Resistant Pest Management Newsletter

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

Vol. 16, No. 1 (Fall 2006)

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Resistance Management from Around the Globe

Baseline Resistance

Need for Generating Baseline Data for Monitoring Insecticide Resistance in leaf Webber cum Fruit Borer, *Pempelia morosalis* (Saalm Uller), the Key Pest of Biofuel Crop, *Jatropha curcas*.

While exploring the energy alternatives, biofuels, *viz*., ethanol from sugarcane and biodiesel derived from the oil bearing plants like sunflower, canola, rapeseed, *Pongamia*, mahua etc. can be used as a substitute or an additive to diesel. Out of the current annual consumption of 127 million tonnes of crude oil, 70 percent is imported. Forty million tonnes of diesel are consumed in a year constituting 40 percent of all petro-products (Soumitra Biswas *et. al*., 2006). Due to spiraling price of crude oil many nations including India are looking for alternatives. Out of various biofuel sources, the *Jatropha curcas* is considered to be the best-bet as far as India is considered for the following reasons:

1. It is adapted to a wide range of climates .It is grown as live fence to protect agricultural fields from damage by live stocks in most of the states in a variety of agro climatic conditions .

2. It can grow almost in any type of soil *viz*., gravelly, sandy, saline and even on the poorest stony soils and rock crevices (ITCOT, 2004).

3. The gestation period is low. The plant start yielding in second year and the economic yield stabilizes from the 4-5th year onwards. The plant is a perennial shrub and may live for 50 years with an average effective yielding time of 50 years (Soumitra Biswas *et. al*., 2006).

4. It is drought resistant, and does extremely well even with low rainfall. Reasonable production with little inputs in marginal lands is ensured. It is estimated that out of 146 million hectares of wasteland , 33 million hectares can be reclaimed for Jatropha cultivation. (NBSSLP, 2004) apart from the arable land that could be used for Jatropha cultivation.

5. Scope for more employment opportunities for the millions of farmers in small scale farming system vogue in India.

The Planning commission recommends allocation of INR1400 crores (US $ 3,000 million) for the promotion for three years (Chitra shanker and Dhyani, 2006). Joint ventures between UK- India based corporate sectors are promoting Jatropha cultivation in India. At present they have plans to bring one lakh (0.1 million) hectares under Jatropha cultivation in Tamil Nadu, Andhra pradesh and Chattisgarh states. The joint ventures supply seedlings, provide technical guidance and assure buy back of seeds (Soundariya Preetha. 2006). To provide technical guidance, they have set up Research and Development Centres to take up research activities to tackle emerging problems with large scale cultivation.

In Virudhunagar district of Tamil Nadu, one million seedlings are raised this year in eight centers for distribution under India Government Afforestation project to promote Jatropha for biodiesel (Anonymous, 2006).

The crop was earlier projected as less prone to damage, perhaps due to use of wild varieties as live fence especially in paddocks and dry lands as cheaper means of fencing in scattered patches. Serious damage by *Nephopteryx larvae* was observed and first time was reared at Pusa and Mandalay (Hampson, 1912). Scutellarid bug, *Chrysocoris purpureus* was recorded in North western provinces, Sikkim, Calcutta, and Assam and several parts of South India including Pondicherry (Puducherry) (Kershaw and Kirkaldy, 1908). The garden Jatropha was reported to be affected by sucking pests like tailed mealy bugs *Ferrisaina (Ferrisia) virgata,* C., Jatropha Scale : *Hemilecanium imbricans N*. and neem Scale: *Pulvinaria maxima G* (Ayyar , 1940), leaf and flower webber cum fruit borer, *Salebria (Pempelia) morosalis* (Saalm Uller), the castor semilooper, castor semilooper, *Achaea janata* L. (on *Euphorbia pilulifera*) (Beeson, 1941). More than 40 species of insects affecting Jatropha has been reported (Grimm and Fuhrer, 1998)

With taking up Jatropha as regular monocrop in continuous stretches not only in marginal lands but also in arable lands with high yielding, high fertilizer responsive cultivars, outbreak of pests can be anticipated. Reports of pest occurrence started appearing. Apart from *P.morosalis* and *A. janata*, the polyphagous tobacco cut worm, *Spodoptera litura* (Fabricius) is found to affect this crop (Robinson *et. al.*, 2004) and the incidence was to the extent of 60 to 70 percent in Madhya Pradesh (Meshram and Joshi, 1994). In Jhansi, *P.mormoralis* and Scutellarid bug, *Scutellera nobilis* Fabr. are considered as major pests causing great concern (Chitra shanker and Dhyani, 2006). While visiting the farmers fields in Virudhunagar district and crops maintained by Research and Development Centers of Corporate sectors in Coimbatore, the senior author observed the incidence of *P. morosalis, S.nobilis, F.virgata*, Calotropis leaf hopper bug (Green striped leaf hopper bug), *Eurybrachis tomentosa* F., grape-vine thrips, *Rhipiphorothrips cruentatus* H, and Chilli muranai mite, *Pol phagotarsonemus latus* Banks. Among these *P. latus* and *P. morosalis* are emerging as major problems in Tamil Nadu. Manoharan *et. al.* (2006) reported more than a dozen pests occurring in Jatropha planatations in Udumalpettai, Erode and Mettupalayam areas of Tamil Nadu.

