorado potato beetle were exposed by residual leaf-disc bioassay to a diagnostic dose (DD98) for several insecticides. Three days after treatment, mortality was ≤75% for 41.7%, 97.9%, 72.3% and 80.9% of populations treated respectively with imidacloprid, lambda-cyhalothrin, azinphos-methyl or spinosad.

KEYWORDS Colorado potato beetle, Canada, susceptibility, imidacloprid, lambda-cyhalothrin, spinosad, azinphos-methyl.

INTRODUCTION With a farm value exceeding $882 million in 2003, potato continues as an extremely important field vegetable crop in Canada. For a number of years the Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say), has been the most damaging and recalcitrant foliage-feeding, insect pest of potatoes in Canada. Infestation and defoliation of plants in the field could sharply reduce potato yields (Tolman *et al*. 1986; Noronha *et al*. 2002). If the problem was not addressed promptly, repeated applications of insecticides often proved necessary. Adequate control of this pest has been a major concern for potato growers due to repeated development of resistance of CPB populations to conventional insecticides. By 1995 only foliar insecticides containing the δ- endotoxin of the bacterium *Bacillus thuringiensis var. tenebrionis* remained widely effective in eastern Canada. In 1995, imidacloprid (Admire 240F), a chloronicotinyl insecticide with a new mode of action, was registered in eastern Canada. Management programs utilizing Admire 240F have subsequently generally provided excellent CPB control. Localized resistance to imidacloprid has, however, developed. By 1999 as much as 90-fold resistance had been demonstrated on Long Island, NY (Moyer 1999). Subsequent research on two CPB strains from Long Island confirmed resistance of as much as 150-fold in adults (Zhao *et al*. 2000; Hollingsworth *et al.* 2002) and 13-fold in larvae (Zhao *et al.* 2000).

In Canada in 2002, less than 75% mortality at an imidacloprid Diagnostic Dose lethal within 3 days to 98% of the reference insecticide-susceptible population (DD98) was observed in 13 of 35 populations of larvae reared from CPB collected in Ontario, Québec, New Brunswick and Nova Scotia (Tolman *et al*. 2003). Control-failures were not, however, associated with these populations. The survey was expanded in 2003 to further study possible developing tolerance to imidacloprid. In addition, to identify possible reversion (Kristensen *et al*. 2000) of previously identified tolerance to lambda-cyhalothrin and azinphos-methyl, CPB larvae were exposed to the DD98 for each insecticide. Finally, in order to determine variation in baseline susceptibility to spinosad, registered in 2003 for CPB control in Canada, additional larvae were also exposed to the DD98 for spinosad. Results of the 2003 Canadian survey of larval susceptibility to these insecticides are herein summarized.

MATERIALS AND METHODS

Insects - All bioassays were undertaken using second instar larvae. An insecticide susceptible (LAB-S) strain, reared on potato plants in the laboratory (Harris and Svec 1976) for over 45 generations served as the reference strain. Depending on the insecticide, 47 or 48 populations collected from six Canadian provinces were surveyed for susceptibility (Prince Edward Island - 6; Nova Scotia - 1; New Brunswick - 12/13; Québec - 9; Ontario - 17; Manitoba - 2). Approximately 300-500 adult CPB from each population were collected directly from infested potato fields and shipped to the laboratory. For each population, approximately 20-25 pairs of field-collected adults were held for oviposition on potted potato plants in the laboratory. Eggs were collected daily to produce second instars for bioassay.

Insecticides - Formulated imidacloprid (Admire 240F - 240 g a.i./L), spinosad (Tracer 480SC - 480 g a.i./L), azinphos-methyl (Guthion 240SC - 240 g a.i./L) or lambda-cyhalothrin (Matador 120EC - 120 g a.i./L) was suspended in reverse-osmosis (RO)-water to give stock solutions containing 2.0 ppm active insecticide; dilutions were subsequently made as required with RO-water.

Residual Bioassay - Leaf discs (43 mm diameter), punched from fresh potato leaves, were immersed in the desired concentration of insecticide and allowed to dry. Dry, treated discs were individually transferred to labelled, sterile Gelman® 47 mm microbiological dishes. Five second instars were placed on the leaf disc. The dish was then covered and transferred to a holding room at 27°C and 65% RH under continuous light. Mortality was counted three days after treatment (3 DAT) and corrected for natural mortality using Abbott's correction (Abbott 1925). For each series of bioassays for each population, control insects were placed on leaf-discs dipped in RO-water.

Diagnostic Dose - For each insecticide, using the LAB-S strain, at least 3 separate series of bioassays were run at each of 7 concentrations with a minimum of 60 larvae (3 bioassays x 4 replicates/bioassay x 5 larvae/replicate) for each concentration tested. Probit analysis (SAS Institute 2001) of the data generated for each insecticide was then completed to develop dose-mortality regression lines. For each insecticide the LC98, lethal to 98% of the LAB-S population 3 DAT

was selected as the Diagnostic Dose (DD) for the susceptibility survey. To complete the survey for each population, two replicates of 5 second instars were treated on each of 3 separate days and mortality counted 3 DAT. Results for each population were then averaged for presentation. Possible correlation in susceptibility between insecticides was also determined (MSTAT Development Team 1991).

OBSERVATIONS AND DISCUSSION The average responses of second instars from 47-48 populations exposed in 2003 to the DD98 for the studied insecticides are illustrated in Figure 1 and summarized in Tables 1 and 2. For imidacloprid, mortality at the DD98 was ≤ 90% for only 16 (33.3%) populations exposed by residual contact/ingestion to 0.36 ppm imidacloprid. Larval mortality ≤ 50% was recorded for 5 (10.47%) collected populations. For one population collected in Québec, mortality barely exceeded 25%, a response level considered to represent a resistant population (Ellis 1989, *in* Olson *et al*. 2000). While susceptibility to imidacloprid of Canadian CPB in 2003 appeared lower than that recently reported in a European survey (Nauen and Denholme 2005), Canadian growers have not yet reported wide-spread control failures in the field. Admire 240F remains the dominant insecticide applied for CPB control in Canada.

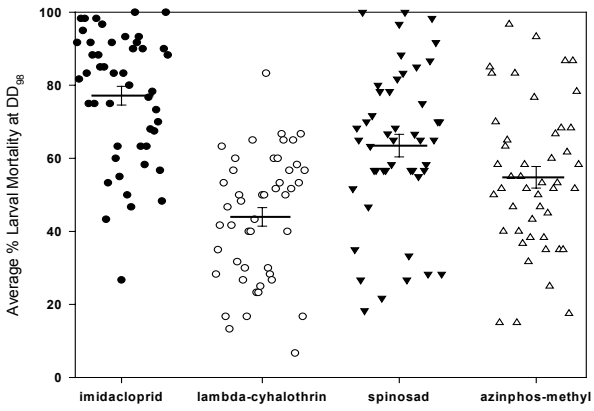

Figure 1: Scatter dot plot of susceptibility in 2003 of 2nd instar larvae of representative Canadian populations of Colorado potato beetles to diagnostic doses (LC_{98}) for imidacloprid (0.36 ppm), lambda-cyhalothrin (0.125 ppm), spinosad (0.20 ppm) and azinphos-methyl (25.0 ppm) via residual, leaf-disc bioassay.

Mortality of second instars exposed to residual deposits of 0.125 ppm lambda-cyhalothrin was ≤ 75% for 46 of 47 tested populations and below 25% for 7 (14.8%) collections. Permethrin, the first of the pyrethroids, was registered in Canada in 1978. By 1982 significant resistance to several pyrethroids, including permethrin, had been documented in CPB collected in Québec (Harris and Turnbull 1986). Resistance to pyrethroids continued to spread in Canadian CPB populations to the extent that by the time Matador was registered in Canada, it was not recommended for CPB control in Ontario (OMAFRA 1997). While the spray-histories of individual collections is not fully known, it would appear that there has been little reversion of tolerance to lambda-cyhalothrin in any tested population.

Mortality of second instars exposed to residual deposits of 25.0 ppm of azinphos-methyl was ≤ 75% for 38 of 47 tested populations and ≤ 50% for 19 (40.4%) collections. Larval mortality ≥90% at the DD98 was, however, recorded for 2 populations. First registered in Canada for CPB control in 1962, azinphos-methyl

Table 1: Summary of relative mortality of second instar larvae from selected Canadian populations of Colorado potato beetle (CPB) at a diagnostic dose lethal to 98% (DD_{98}) of an insecticide-susceptible strain, 2003.

	Average % Mortality at DD_{98} for Indicated Insecticide			
	imidacloprid	lambda-cyhalothrin	spinosad	azinphos-methyl
DD_{98} (ppm)	0.36	0.125	0.2	25
n[1]	48	47	47	47
Mean	77.2	44	63.5	54.8
Range	26.7-100.0	6.7-83.3	21.7-100.0	15.0-96.7

1 - Number of field populations.

Table 2: Distribution of mortality of second instar larvae from selected Canadian populations of Colorado potato beetle (CPB), at a diagnostic dose lethal to 98% (DD_{98}) of an insecticide-susceptible strain, 2003.

Test Insecticide (n)[1]	No. (%) Strains with Indicated % Mortality at DD_{98}			
	$ 90%	# 75%	# 50%	# 25%
imidacloprid (48)	16 (33.3%)	20 (41.7%)	5 (10.4%)	0 (0.0%)
lambda-cyhalothrin (47)	0 (0.0%)	46 (97.9%)	28 (59.6%)	8 (17.0%)
spinosad (47)	5 (10.6%)	34 (72.3%)	9 (19.1%)	2 (4.3%)
azinphos-methyl (47)	2 (4.3%)	38 (80.9%)	19 (40.4%)	4 (8.5%)

1 - Number of field populations.

remained effective for a number of years. Not until 1979 was resistance to Guthion 2SC confirmed in CPB collected near Sherbrooke, Québec (Harris and Svec 1981). In this study, in contrast with results reported for lambda-cyhalothrin where larval mortality at the DD98 remained below 75% for all but 1 population, larval mortality for azinphos-methyl at the DD98 was ≥ 75% for 20% of 47 populations. Thus while resistance to azinphos-methyl remains a very serious problem, we recommend growers undertake a dip test (Banks and Squire 1992) to determine insecticide resistance of early instar larvae in their fields. Use of azinphos-methyl against susceptible larvae would reduce selection for imidacloprid-resistant CPB, preserving effectiveness of imidacloprid for CPB control.

As was the case for imidacloprid prior to its introduction and widespread use (Olson *et al*. 1996), there was considerable variation in susceptibility to spinosad among CPB populations never exposed to the insecticide. For second instars, only 5 (10.6%) populations exhibited ≥ 90% mortality when exposed to residual deposits of 0.20 ppm, the DD98 for spinosad; for 34 (72.3%) collections of larvae exposed to the DD98 mortality was ≤ 75%. Representative Canadian CPB populations have also shown significant variation in susceptibility to a DD98 for novaluron, an insect growth regulator not yet registered for CPB control in Canada (Cutler *et al*. 2005).

Potential correlation in larval susceptibility at the DD98 was compared across the four insecticides in the study for 47 different populations (Table 3). In contrast to Olsen *et al.* (2000) who found that CPB populations were generally less susceptible to imidacloprid if they were resistant to other classes of insecticides, we identified no significant correlations between susceptibility of second instars to imidacloprid and susceptibility to any other tested insecticide. There was, however, positive correlation between susceptibility to lambda-cyhalothrin and susceptibility to azinphos-methyl (Table 3).

Table 3: Correlations of average % mortality at a DD_{98} for selected insecticides for second instar larvae from selected Canadian populations of Colorado potato beetle, 2003.

Insecticide Correlation	slope	r	p
imidacloprid - lambda-cyhalothrin	0.063	0.063	0.67
imidacloprid - azinphos-methyl	0.118	0.101	0.5
imidacloprid - spinosad	-0.094	-0.077	0.61
lambda-cyhalothrin - azinphos-methyl	0.336	0.288	0.05
lambda-cyhalothrin - spinosad	-0.011	-0.009	0.95
azinphos-methyl - spinosad	-0.034	-0.036	0.81

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Generating baseline data for insecticide resistance monitoring in sugarcane woolly aphid, *Ceratovacuna lanigera* Z.