Among the various pests listed above, *P. morosalis* is specific to Jatropha and reported to affect a few forest species like *Desmodium gangeticum*, *Flemingia sp*., and *Uraria lagopides,* (Beeson, 1941) and not occurring on field crops grown in India. Considering the history of forest pests becoming the pests of field crops subsequently due to reduction of forest habitat and monoculture, *P. morosalis* may likely to become regular pests with extension of area and perennial nature of Jatropha plantations. The adult moth is gray in colour with snout like labial palpi in the head with hyaline hind wings. The male is slightly smaller than the female with pointed abdominal tip. The total oviposition period of the female was recorded as 4.61 days. The sex ratio of leaf webber was 1.2:1 (male: female). On an average the developmental period from egg to adult emergence was 42.34 days with a high adult emergence of 80.17 percent. Adult longevity of female was 7.25 days while male 5.67 days exhibiting a sex ratio of 1.2:1 (male: female) (personal communication from Dr. Gunathilagaraj). The pupal period is 7-9 days in June to August (Beeson, 1941).

During our regular field visits the greenish brown/brownish green caterpillars were observed webbing the leaves and feeding on leaves remaining in the leaf web. At flowering they bore into peduncle and fruits which show galleries made of silk and frass. The caterpillar bores into the fruits throwing out faecal matter. The greenish larvae turn pinkish at the time of pupation. It pupates on the fruits. The larvae are seen under a cover of silk, frass or excreta, which extend between flowers/fruits.

For management of these pests at present major focus falls on chemical insecticides, though a dipteran parasite and spider *Stegodyphus* sp. were reported as natural control agent in Jhansi (Chitra shanker and Dhyani, 2006). The mass culture and release technology are yet to be perfected. At present Tamil Nadu Agricultural University (Paramathma *et. al*., 2004) recommends spray of endosulfan. However most of the farmers under the influence of pesticide dealers may apply an array of insecticides as is in vogue for the management of pests affecting other field crops. Repeated use of chemicals may likely to induce insecticide resistance. For effective pest management, detection and continuous monitoring of insecticide resistance are essential. Monitoring of resistance involves comparison of LD50 or the slopes of dose-response curves between field and susceptible reference populations. The reference populations are maintained in the laboratory without exposure to insecticides. In most practical situation the best monitoring method is the use of discriminating/ diagnostic dose (DD) i.e. the dose that kills 99 percent of susceptible individuals (Roush and Miller, 1986.). Sometimes a susceptible (local) population is not available, DD fixed elsewhere is taken as reference as with the case with *H. armigera*. The DD based on LD 99 for susceptible NRI (Natural Resources International, U.K. and Australia susceptible strains are used ( ). This situation can be avoided in the case of *P. morosalis* as the pest exposure is nil.

The objective in resistance monitoring is to exaggerate the differences between susceptible and resistant individuals such that frequency of misclassification is greatly reduced (ffrench-constant and Roush, 1990). For estimating resistance level in a population, the initial base-line level of susceptibility is essential so that, comparisons can be made in future (Hopkins *et al*., 1984). For successful monitoring programme the base-line susceptibility to different insecticides must be estimated separately for each species in the complex. The laboratory population which has not undergone any exposure to insecticides provides the advantages of totally susceptible bench mark for calculating resistance ratios. Resistance in insect pests can be detected by the use of discriminating dose tests. Such detection should be done at the earliest stage of effective pest management. Hence, a suitable monitoring technique is to be developed not only to detect the presence of resistance but also to monitor the changes in resistance frequency to determine whether a programme is effective or not. Information on the the discriminating doses fixed for the important pests like, *Helicoverpa armigera* Hub., *Plutella xylostella* L., *S.litura* , rice leaf folder *Cnaphalocrocis medinalis* Guene, rice brown plant hopper *Nilaparvata lugens* Stal., cotton leaf hopper, *Amrasca devastans* (Distant) and cotton aphid, *Aphis gossypii* (Glov.) had been compiled by Regupathy *et. al.* (2004.) and subsequently for coffee green scale, *Coccus viridis* Green (Senthilkumar and Regupathy, 2004) *Thrips tabaci* (Lindeman) (Deepa,et.al., 2005), sugar cane wooly aphid, *Ceratovacuna lanigera* Z. (Vijayaraghavan and A. Regupathy, 2005), At present the pesticide application is nil or at least, minimal, the exposure of *P. morosalis* may be nil or negligible. This is the ideal time to get the susceptible population for generating base line susceptibility.

Out of number of bioassay techniques *viz*., topical assay, leaf residue/ leaf disc, foliar application bioassay, thin layer exposure bioassay/ surface residue vial bioassay, sticky card technique, slide dip bioassay and glass vial technique available, the topical assay will be more suitable for *P. morosalis* considering the shielded habitat. The topical assay is an old and reliable method. Technical grade insecticide dissolved in less toxic carrier/solvent (acetone) is applied topically (usually 0.5µl) to the dorsal thoracic segments of the test insect with the help of a microapplicator and transferred to petridishes provided with feed. Mortality is assessed after 24h. Control insects were treated with acetone alone. This technique is used for assessing the toxicity fixing diagnostic doses and monitoring insecticide resistance in Helicoverpa armigera and shoot and capsule borer *Conogethes punctiferalis* Guenee (Renuka, 2003; Rajabaskar, 2003). Topical application used to assess the toxicity to *C. punctiferalis* can be extended to *P. mormoralis* as the damage and pest behaviour is similar to *C. punctiferalis*. Both belong to Family Pyralidae and remains inside the webbings. The method is briefly described below.

Field collected *P.morosalis* larvae weighing 18 - 22 mg (length 1.2 cm) reared on Jatropha flower heads capsules could be used for bioassay. An aliquot of 1µl of a known dilution of insecticide is placed on the thoracic dorsum of each larva using 1µl repeating dispenser (PB 600 -01, Hamilton Co. Ltd.,) fitted with a 50µl syringe and Rheodyne needle . The control is treated with acetone alone. Not less than 50 larvae per dose need to be used per dose/treatment. Mortality counts are taken 24 h after treatment. To assess the relative toxicity of insecticides median lethal dose (LD_{50}) to *P. morosalis* is determined. Preliminary range finding tests are made to fix the appropriate dosage range. The log -dose / concentration - response curves are fitted. The DD can be arrived at as detailed by Roush and Muller (1986).

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Arthropod Resistance

Mobile Inspection and Diagnosis Service of Sprayers in Resistance Prevention

Results Obtained Out of Inspections Performed by a Group of Producers During the 2004-2005 Season

INTRODUCTION Misaplication of pesticides is a serious problem in Agriculture. Poorly calibrated sprayers result in over-dosing or under-dosing of pesticides. Both situations are undesirable, because of environmental and commercial reasons for the former and poor control and resistance development concerns for the latter. Continuous under-dosing in a control program can exacerbate resistance problems. Our commercial and professional activity related both to pest control and the appropriate use of pesticides has enabled us, from the very start, to obtain and evaluate suggestions from a growing number of producers who have shown to be very interested in both improving and optimizing the whole process related to those two subjects. Producers are especially interested in learning about sprayers and in the techniques applied in their handling.

Aspects like reducing the volume of water used and minimizing the damages brought about both by overdosing and under-dosing, the use of new products with extremely reduced doses, the need to cut down damage risks in adyacent fields, etc.. All these aspects are related to the need to control the equipment carefully before use and to perform a close regulation and calibration of all devices. This has enabled us to visualize a demand for a fully specialized kind of service. Such a service must be carried out by highly specialized technicians who must be able both to diagnose and give solution to a countless number of failures and defects which are usual in spraying equipments. These technicians must also be able to solve problems on the spot, where the sprayer is at work and at the time required. As a result, we have decided to devise and put into practice a specific inspection service of each component of the spray hydraulic system as well as of the part that contributes to put the system at work both efficiently and safely. The aims are: to evaluate the work done by each component of the equipment, to diagnose its corresponding state and to solve any problems immediately and on the spot.

In order to develop this project, we have done some research so as to collect information which would contribute to optimize the service from various experiences of technical inspections in use, especially from European countries. We have also got some information from some articles issued by authors such as Santiago Plana de Martí (1999), Daniel Vannuci and Marcelo Biocca (1999) and Jozef Sawa (1999). Through a careful analysis and studies carried out abroad, we can pick up three basic aims to follow up in the inspection of sprayers in use. They are as follows:

a. Increase farmers' profitability: By identifying and repairing sprayers' failures he can carry out more efficient tasks, cutting down risks of over-dosing.
b. Reduce under-dosing: Increase pesticide use efficiency by ensuring the right dose application, reducing the risk of lack of control and resistance development.
c. Maximize workers' safety: By identifying possible causes of intoxication, poissoning or accidents.
d. Cut down pollution: By avoiding deficient distribution of pesticides and eventual contamination.

Our company started providing this service in 1999 by focusing attention on the Province of Córdoba, Argentina. We have provided our service to highly technically developed producers who are quite ready to adopt new technologies in their activities. At present, inspections and diagnosis work are being carried out in most areas with extensive crops in our country. This task is conducted by the previously mentioned producers.

MATERIALS AND METHODS The service is performed where the sprayer is at work. For this purpose, a utility van equipped with special instruments is used. Besides, accessories and parts of the sprayer are carried to solve any emerging problem in the machine. The technical instruments mounted on the van are the following: - Nozzle testing bench, Gauge inspection bench, Electronic flow-meters, Digital tachometer, Manometers, Electronic tester, GPS, Gauge computer, 50 m measuring tape, Graduated bars, Stakes, and Various tools. It is important to emphasize that the technical instruments used need to have the utmost grade of accuracy due to the kind of controls to be made. For this reason, the standards with which our instruments are permanently measured, are also examined and certified periodically by a certifying organization. In order to achieve the aims mentioned above, some procedures and directives have been developed in ISO 9000 Norm formats. They are based on worldwide European normatives because they are widely spread and highly accepted. These technically based normatives are meant to be the foundations of our procedures. They are as follows:

1. Security - EN 907
2. Environmental Protection - EN 12761
3. Equipments in Use Inspection - EN 1790

Inspection and diagnosis of the state and operation of each sprayer component such as: tank, accessory tanks, agrochemical charger, pump, flybacks, mixers, manual and electrical controls, cut off locks, pressure regulators, manometers, flow gauge, computers, filters, hoses, nozzles, disks, etc., are carried out by following instructions and procedures developed according to international requirements and normatives using the above mentioned instruments. To avoid workers' accidents we also verify the presence of personal protection equipment such as masks, gloves, helmets, boots, overalls and the protection of the power takeoff, of the drive belts, the signal protection, etc.. Once the inspection is finished, the certification is either given or not according to the state of equipments' final diagnosis.

ACHIEVED RESULTS The inspections made during 2004-2005 were applied to a segment of producers characterized by working in wide areas and the intensively use of the sprayer. The following data are obtained from the total inspections. The achieved results are as follows: Table 1, Table 2, and Table 3.

Table 1.

Inspected Machinery Percentage		
Machines	Tractor Mounted	19%
	Self-Propelled	81%

Table 2. Geographical areas distribution

Provinces	Percentages
Córdoba	**64%**
Santa Fe	
Buenos Aires	
Salta	**29%**
Jujuy	
Santiago del Estero	
Tucumán	
Chaco	
Entre Ríos	**7%**
Corrientes	
San Luis	

Percentage of the total inspected machines according to provinces/regions.