White sugarcane woolly aphid *Ceratovacuna lanigera* Z. is recently emerged as one of the most serious pests of sugarcane in India. It was reported from Philippines, Indonesia, Taiwan, China, Japan, Korea, India and Pakistan (Takara and Azuma, 1968; Hill, 1993; Gupta and Goswamy, 1995; Anon, 2001) whereas *C. japonica* and *C. graminum* were recorded in Japan, Korea (Anon, 2002) and India (David and Nandagopal, 1986). In Tamil Nadu Woolly aphid has entered border areas of Vellore District from Andhra Pradesh during April 2004. It has spread into all the taluks of Vellore District and to the adjoining districts, *viz*., Tiruvannamalai, Dharmapuri and Krishnagiri (Thirumurugan *et al*., 2005).

The roving survey indicated that the incidence of this pest in Tamil Nadu was found in isolated patches (Kalaiyarasan,2005) The pest is controlled by using the insecticides monocrtophos, dichlorovos, fenitrothion, dimethoate and endosulfon (Patil *et al.*, 1980; Verma and Bindra, 1980; Ananthanarayana *et al*., 1984; Regupathy *et al*.,2003; Lingappa *et al*.,2003; Shankar and Shitole 2004). Neonicotinoids like thiamethoxam, imidacloprid and acetamiprid were found to be effective (Shinde *et al.*, 2003). Base line data on the susceptibility of the target pest to the toxicant is the most important factor for the insecticide use especially for monitoring the development of resistance.

Acute toxicity studies were carried out for thiamethoxam, imidacloprid, dimethoate and methyldemeton against the sugarcane *C.lanigera* for three successive generations by using the cages of 3x2.1 cm size made by cutting polyvinyl tube of 2.1 cm dia in to 3cm length, one end of which covered with muslin cloth. The aphids were confined within the cage by stretching parafilm membrane across open end of the cage. Different concentrations of insecticides were prepared and mixed with equal quantity of 10 per cent sucrose solution. Parafilm sachet containing the various concentrations of insecticides in the 10 per cent sucrose solution was covered by stretching second piece of parafilm over the first. Ten third instar aphids were caged and covered by Parafilm sachet described by Mitler and Dadd (1964). The aphids were allowed to feed and observations on the mortality of aphids were taken 24 hrs after feeding in various treatments. The LC50 and LC90 values, susceptibility index and rate of resistance decline (Regupathy and Dhamu,2001) were worked out. Among the chemicals tested, thiamethoxam and imidacloprid were found to be highly toxic. The highly toxic nature of thiamethoxam was reported by Mathirajan and Regupathy (2001) to *A.gossypii.*

The LC50 was 0.1469 ppm for thiamethoxam (Table 1); 0.1352 for imidacloprid (Table 2); 94.0850 for dimethoate (Table 3) and 73.2060 for methyl demeton (Table 4). LC95 was 1.6532 for thiamethoxam (Table 1); 1.3068 for imidacloprid (Table 2); 1127.2576 for dimethoate (Table 3) and 890.5753 ppm for methyl demeton (Table 4). Thiamethoxam was found to be highly toxic to aphids as indicated from low LC50 values

Table 1. Acute toxicity of thiamethoxam to *C.lanigera*

Generation	Regression equation	χ^2	LC_{50} (ppm)	Fiducial limits		LC_{95} (ppm)	Fiducial limit	
				LL	UL		LL	UL
1	Y = 1.6086 + 1.5648 x	1.1405	0.1469	0.1029	0.2098	1.6532	0.7553	3.6183
3	Y = 1.6907 + 1.6053 x	0.2526	0.1152	0.0782	0.1697	1.2193	0.5909	2.5159

Table 2. Acute toxicity of imidacloprid to *C. lanigera*

Generation	Regression equation	χ^2	LC_{50} (ppm)	Fiducial limits		LC_{95} (ppm)	Fiducial limit	
				LL	UL		LL	UL
1	Y = 1.4421+ 1.6695 x	2.1846	0.1352	0.0961	0.1903	1.3068	0.6501	2.6272
3	Y = 1.4169 + 1.7313 x	1.3724	0.1174	0.0827	0.1665	1.0462	0.5416	2.0211

Table 3. Acute toxicity of dimethoate to *C. lanigera*

Generation	Regression equation	χ^2	LC_{50} (ppm)	Fiducial limits		LC_{95} (ppm)	Fiducial limit	
				LL	UL		LL	UL
1	Y=-2.6018 + 1.5284x	1.8707	94.085	66.218	133.6782	1121.258	478.21499	2628.9829
3	Y=-2.56299 + 1.5246x	0.1335	91.2849	63.9	130.57	1094.593	469.6429	2551.1607

Table 4. Acute toxicity of methyl demeton to *C. lanigera*

Generation	Regression equation	χ^2	LC_{50} (ppm)	Fiducial limits		LC_{95} (ppm)	Fiducial limit	
				LL	UL		LL	UL
1	Y=-2.3740+1.5159 x	1.1642	73.206	53.4723	100.2225	890.5753	438.5936	1808.3354
3	Y=-2.4884+1.5422 x	0.6742	71.7164	50.034	102.7949	836.0015	372.4607	1876.4358

The present observation of low LC50 is when compared to OP's in concurrence with the LC50 of *A.gossypii* to thiamethoxam (1.0414 - 35.2153) (Praveen and Regupathy 2004); imidacloprid (0.05642 - 0.07793) (Kumar, 1998), *A.devastans* to thiamethoxam (0.0087 - 0.06721); imidacloprid (0.00101 - 0.08861) (Praveen and Regupathy, 2004a); imidacloprid (0.00056 - 0.00457) (Jeyapradeepa and Regupathy, 2002) and *T.tabacci* to thiamethoxam (0.0971 - 2.6076); imidacloprid (0.0713 - 1.9650) (Praveen and Regupathy, 2004b).

The susceptibility of *C.lanigera* was tested without selection pressure to insecticides by rearing the population in glass house condition for three generations. The results indicated that the susceptibility increased as indicated from low LC50 and LC95 values.

The LC50 values at third generation declined to 0.1152 ppm for thiamethoxam; 0.1174 ppm for imidacloprid; 91.2849 for dimethoate and 71.7164 ppm for methyl demeton. The values for third generation with out selection pressure were comparable with the data obtained by earlier workers; 0.3412 for thiamethoxam and 0.4583 for imidacloprid (Praveen and Regupathy, 2004a); 0.3488 for thiamethoxam (Mathirajan and Regupathy, 2002); 0.309 for imidacloprid to *A.gossypii* (Kumar, 1998). Further the LC50 value obtained by Elbert *et al.*, (1991) (0.30 ppm) also indicated that the values obtained in the present investigations could be used as baseline data for fixing discrimination dose for thiamethoxam and imidacloprid.