Table 3. Life time of inspected

Life Time	
Year	Percentage
2005	52%
2004	
2003	
2002	
2001	
2000	
1999 and the previous ones	48%

Percentage of inspected machines according to manufactured date.

The information of the inspected elements and components of each sprayer is presented in the two following charts. In figure 1, we can see the result of the elements' inspection related to the workers' security. At first side, we can infer the lack of conscience of the serious risk that the group of workers' work involves. In these inspections, we could verify that the workers do not wear helmets in 97% of the sprayers; 61% do not wear gloves; 39% work without masks, and 67% do not wear overalls.

In figure 2, we can see the state of the sprayer's components which directly affect the quality of pesticides controls carried out. The details provide what happens in Argentina with the use of pesticides, treatment cost, outcome losses, etc.

The results shown in the previous chart enable us to notice five essential problems as seen from application quality. Because of their incidence upon the desired results they should be both analyzed and corrected as soon as possible. The problems are the following: Agitators, Boom's condition and stability, Worn-out nozzles, Hydrical balance, Manometers and sensors. The solution to these problems will depend on owner's decision regarding investment on professional specialization and sprayer maintenance and repair. The results shown here indicate that most of the sprayers analyzed cannot provide an appropriate operation. It is important for those providing this type of service to emphasize the environmental and commercial importance of having a properly calibrated sprayer to optimize control and reduce the chances of pesticide resistant pest development due to under-dosing.

ACKNOWLEDGEMENTS The author wants to thank Dr. Pablo Kálnay for his collaboration with this article.

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Figure 1. Results of the personal safety elements inspection.

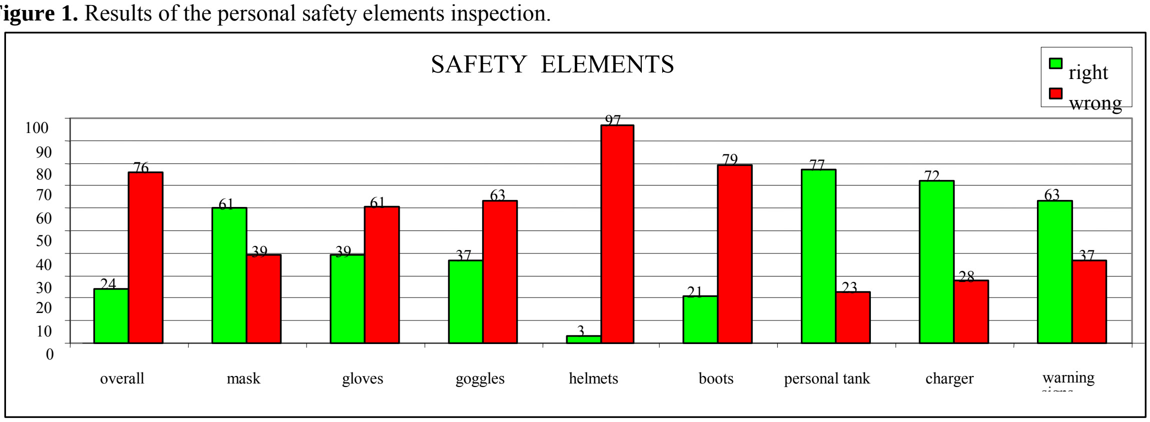
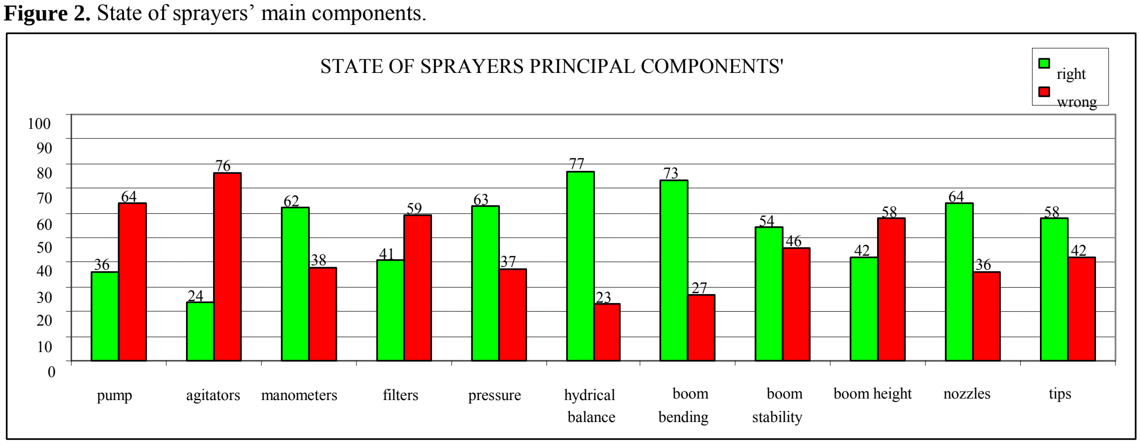


Figure 2. State of sprayers' main components.



Comparative Efficacy of Candidate Termiticides Against Worker Termites, *Odontotermes obesus* (Rambur)

ABSTRACT In Central and North India termites, *Odontotermes Sp* .is reckoned as a pest of economic importance for several economically important crops like Groundnut, Maize, Wheat, sugarcane and cotton inflicting damage up to 60 percent. Adverse effect of persistent organochlorine and Cyclodienes, primarily used for termite control on environment provoked the need to screen ecologically safer insecticides against termites, *Odontotermes obesus* (Rambur).

Application of Chlorpyriphos + Cypermethrin (Nurelle D) recorded the lowest median lethal concentration (0.0017%) and the highest relative toxicity of 2.29 as significant over application of Chlorpyriphos, 1.0, the standard check with LC_{50} value of 0.000395. Application of Cypermethrin + Chlorpyriphos and Cypermethrin alone proved superior over Chlorpyriphos in terms of the lowest median lethal time. For 50 percent mortality of assorted worker termites population.

KEY WORDS *Odontotermes obesus*, Termiticide

INTRODUCTION Subterranean termites are the major pest of agriculture and human structures throughout tropical and subtropical climatic zones causing damage worth billions of dollars worldwide. As per the pest ranking system (based on severity of damage inflicted) developed by Natural Resource Institute, United Kingdom,the termite was rated as rank one pest in wheat not only at Indian but also at the South Asian level. (Geddes and Iles, 1991). *Odontotermes* is identified as an important pest in Central and North India causing damage to the extent of 60 percent in several economically important crops like Groundnut, Maize, Wheat, Sugarcane and Cotton (Mishra, 1999).

Management of Subterranean termites primarily relies on treatment of soil at the site of active infestation. Offlate, increased environmental concern due to several unintended repercussions of persistent Organochlorines and Cyclodienes like Aldrin, Dieldrin, Chlordane and Heptachlor, though the most effective and long lasting insecticides for the control of termites has prompted the need for screening newer potent insecticides against termites (Khoo and Sherman, 1979).

Thus, the present study was framed with a view of screening five candidate termiticides belonging to different classes viz., Cypermethrin (Synthetic Pyrethroid), Chlorpyriphos (Organophosphate), Nurelle D (Chlorpyriphos + Cypermethrin, a Combination Product), Carbaryl (Carbamate) and Lindane (Organochlorine) for their efficacy against assorted population of worker termites, *Odontotermes obesus*.

MATERIALS AND METHODS Collection of termites, *Odontotermes* was done with the help of modified bucket trap as described by Su and Scheffrahn, 1986, after location of live termitoria in the fields from 4 villages of Nagpur district (Maharashtra). The assorted population of collected worker termites was maintained in the laboratory, Department of Plant Pathology, College of Agriculture, Nagpur on fungus comb (the natural food collected from termitoria). The culture was maintained at 25 ±2 ºC with relative humidity of 80 - 90 percent. Due care was taken for acclimatization of worker termites before proceeding for the bioassay (Gurusubramanian *et. al.*, 1999).

Five dilutions of each insecticide were prepared by serial dilution method in distilled water. Insecticides in powder formulations were diluted with talk powder. The bioassay was carried out in plastic cup (4.5cm x 6.5cm x 5.0cm) covered with a black cloth. A wet filter paper was placed at the bottom of each cup to maintain necessary humidity along with a layer of sterilized soil and fungus comb as a food source. A set of 12 worker termites replicated thrice was subjected to each concentration of five insecticides. The insecticide dilutions were applied to the worker population @ 2 ml/cup with the help of hand atomiser, whereas, the control set was sprayed with same quantity of distill water to record any natural mortality in worker population. Dilutions of insecticides in powder form were applied @ 2 gm/cup on the soil surface with workers and the control set was given the same quantity of talk powder to record any natural mortality.

Treated as well as termites in control set were maintained at 25 ±2 ºC temperature and 80-90 percent Relative Humidity. The mortality count was recorded every 24 hours for 7 days after application of treatment. The moribund workers (unable to produce coordinated movement of body when prodded) were considered as dead. Bioassay data was pooled and concentration mortality and time mortality regression was computed by POLO - PC software for Probit analysis (Anonymous, 1987). Relative toxicity of insecticides under consideration was calculated by dividing LC_{50} due to Chlorpyriphos by LC_{50} of the insecticide to ascertain the potency of candidate termiticides over Chlorpyriphos, the standard check.

RESULTS AND DISCUSSION For nearly five decades, management of Subterranean termites have extensively relied on treatment of soil with persistent insecticides. Though, Chlorpyriphos became available in the 1980`s, people mostly employed the use of persistent Organochlorines and Cyclodienes as a termite management strategy until the ban was imposed on these insecticides. As a consequence of the adverse effects of these chemicals on environment, research focus was shifted towards screening of new, less harmful candidate termiticides with long residual activity that is highly repellent or inflicting rapid kill like Organophosphtes and Pyrethroids (Su and Scheffrahn, 1992).

Concentration mortality regression analysis (Table 1) indicates the superiority of Nurelle - D (Chlorpyriphos + Cypermethrin) in terms of lowest median lethal concentration, 0.0017 percent against the assorted population of worker termites followed by Chlorpyriphos (0.0039 %), the standard check. These treatments were superior when compared with other insecticide treatments like Cypermethrin (0.0155 %), Carbaryl (0.0327 %) and Lindane (0.1183 %), respectively. There were significant toxicity differences between Chlorpyriphos + Cypermethrin (Nurelle D) and Chlorpyriphos also between Chlorpyriphos and Cypermethrin determined by non overlap of 95 percent Fiducial Limits at LC_{50} values of these insecticides.

Higher efficacy of Chlorpyriphos against the termite's population was reported by Khoo and Sherman, 1979, Su *et. al.*,1982, Su and Scheffrahn,1990 and 1992, Goyal,1994 along with Mishra,1999 whereas, higher potency of Cypermethrin (Su and Scheffrahn, 1990) and lower efficacy of Carbaryl (Smith,1979 and Su *et. al.*,1982) and Lindane was reported by Arora and Arora,1995 which are in corroboration with the present findings. Estimation of relative toxicity values (Table 1) is indicative of the fact that the combination product, Nurelle D was 2.29 times effective over Chlorpyriphos, whereas, rest of the insecticides (Cypermethrin - 0.25, Carbaryl - 0.12 and Lindane - 0.03) were not comparable with efficacy of the Chlorpyriphos (1.0), the standard check.