The LC50 value for other pests is in close range. The LC95 values of *C.lanigera* were 1.2193 for thiamethoxam; 1.0462 for imidacloprid; 1094.5928 for dimethoate and 836.0015 ppm for methyl demeton. The LC95 values of 3745.0 ppm of methyl demeton (Hollingworth et al., 1994) and 49.1667 and 418.4538 ppm for dimethoate and methyl demeton for *A.gossipii* (Praveen and Regupathy, 2004) and 2722.7 and 4466.8 ppm for dimethoate, methyl demeton respectively for *A.devastans* (Jeyapradeepa and Regupathy, 2002) were comparable. The LC50 and LC95 values declined when the generation proceeds. This results were in line with that obtained for *A.gossypii* by Praveen and Regupathy(2004), for *A.devastans* by Jeyapradeepa and Regupathy (2002) and for *N.lugens* by Sujatha and Regupathy (2003).

It was proven to be very toxic and effectiveness of thiamethoxam and imidacloprid was comparable under field conditions. Imidacloprid was found to be more toxic to *A.gossypii* (Kumar, 1998) and *Aphis craccivora* Koach than methyl demeton (Ramesh babu and Santharam, 2000) and to apple aphids *Nasonovia ribisnigri* and *M.persicae* than lamdacyhalothrin, deltamethrin, cypermethrin, dimethoate, methyl demeton, primicarb and heptanophos (Barber *et al*., 1999). The susceptibility index varied between 1.02 and 1.27 based on LC50 and it was in the range of 1.02 - 1.35 based on LC95 values for all the chemistries tested. The rate of resistance decline was negative for all the insecticides tested, indicating the susceptibility of the test insect. In terms of number of generations required for 10 fold decrease in LC50, a numerically high value was obtained for, imidacloprid (49.01), methyldemeton (34.48), thiamethoxam (28.40) and dimethoate (23.25) (Table 5).

Table 5. Susceptibility index and rate of resistance decline in *C. lanigera*

Insecticides	Susceptibility index		Rate of resistance decline	
	LC_{50}	LC_{95}	R	G
Thiamethoxam	1.27	1.35	-0.0353	28.4
Imidacloprid	1.15	1.24	-0.0204	49.01
Dimethoate	1.03	1.02	-0.0044	23.25
Methyl demeton	1.02	1.06	-0.0029	34.48

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Development of pest resistant *Bt* sorghum: Need of the hour

Among the various constraints, insect pests, especially, lepidopteron, coleopterans and Dipterans are major threat to agriculture. Recombinant DNA technology and genetic engineering have opened a new era in insect pest management through the production of Bt transgenics, which proved very effective against such

pests. After Bt cotton, it's the time to develop and introduce Bt sorghum (*Sorghum bicolor* (L). Moench) for cultivation, as its an important crop in diet of millions of people in arid & semi-arid tropics including India where drought stress, pest and disease attack causes frequent failure of crops. India is the largest sorghum grower (11.89mha (8.20 % of total cultivated area) in the world and ranks seventh in production. In India, sorghum is grown as either grain or fodder in almost all states especially in Maharashtra and Karnataka. Sorghum is grown both during southwest monsoon (*kharif*) and post monsoon-season (*rabi*). Rabi crop produces high quality grains as they mature during winter season with clear, dry and rain-free climate. Through out the Deccan Plateau Rabi sorghum is preferred for human consumption. Among the various insect pests that can attack sorghum from seedling stage till harvest, lepidopteran and dipteran insect pests are major ones. Sorghum of one month old is attacked by a dipteran insect pest sorghum shootfly, *Atherigona soccata* (Muscidae: Diptera) that bore into the young seedling and cause dead heart (death of seedling) symptom. Another dipteran pest called sorghum midge, *Contarania sorghicola* can attack at milky stage of grain formation. After one month, it's prone to attack by a lepidopteran stem borer, *Chilo partellus* (Pyralidae: Lepidoptera), followed by a lepidopteran defoliator commonly called as *Mythimna seperata* (Noctuidae: Lepidoptera), a deadly pest particularly endemic in dharwad region of Karnataka which can devour whole plant at a density of 3 larvae per plant. Later, towards crop maturity/grain maturation, three lepidopteran head borers like *Helicoverpa armigera*, *Stenochroia elongella* and *Cryptoblabes angustipenella* can be noticed. More than 50% of the plant protection costs goes only towards these lepidopteran and dipteran pests. Other sorghum pests include sucking pests like sorghun shoot bug, *Rhaphalosiphum maidis*. Most of the organophosphate chemical insecticides that are commonly used *viz*., chlorpyriphos, monocrotophos, quinalphos, etc., for most of the field crop pest management, cannot be used on sorghum as they are phytotoxic and hence only safer insecticide that is recommended is endosulfan, a cyclodeine compound, belonging to organochlorines.

In this context, *Bt* being an effective tool especially against lepidopteran, coleopteran and dipteran insect pests, it's the need of the hour to develop *Bt* transgenic sorghum, which will serve as a protective umbrella for sorghum crop against these pests as a best alternative to chemical control and can make the sorghum, a more profitable crop with reduced frequency of single (endosulfan) pesticide sprays.

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Natural refugia for Bt resistance management vis-à-vis mating synchrony of *H.armigera* from different hosts