Time mortality response data (Table 2) of insecticide dilutions constituting LT_{50} and LT_{90} (range) reflects the higher potency of Nurelle D in terms of lower time (0.4 days for 0.05 %, 0.6 days for 0.005 % and 1.3 days for 0.0005 %, respectively) to record 50 percent mortality in worker termites. The former treatment was followed by Cypermethrin (LT_{50} values, 0.5 days for 1.0 %, 0.9 days for 0.1% and 1.5 days for 0.01% concentration, respectively) superior over the standard check, Chlorpyriphos which registered LT_{50} values of 0.8 days for 0.2 percent, 1.2 days for 0.02 percent and 2.4 days 0.002 percent concentration respectively, strongly supported by the findings of Su and Scheffrahn, 1990.

Rest of the treatments viz., application of Carbaryl and Lindane followed the low efficacy trend as in case of concentration mortality response. On the contrary application of Nurelle D and Cypermethrin shows promise to be used as termiticides besides Chlorpyriphos, the standard check.

Table 1: In Vitro Concentration Mortality Response (LC50) of Worker Termits, *Odontotermes obesus* (Rambur).

Sr. No.	Treatment Details	n	(LC50) (95 % FL)	(LC90) (95 % FL)	Slope ± SE	Heterogeneity	x2	Relative Toxicity
1	Cypermethrin 10 EC	216	0.0155 (0.0068 - 0.0370)	0.463 (0.1553 – 1.7108)	1.46 ± 0.17	0.41	2.5	0.25
2	Chlorpyriphos 20 EC	216	0.0039 (0.0019 – 0.0080)	0.0418 (0.0180 – 0.1695)	1.25 ± 0.21	0.29	1.7	1
3	Nurelle D EC	216	0.0017 (0.0008 – 0.0037)	0.0281 (0.0109 – 0.1321)	1.32 ± 0.18	0.49	3	2.29
4	Carbaryl 10 WP	216	0.0327 (0.0147 – 0.0738)	0.716 (0.2622 – 2.6581)	1.36 ± 0.15	0.12	0.7	0.12
5	Lindane 10 WP	216	0.1183 (0.0046 – 0 .2237)	1.9701 (0.7053 – 8.3790)	1.21 ± 0.16	0.5	3	0.03

n - Number of worker treated, LC_{50} - Median Lethal Concentration, FL - Fiducial Limit, X^2 - Chi Square

Relative Toxicity = LC_{50} of Chlorpyriphos by LC_{50} of insecticides to be compared.

X^2 table (7.81) at 0.05 probability (3 d.f.). Thus, the deviation of observed data from expected frequencies are purely due to chance.

Table 2: In Vitro Time Mortality Response (LT50) of Worker Termites, *Odontotermes obesus* (Rambur).

Sr. No.	Treatment Details	Conc. (%)	n	(LC50) (95 % FL)	(LC90) (95 % FL)	Slope ± SE	Heterogeneity	X2
1	Cypermethrin 10 EC	0.01	36	1.5 (0.93 – 4.94)	6.4 (4.77 – 10.59)	2.66 ± 0.21	1.04	5.2
		0.1	36	0.9 (0.38 – 1.31)	3.1 (2.02 – 4.93)	3.28 ± 0.28	1.3	6.5
		1	36	0.5 (0.42 – 0.74)	1.2 (0.82 – 3.71)	3.46 ± 0.29	0.98	4.9
2	Chlorpyriphos 20 EC	0.002	36	2.4 (2.07 – 2.73)	11.6 (10.95 –18.22)	2.03 ± 0.19	1.24	6.2
		0.02	36	1.2 (0.87 – 1.69)	5.8 (2.51 – 8.89)	2.49 ± 0.37	0.16	0.8
		0.2	36	0.8 (0.70 – 0. 99)	2.4 (1.71 – 3.91)	2.74 ± 0.32	0.26	1.3
3	Nurelle D	0.0005	36	1.3 (0.74 – 3.91)	7.1 (2.80 – 17.34)	1.69 ± 0.18	0.09	0.4
	[Cyper (50) + Chlor (5)] 505 EC	0.005	36	0.6 (0.45 – 0.63)	2.3 (1.39 – 2.71)	2.35 ± 0.26	0.34	1.6
		0.05	36	0.4 (0.35 – 0.43)	1.2 (1.10 – 1.82)	2.81± 0.21	0.69	3.5
4	Carbaryl 10 WP	0.01	36	3.8 (3.54 – 4.31)	14.3 (10.31 – 26.74)	1.67 ± 0.22	0.1	0.5
		0.1	36	1.7 (1.28 – 2.11)	12.7 (9.15 – 18.93)	2.13 ± 0.18	0.4	1.8
		1	36	1.1(0.92 – 1.70)	3.7 (2.32 – 8.89)	2.49 ± 0.37	0.16	0.8
5	Lindane 10 WP	0.1	36	4.7 (4.03 – 5.23)	21.6 (15.61 – 35.18)	1.49 ± 0.18	1.02	5
		1	36	2.5 (2.09 – 2.89)	19.1 (11.34 – 30. 56)	1.60 ± 0.19	0.29	1.5
		10	36	1.7 (1.25 – 1.93)	10.4 (7.88 – 14.84)	1.89 ± 0.26	0.34	1.7
6	Control	0	108	16.8(11.37 – 51.01)	43.2 (21.62 - 328.02)	3.11 ± 0.78	0.61	3

Control Mortality to the extent of 12 percent is quite normal (Gurushubramaniam *et. al.* , 1999).