The old-world bollworm, *Helicoverpa armigera* is a polyphagous pest causing extensive damage on many important agricultural and horticultural crops, including cotton, in India and several parts of the world. Approval of the Bt cotton for commercial cultivation in India, since 2002, is an important development. More than 20 hybrids are presently under cultivation in different parts of the country. Many more hybrids by other seed companies, which carry the same gene, are likely to be approved for commercial cultivation in due course of time. With the available reports, the gene currently employed is at a high enough level of expression to create strong pressure on the bollworms at least for 100-120 DAS. It is expected that any competitive biological system would respond to high level of selection pressure by mechanisms that would either avoid or mitigate it. Random genetic changes that keep happening in a population of insect might include resistance alleles at a very low frequency, which can rapidly increase when challenged. *H.armigera* has already developed resistance to many potent insecticides, especially to pyrethroids. Like with chemical insecticides, *H.armigera* has every potential to develop resistance to cry toxins under field conditions due to continued selection pressure, throughout the crop growth period if proper resistance management tactics are not impleted. Development strategies to delay development of resistance to cry toxin by the worm has been therefore felt necessary in India and elsewhere. Like in other countries Genetic Engineering Approval Committee (GEAC) has made it mandatory for every field of Bt cotton to be surrounded by a belt of non-Bt cotton, called refuge crop, equivalent to 20% of the total crop area. The refuge strategy is expected to delay resistance development in bollworms by dilution of resistance allele frequency in the population due to intermating between susceptible individuals from refuge and possibly a few resistant ones arising on Bt crop. Considering the large acreage under different alternative hosts of *H.armigera*, Small land holdings, high seed cost, coincidence of insect infestation on Bt cotton and other alternate hosts, overlapping generations and compatible cross fertilization between strains from different crops, many workers felt that cultivation of Bt cotton with mandatory refuge may not be enforceable under the Indian (and other related countries) conditions. It is very obvious to note

differential growth period (longer in Bt fed insects) between the insects from Bt and non-Bt cottons (under laboratory conditions) due to the direct effect of Bt toxin on the physiology of insect which inturn questions the possibility of synchronized and random mating between the insects from Bt and other normal hosts.

It is very important to determine under field condition, whether available cropping system (including non-Bt) in a particular area serve as good refugia that supplement the susceptible insects in synchrony with that of resistant moths emerging from Bt cotton ultimately ending in random mating between the two and serving the purpose of resistance dilution and if not, whether different sowing dates of Bt and normal crops can serve the purpose? The answer will definitely strengthen the concept of refugia for Bt resistance management.

Moreover, Pink bollworm (PBW), *Pectinophora gossypiella* is next major bollworm pest on cotton has narrow range of host plants and infests only cotton crop in India and that too late in the season causing severe hidden infestation. The pest is known to undergo obligatory diapause in Indian conditions when there is no cotton crop in the field. As it is proven fact that the Bt protein levels in the whole plant drastically reduce as age advance, especially when PBW is active, the chances for resistance development are more for PBW due to sub lethal dose and if there is no non-Bt cotton refuge, frequency of resistant individuals will go increasing. Hence, in areas where PBW is major pest and if Bt toxin is targeted against it, structured non-Bt cotton refugium is an absolute component for Bt resistance management.

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Assessment of acute toxicity and resistance monitoring of lambda cyhalothrin (Karate Zeon 5 CS) to *Thrips tabaci* (Lindeman) in Cotton

ABSTRACT The median lethal concentration (LC50) of lambda-cyhalothrin was 35.6142 ppm for F1 generation of *T. tabaci* and 19.0175 ppm for F7 generation. The susceptibility increased with succeeding generation. The susceptibility index was 1.8727 based on LC50 and 1.0650 based on LC95. The rate of resistance decline and the number of generations required for a 10-fold decrease in LC50 was calculated as -0.0389 and 25.71 respectively. Discriminating dose of 0.7 ppm for imidacloprid, 1.0 ppm for thiamethoxam and 1000 ppm for dimethoate and a tentative dose of 150 ppm (based on acute toxicity studies) for lambda-cyhalothrin were followed for monitoring resistance in *T.tabaci.* The per cent survival varied from 12.50 to 17.39 for lambda cyhalothrin, 11.11 to 16.67 for imidacloprid, 12.00 to 16.42 for thiamethoxam, 13.24 to 19.12 for dimethoate in Thrips Insecticidal Bioassay System. The per cent survival varied from 13.33 to 17.33, 13.33 to 16.00, 12.00 to 17.33 and 14.67 to 17.33 for lambda-cyhalothrin, imidacloprid, thiamethoxam and dimethoate respectively in leaf dip method. The order of resistance in insecticides to *T.tabaci* in both the methods was dimethoate>lambda-cyhalothrin>thiamethoxam>imidacloprid.

INTRODUCTION The advent of new chemical and new, safer formulations necessitate to determine the selective toxicity and generation of baseline data for monitoring resistance to insect pests for sustained control. Of the new generation molecules, lambda cyhalothrin, an ecofriendly and photostable synthetic pyrethroid has been used against a wide array of pests. However, it has also been reported that many insects have become resistant to lambda cyhalothrin (Cho and Lee, 1984; Cameron and Walker, 1998)

Monitoring of insecticide resistance is a pre-requisite for any insecticide resistant management programme. The chief objective of resistance monitoring is to exaggerate the differences between susceptible and resistant individuals so that the frequency of misclassification is greatly reduced (Ffrench constant and Roush, 1990). Tabashnik and Croft (1982) opined that monitoring of resistance levels is essential in deciding management strategy against a pest. Suitable monitoring technique should be used not only to detect the presence of resistance but also to monitor the changes in resistance frequencies to determine whether a management programme is effective or not. In most practical situations the best monitoring method is the use of discriminating dose *i.e*., the dose that kills 99% of the susceptible individual (Roush and Miller, 1986). The resistance level in *T.tabaci* was monitored for insecticides *viz*., lambda-cyhalothrin, imidacloprid, thiamethoxam and dimethoate using discriminating dose screen during December 2004 to March 2005 continuously at weekly intervals in Coimbatore following leaf dip method and Thrips Insecticidal Bioassay System (TIBS).

MATERIALS AND METHODS *Thrips tabaci* was reared on potted plants of MCU 12 cotton without exposure to insecticides under laboratory conditions at 36 ± 1oC.

The cotton seeds were sown at the rate of four to five per pot (25 x 25 cm) in a staggered manner at weekly intervals and seedlings were thinned to two plants per pot after 15 days. Five to ten adult thrips were introduced on potted plants on 50 days after sowing and allowed to multiply. The seedlings were covered with mylar film cages in order to prevent the egg laying by insects other than the test insects and to prevent from natural enemies. Cylindrical Mylar film cages of size 30 cm diameter and 70 cm height were used for studies. All the cages were provided with proper ventilation by covering the top of cages with muslin cloth and second instar nymphs were used for bioassay. The adults of this F1 generation were transferred to fresh cotton plants for egg laying for development of subsequent generations.

To study the level of insecticide resistance in *T. tabaci* to insecticides *viz*., lambda-cyhalothrin, imidacloprid, thiamethoxam and dimethoate, leaf dip method of Elbert and Nauen (1996) and Thrips Insecticidal Bioassay System (TIBS) developed by Rueda and Shelton (2003) were followed. Thrips population from TNAU campus, Coimbatore were collected at weekly intervals.

In leaf dip method, leaf discs (45 mm dia) were cut from medium sized cotton leaves (MCU 12) using cork borer and dipped in different concentrations of formulation prepared using water for five seconds, then dried on filter paper in open air for 20 minutes. The bases of small ventilated polythene petri dishes (50mm dia) were filled with agar gel (12 g lit^{-1}, 5ml) to maintain the turgidity of leaf. The leaf discs were placed on the agar with their adaxial surface downwards. Second instar nymphs were collected from the pot culture cotton plant using aspirator and placed on a black cloth. Using a fine camel hairbrush, twenty thrips were then transferred on to the treated leaf disc and set up was covered with a lid. Leaf disc immersed in water alone served as control. The test was replicated thrice. Mortality was recorded 48 h after exposure to the insecticides.

Thrips Insecticidal Bioassay System (TIBS) allowed thrips to be collected from field and transferred directly in a plastic 0.5 ml centrifuge tube previously treated with insecticide. Thrips mortality was assessed after 24 h. The tube had a flexible plastic cap and on the inside of the cap was a small well into which 0.08 ml of 10 per cent sugar water solution was deposited. The solution was sealed into the well with a small piece of stretched parafilm through which thrips fed on the solution. The tube but not the cap was treated with an insecticide solution by filling the tube to its top. After 4 hrs the insecticide was poured out and the tube was allowed to dry overnight. A small opening at the end of the tube was then made using a heated sewing pin. It was through this opening the thrips were released into the tube using suction device. Once the thrips were released, this opening was sealed with a small piece of parafilm. Discriminating doses developed by Praveen and Regupathy (2003) for imidacloprid (0.7 ppm), thiamethoxam (1.0 ppm) dimethoate (1000 ppm) and a tentative dose of 150 ppm (based on acute toxicity studies) were followed for monitoring resistance in *T.tabaci.*

The median lethal dose (LD50) and median lethal concentration (LC50) of insecticide used were determined by Finney's probit analysis (Regupathy and Dhamu, 2001). Susceptibility indices, the rate of resistance decline, and resistance frequency were also calculated (Regupathy and Dhamu, 2001).

RESULTS AND DISCUSSION The median lethal concentration (LC50) of lambda-cyhalothrin was 35.6142 ppm for F1 generation and 19.0175 ppm for F7 generation. (Table 1). The susceptibility index was 1.8727 based on LC50 and 1.0650 based on LC95. The rate of resistance decline and the number of generations required for a 10-fold decrease in LC50 was calculated as -0.0389 and 25.71 respectively. (Table 1).

Table 1. Acute toxicity of lambda-cyhalothrin 5.0 CS to T. tabaci by leaf dip method

Generation	Regression Equation	Chi square χ^2	LC_{50} ppm	Fiducial limits		LC_{95} ppm	Fiducial limits	
				LL	UL		LL	UL
1	Y = -6.9678 + 2.6293x	1.2444	35.6142	28.115	45.1193	150.3888	82.949	272.6589
2	Y = -5.0973 + 2.2742x	1.4663	27.5422	20.735	36.5842	145.6434	76.1067	278.714
3	Y = -4.7652 + 2.2226x	1.8604	24.7499	18.3378	33.4043	136.0359	70.6908	261.7847
4	Y = -3.4888 + 1.9085x	1.5577	23.006	16.217	32.622	167.3531	73.9281	378.8417
5	Y = -3.4263 + 1.9448x	1.7262	21.5222	15.0699	30.7372	150.907	68.9252	330.4002
6	Y = -2.9261 + 1.8470x	1.2842	19.5599	13.2952	28.7765	152.0361	66.8688	345.6767
7	Y = -2.0840 + 1.8891x	0.814	19.0175	12.9494	27.9292	141.2094	64.6525	308.4199

Susceptibility Index

(LC 50) – 1.8727; Rate of resistance decline (R) = -0.0389 ; Generations for 10 fold resistasnce decline – 25.70

(LC 95) - 1.0650

In the present study both the methods were compared for three weeks. As there was no appreciable difference between the two methods, the TIBS method was followed. Shelton *et al*. (2003) used TIBS to evaluate the susceptibility of onion thrips, *T. tabaci* to lambda-cyhalothrin and reported that it could be instrumental for developing resistance management strategy for onion thrips. A common approach for conducting resistance assays for thrips is to culture the insect populations until sufficient number are available for testing. This approach presents several problems *viz*., difficulty of containing thrips in cages because of their small size, contamination by other colonies in the greenhouse and finally change of resistance over time. Hence, field level monitoring ideally provides the grower with the reliable and timely knowledge needed to make an informed decision on using an insecticide. As the discriminating dose (DD) can kill 95 per cent of the subjected normal population, based on lethal concentration obtained for lambda-cyhalothrin on F1 population of *T.tabaci*, tentative discriminating dose of 150 ppm was fixed for lambda-cyhalothrin based on acute toxicity studies (Table 1) and DD developed by Praveen and Regupathy (2003) for imidacloprid (0.7 ppm), thiamethoxam (1 ppm) and dimethoate (1000 ppm) were used. Second instar nymphs collected from field were tested for resistance to lambda-cyhalothrin. The per cent survival at 150 ppm (DD) varied from 12.5 to 17.4 (Table 2) in TIBS. Maximum survival was observed during second week of February and minimum survival was noticed during second week of January. Per cent survival varied from 13.3 to 17.3 (Table 4) in leaf dip method. For imidacloprid maximum survival was observed during second week of February and minimum survival was noticed during second week of January. The level of resistance varied from 11.1 to 16.7 per cent in TIBS (Table 2) at discriminating dose of 0.7 ppm. Maximum level was observed during fourth week of February and minimum level during first week of February. Per cent survival varied from 13.3 to 16.0 in leaf dip method (Table 4). The level of resistance for thiamethoxam ranged between 12.0 to 16.4 per cent in TIBS (Table 3) at 1.0 ppm (DD). Maximum survival was observed during first week of March and minimum survival during first week of December. The level of resistance in leaf dip

Table 2. Mean weekly resistance of *T. tabaci to* lambda-cyhalothrin (Karate Zeon 5.0 CS) and imidacloprid(Confidor) in Coimbatore during 2004-05 (TIBS)