LT50 - Median Lethal Time

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Variation in the Susceptibility and Resistance of *Spodoptera litura* (Fab) (Delhi and Punjab Populations) to Various Synthetic Pyrethroids

ABSTRACT The third instar larvae of *S. litura* obtained from Punjab was 20.74, 15.46, 12.9, 9.59 and 7.05 and 16.74, 13.22, 21.18, 11.50 and 4.83-fold resistance to deltamethrin, alphacypermethrin, cypermethrin, betacyfluthrin and fenvalerate, respectively, when applied by direct spray and leaf dip method, respectively as compared to Delhi population. Regardless of method of application betacyfluthrin was most effective against Delhi and Punjab population.

KEY WORDS *Spodoptera litura*, Susceptibility, Relative Resistance and Synthetic Pyrethroids

INTRODUCTION *Spodoptera litura* (Fab), the tobacco caterpillar earlier known to be a sporadic pest has emerged as major polyphagus pest in recent past. This pest is reported to attack a wide range of vegetables, cash and fiber crops. Because of their ability to inflict serious economic damage to these crops, the pest has been subjected to heavy dose of insecticide treatments. Typical field control of this pest includes use of several synthetic pyrethroids, organophosphates and carbamates. Amongst different group of insecticides, synthetic pyerthroids constitute 8-75 % and are widely used allover India to control this pest. Cypermethrin is most commonly used followed by fenvalrate, deltamethrin, lambdacyhalothrin and betacyfluthrin. Due to indiscriminate and non-judicious use of these insecticides has resulted in the development of high degree of resistance to these compounds. The level of resistance to synthetic pyrethroids vary/differ significantly and constitute a serious threat to crop production in many regions[1-7]. Thus, to know the level of susceptibility and resistance of field collected populations of third instar larvae of *S. litura* from Delhi and Punjab, to synthetic pyrethroids, the present investigation was carried out a series of dosage mortality studies to provide base line data and any change in ld-p lines for several synthetic pyrethroids between the two populations by direct and spray and leaf dip method.

MATERIALS AND METHODS The larvae of *S. litura* were collected from the fields of Indian Agricultural Research Institute, New Delhi and Phagwara district. of Punjab were reared in laboratory on castor leaves at 27±1º C and 60±5 % RH under laboratory conditions. The insecticides viz., Betacyfluthrin (Bayer India Ltd), Alphamethrin (BASF India Ltd.), Cypermethrin (Zeneca ICI Agrochemicals), Deltametnrin (Aventis crop science Ltd.) and Fenvalerate (Rallis India Ltd.) evaluated in the present studies were obtained as commercially available formulations from their respective source of supply. Different concentrations of various insecticides were prepared by using distilled water for the dilution of emulsifiable concentrates

(EC). Direct Spray: Ten third instar larvae (7 day old) of S. litura were randomly selected and placed in the petri-dishes and one ml of each concentration of the test emulsions was directly sprayed at a pressure of 340 g cm^{-2} under Potter's tower. In case of control the larvae were sprayed with distilled water. The sprayed petri-dishes containing treated larvae were air dried for about 5 minutes and then transferred to separate glass jars (15 X 10 cm) containing fresh untreated castor leaves as food. Leaf Dip: The larvae of S.litura were exposed to insecticide residue on castor leaves. Leaf discs of approximately 6cm diameter were cut from the castor leaves and were dipped in the required concentrations of different insecticides for 20 seconds and then air-dried. The treated leaf discs were then transferred to clean jars (15 X 10 cm) and ten third instar larvae were released in each jar. For control the leaf disc was dipped in the sterile water. In both the bioassay operations the jars were covered with muslin cloth and kept in laboratory at 27±1º C and 60±5% RH. There were 3 replications for each concentration and untreated control. Mortality was recorded 24 h after treatment. The moribund insects were counted as dead. The data so obtained was subjected to probit analysis[8]. The relative susceptibility of various insecticides was found out by calculating the LC_{50} of cypermethrin as unity. The relative resistance was calculated as LC_{50} of Punjab population / LC_{50} of Delhi population.

RESULTS AND DISSCUSSION The results presented in table 1 indicate that betacyfluthrin was most effective against third instar larvae of both the population of *S. litura* by direct spray. The LC_{50} values of betacyflutrin, alphamethrin, delatmethrin, cypermethrin and fenvelrate were 0.00054, 0.00519, 0.0071, 0.0159 and 0.0368 to Delhi population and 0.00518, 0.08028, 0.1473, 0.2056 and 0.2597 to Punjab population, respectively.

Table 1. Relative susceptibility of 7-day old larvae of *S. litura* to various synthetic pyrethroids by direct spray method

Insecticides	Heterogenity		Regression	LC_{50}	Fiducial limits	Relative
	df	X^2	Equation Y=	(%)		Susceptibility
Delhi Population						
Betacyfluthrin	3	5.39	8.7805+1.1558	0.00054	0.0036-0.00072	29.44
Alphamethrin	3	2.38	8.5574+1.6838	0.00519	0.0044-0.0061	3.06
Deltamethrin	4	2.72	9.2734+1.9903	0.0071	0.0062-0.0081	2.23
Cypermethrin	3	3.63	7.2029+1.2260	0.0159	0.0128-0.0984	1
Fenvalerate	4	3.39	6.6180+1.1283	0.0368	0.0296-0.0457	0.43
Punjab Population						
Betacyfluthrin	3	5.28	9.5793+1.9967	0.00518	0.0044-0.0058	39.7
Alphamethrin	4	7.09	6.3746+1.2543	0.08028	0.0653-0.0984	0.25
Deltamethrin	3	4.37	6.6480+1.9814	0.1473	0.1285-0.1687	1.39
Cypermethrin	3	0.4	6.8517+2.6960	0.2056	0.1841-0.2297	1
Fenvalerate	3	5.23	6.3917+2.3773	0.2597	0.2318-0.2910	0.8

In none of the cases, the data were found to be significantly heterogeneous at P=0.05, Y=Probit kill and X= log concentration; LC50 = Concentration calculated to give 50 per cent mortality

Respo nse *S. litura* to five pyrethroids by leaf dip method shows (Table 2) that Delhi population was 37.50, 4.45, 1.04, and 0.26 and Punjab population was 17.57, 0.56, 0.33, and 0.29 times more susceptible to betacyfluthrin, alphamethrin, deltamerthrin and fenvelrate as compared to cypermethrin respectively. Regardless of method of application both the population of *S. litura* were highly susceptible to betacyflutrin.