Weeks	1 Dec'04	2 Jan'05	3 Jan'05	4 Jan'05	5 Jan'05	6 Feb'05	7 Feb'05	8 Feb'05	9 Feb'05	10 Mar'05	11 Mar'05	12 Mar'05
Lambda-cyhalothrin (Karate Zeon 5.0 CS)												
No. of insect dosed	74	73	72	68	71	70	69	74	75	69	66	73
No. dead	64	61	63	57	62	61	57	63	62	60	56	62
% Mortality	85.3	83.6	87.5	83.8	87.3	87.1	82.6	85.1	82.7	86.9	84.8	84.9
% Resistance ± SE	14.7± 4.1	16.4 ± 4.4	12.5 ± 3.9	16.2 ± 4.5	12.7 ± 3.9	12.9 ± 3.6	17.4 ± 3.8	14.9 ± 3.6	17.3 ± 4.4	13.0 ± 4.1	15.2 ± 4.4	15.1± 4.2
Imidacloprid (Confidor[â])												
No. of insect dosed	75	74	65	62	68	72	71	68	66	70	74	71
No. dead	66	64	55	52	57	64	60	59	55	62	66	62
% Mortality	88	86.5	84.6	83.9	83.8	88.9	84.5	86.8	83.3	88.6	89.2	87.3
% Resistance ± SE	12.0± 3.8	13.5 ± 4.0	15.4 ± 4.5	16.1 ± 4.7	16.2 ± 4.5	11.1 ± 3.7	15.5 ± 4.3	13.2 ± 4.1	16.7 ± 4.6	11.4 ± 3.8	10.8 ± 3.6	12.7± 3.9

Table 3. Mean weekly resistance of *T. tabaci* to thiamethoxam (Actara[â]) in Coimbatore during 2004-05 (TIBS)

Weeks	1 Dec'04	2 Jan'05	3 Jan'05	4 Jan'05	5 Jan'05	6 Feb'05	7 Feb'05	8 Feb'05	9 Feb'05	10 Mar'05	11 Mar'05	12 Mar'05
Thiamethoxam (Actara[â])												
No. of insect dosed	75	72	70	74	68	65	72	73	75	67	69	72
No. dead	66	61	61	62	59	55	63	62	64	56	58	61
% Mortality	88	84.7	87.1	83.8	86.8	84.6	87.5	84.9	85.3	83.6	84	84.7
% Resistance ± SE	12.0 ± 3.8	15.3 ± 4.2	12.9 ± 4.0	16.2 ± 4.3	13.2 ± 4.1	15.4 ± 4.5	12.5 ± 3.9	15.1 ± 4.2	14.7 ± 4.1	16.4 ± 4.6	15.9 ± 4.4	15.3 ± 4.3
Dimethoate (Rogor)												
No. of insect dosed	75	74	75	70	68	70	72	73	75	68	69	70
No. dead	65	64	62	59	55	60	62	61	63	59	58	59
% Mortality	86.7	86.5	82.7	84.3	80.9	85.7	86.1	83.6	84	86.8	84	84.3
% Resistance ± SE	13.3 ± 3.9	13.5 ± 4.0	17.3 ± 4.4	15.7 ± 4.4	19.1 ± 4.8	14.3 ± 4.2	13.9 ± 4.1	16.4 ± 4.4	16.0 ± 4.3	13.2 ± 4.1	15.9 ± 4.4	15.7 ± 4.4

Table 4. Mean weekly resistance of *T. tabaci* to different insecticides in Coimbatore during 2004-05 (leaf dip method)

	Lambda-cyhalothrin			Imidacloprid			Thiamethoxam			Dimethoate		
Weeks	Dec'04	Jan'05	Jan'05	Dec'04	Jan'05	Jan'05	Dec'04	Jan'05	Jan'05	Dec'04	Jan'05	Jan'05
No. of insect dosed	75	75	75	75	75	75	75	75	75	75	75	75
No. dead	65	63	62	65	64	63	63	62	66	64	62	62
% Mortality	86.7	84	82.7	86.7	85.3	84	84	82.7	88	85.3	82.7	82.7
% Resistance ± SE	13.3 ± 3.9	16.0 ± 4.3	17.3± 4.4	13.3 ± 3.9	14.7 ± 4.1	16.0 ± 4.3	16.0 ± 4.3	17.3 ± 4.4	12.0 ± 3.8	14.7 ± 4.1	17.3 ± 4.4	17.3 ± 4.4

method ranged between 12.0 to 17.3 per cent (Table 4). The level of resistance for dimethoate frequency ranged from 13.2 to 19.1 per cent (Table. 3) at 1000 ppm (DD). The maximum level was noticed during fourth week of January and minimum level was observed during first week of March. The level of resistance in leaf dip method ranged between 14.7 to 17.3 per cent (Table 4). Shelton *et al.* (2003) reported the development of resistance by onion thrips, *T. tabaci* to lambda-cyhalothrin in some onion growing regions of New York. Zhao *et al.* (1995) observed the development of resistance to imidacloprid in greenhouse population of *F. occidentalis.* The order of resistance in insecticides to T.tabaci in both the methods was dimethoate>lambda-cyhalothrin>thiamethoxam>imidacloprid. (Table 5). The same order of toxicity *viz*., imidacloprid>thiamethoxam>dimethoate was also observed by Praveen and Regupathy (2003) in Coimbatore population as well as in different locations in Tamil Nadu. This indicates that there is no variation in susceptibility in different location.

Table 5. Insecticide Resistance Frequency of *T.tabaci* to different insecticides

Insecticides	TIBS		Leaf dip method	
	Number tested	Per cent Survival	Number tested	Per cent Survival
Lambda-cyhalothrin	855	14.85	225	15.56
Imidacloprid	836	13.64	225	14.67
Thiamethoxam	852	14.55	225	15.11
Dimethoate	859	15.37	225	16.44

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Abstracts

Irrigation: An abiotic factor influencing efficacy of *Bt* cotton

ABSTRACT Insect bioassay on neonate larvae of cotton bollworm, *Helicoverpa armigera* (Hubner) using Bt cotton (MECH-184) plant parts such as leaves, squares and bolls collected from two contrast ecosystems, (irrigated and rainfed) at various stages (50-130 DAS) of crop growth revealed differential mortality. A mortality ranging from 58-80%, 51-82% and 35-65% was recorded through feeding leaves, squares and bolls respectively sampled from irrigated ecosystem. Whereas, a lower mortality ranging from 42-62%, 31-58% and 22-36% was noticed for leaves, squares and bolls of the same cotton hybrid respectively, collected from rainfed ecosystem, clearly revealing the role of irrigation (An abiotic factor) in influencing the larval mortality and in turn cry toxin expression. The study shows that the efficacy of Bt cotton plants in killing *H.armigera* larvae is higher in irrigated condition compared to the Bt plants grown under rainfed conditions.

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Pattern of cross-resistance of Cry 1Ac toxin selected *H.armigera* to chemical insecticides

ABSTRACT Development of resistance to any xenobiotics imposed against cotton bollworm, *Helicoverpa armigera* (Hubner) is real and it has to be managed with a sound IRM strategy. Limited use of insecticide molecules in case of partial or complete failure of Cry toxin is well thought. As of now, ETL based application of chemical pesticides in Bt-cotton is recommended once after 90 DAS or 1-2 times based on ETL. Accurate prediction and management of resistance requires information on cross-resistance characteristics of the insecticide employed in BT - crops. Study on the pattern of cross-resistance of Cry 1Ac toxin selected (for seven generations) *H.armigera* to chemical insecticides (viz., cypermethrin, fenvalerate, endosulfan, quinalphos, chlorpyriphos, methomyl and spinosad) conducted under laboratory conditions using discriminating doses of insecticides revealed negative cross resistance as Cry1Ac toxin selected H. armigera individuals were more susceptible to all the chemical insecticides tested irrespective of the group, compared to the unselected larvae from non-Bt cotton fields. The study strengthens the concept of "using chemical insecticides" as one of the tools in Bt resistance management strategy to increase the life of Bt technology.

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The Mechanism of Bacterial Insecticide Resistance in Colorado Potato Beetle

ABSTRACT Experiment on the potato beetle IV age larvae organized has shown that under the influence of the bacterial insecticide on base *Bacillus thuringiensis* (Bitoxibacilline, BTB) in larvae occurs the sort of immunization, raising resistance of individuals to insecticide during one generation already. The treatment of 1-dayly larvae per os with BTB in concentration 0.01 % has brought about that the repetition of the treatment with 10-times dose BTB (0.1 %) 6 days later did not become mortal for immunized larvae. In the resistance to BTB formation on the larval stage of ontogeny considerable role play the humoral immunity factors, a ferments of phenoloxydase and antioxidant complexes: DOFA-oxidase, tyrosinase, prophenoloxidase-activating serine protease, peroxidase and catalase. Under the repeated treatment is registered much more quick stabilization of enumerated ferments activities, than in the first hours after the first treatment by low dose of the BTB. Besides, during the electrophoresis in PAAG using Devis system (Ornstein, Devis, 1964) with samples of coverings and guts of larvae was established that repeated treatment by bacterial agent led to the induction of the extended phenoloxidase spectrum. The interesting fact introduces the stable induction in anode zone of new electrophoretic band with phenoloxidase activity, revealled during incubation with both as pyrocatechine, as DOFA (1 mg/ml of incubation medium) (fig. 1).

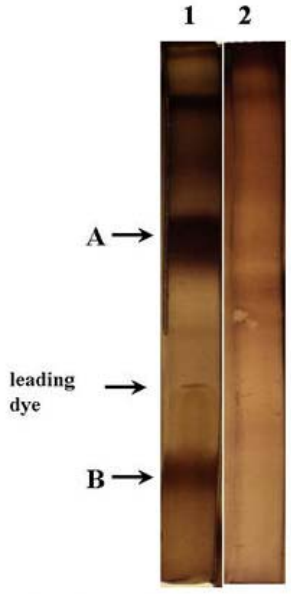

Figure1. Electrophoretic patterns of phenoloxidase bands n the colorado potato beetle larvae:
1 - bands, induced by bitoxibacilline.
Rf A- band = 0.58. Rf B- band = 1.2.
2 - control sample.

Founded molecular form of phenoloxidase had the electrophoretic mobility (Rf) about 1.3 that exceeds the mobility of leading dye bromphenole blue. The induction of similar molecular forms of phenoloxidases was revealled under the effect on colorado beetle larvae not only BTB, but also N-acetil-d-glucosamine. Such a fenomenon revealed also in the house fly.

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Sensitivity profiles of rice blast fungus, *Magnaporthe grisea*, to MBC, IBP and tricyclazole in China

ABSTRACT One hundred and twenty-nine single-conidial isolates of *Magnaporthe grisea* were collected from Guangdong, Guangxi, Anhui and Jiangsu provinces in 2000 to determine the sensitivity profiles of Chinese rice blast fungus population to carbendzim, kitazin p (IBP) and tricyclazole through discriminatory concentrations or detached leaf segments tests. Results showed that the resistance frequency to IBP, which had been withdrawn from practice more than ten years in China, was as high as 79.1%. Meanwhile, one carbendazim-resistant isolate was detected in Gaoyao city, Guangdong province with a frequency of 0.78%. EC50 values of the population for tricyclazole ranged from 0.06 to 1.12 µg/ml with an average value 0.46 µg/ml according to detached leaf segment tests. No significant difference (P=0.05) of sensitivity to tricyclazole was observed between Guangdong, Jiangsu where decreased disease control was reported, and the other two provinces where tricyclazole provided excellent control against blast disease. Tricyclazole could control both the most insensitive strain GY6 and the most sensitive strain DY2 in vivo successfully with EC50 of 13.10 and 0.71 µg/ml , respectively. Moreover, sensitivity of GY6 and DY2 to tricyclazole was both unstable in their asexual single-spored offspring, with mean EC50 values of 5.40 and 4.50µg/ml (all were seedling tests), respectively. EC50 of GY6 did not significantly decrease when continuously selected for twenty generations under the pressure of tricyclazole in vivo. However, the sensitivity of DY2 was decreased 10-fold after selected for twenty generations. These suggested that decreased disease control efficacy of tricyclazole reported in Guangdong and Jiangsu provinces could not be attributed to resistance emergence.

KEYWORDS *Magnaporthe grisea*; Rice blast disease; Fungicide sensitivity; IBP; MBC; Tricicyclazole

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Announcements and Submission Deadlines

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