Table 2. Relative susceptibility of 7-day old larvae of *S. litura* to various synthetic pyrethroids by Leaf dip method

Insecticides	Heterogenity		Regression	LC_{50}	Fiducial limits	Relative
	df	X^2	Equation Y=	(%)		Susceptibility
Delhi Population						
Betacyfluthrin	5	1.16	10.6618+1.5939	0.00028	0.00021-0.0036	37.5
Alphamethrin	5	3.84	8.9336+1.4981x	0.00236	0.0020-0.0027	4.45
Deltamethrin	3	0.48	8.8181+1.9128x	0.01009	0.0086-0.0117	1.04
Cypermethrin	4	1.27	8.1477+1.5910x	0.0105	0.0089-0.0123	1
Fenvalerate	4	1.86	6.0649+0.7564x	0.0391	0.0283-0.0539	0.26
Punjab Population						
Betacyfluthrin	3	4.76	8.1017+1.2448x	0.00322	0.0026-0.0040	17.57
Alphamethrin	3	2.18	6.8026+1.1972x	0.03121	0.0249-0.0391	0.56
Deltamethrin	3	4.94	6.2582+1.6298x	0.16903	0.1418-0.2014	0.33
Cypermethrin	3	2	5.6919+1.0600x	0.22242	0.1489-0.3322	1
Fenvalerate	4	4.82	6.1776+1.6289x	0.18921	0.1508-0.2374	0.29

In none of the cases, the data were found to be significantly heterogeneous at P=0.05, Y=Probit kill and X= log concentration; LC50 = Concentration calculated to give 50 per cent mortality

Comparison of LC_{50} values for synthetic pyrethroids viz., betacyflutrin, alphamethrin, delatmethrin, cypermethrin and fenvelrate of Delhi and Punjab population of *S. litura* indicated differential shift in the level of resistance. It is evident from the data (Table 3). that the third instar of *S. litura* obtained from Punjab showed 20.74, 15.46, 12.93, 9.59 and 7.05-folds resistance to deltamethrin, alphacypermethrin, cypermethrin, betacyfluthrin and fenvalerate, respectively, when compared to Delhi population. Based on the LC90 values obtained, the order to relative resistance was cypermethrin (34.67) > alphacypermethrin (28.28) > deltamethrin (20.86) > betacyfluthrin (3.24) > fenvalerate (1.78)

Table 3. Relative resistance of 7-day old larvae of *S. litura* to various synthetic pyrethroids to by direct spray method

Insecticides	LC_{50} (%)		Relative	LC_{90} (%)		Relative
	Delhi	Punjab	Resistance	Delhi	Punjab	Resistance
Betacyfluthrin	0.00054	0.0052	9.59	0.0068	0.0223	3.24
Alphamethrin	0.00519	0.0803	15.46	0.298	0.843	28.28
Deltamethrin	0.0071	0.1473	20.74	0.0313	0.6532	20.86
Cypermethrin	0.0159	0.2056	12.93	0.1772	0.6144	34.67
Fenvalerate	0.0368	0.2597	7.05	0.5032	0.8988	1.78

Table 4. Relative resistance of 7-day old larvae of *S. litura* to various synthetic pyrethroids to by leaf dip method

Insecticides	LC_{50} (%)		Relative	LC_{90} (%)		Relative
	Delhi	Punjab	Resistance	Delhi	Punja	Resistance
Betacyfluthrin	0.0003	0.0032	11.5	0.0017	0.0345	20.29
Alphamethrin	0.0024	0.0312	13.22	0.0169	0.367	21.71
Deltamethrin	0.0101	0.169	16.74	0.0471	1.033	21.93
Cypermethrin	0.0105	0.224	21.18	0.0671	3.599	53.63
Fenvalerate	0.0391	0.1892	4.83	1.933	1.158	0.59

The relative resistance values based on LC_{50} of the Punjab population when compared to that of Delhi was 21.18 fold to cypermethrin, 16.74 to deltamethrin, 13.22 to alphacypermethrin, 11.50 to betacyfluthrin and 4.83 to fenvalerate, when evaluated by leaf-dip method. The change in the order of relative resistance remained unaltered even at LC_{90} level (Table 4). But there was change in the order to resistance as compared to direct spray method, the order was cypermethrin > deltamethrin > alphacypermethrin > betacyfluthrin > fenvalerate.

Armes *et al*[4] reported resistance levels between 0.2 to 197 and 8 to 121 fold to cypermethrin and fenvelrate respectively in *S .litura* starins collected from Andhra Pradesh. While studying the variation in the efficacy of synthetic pyrethroids against susceptible (Delhi) and resistant (Guntur) level population of *S. litura*, Rao and Dhingra[5] reported similar results. According to them, Guntur population of *S. litura* were 4 and 5 fold resistance to cypermethrin and fenvalerate respectively, when compared with Delhi population. Recently Kranthi *et al.*[6] detected high levels of resistance to cypermethrin ranging from 67 to 148 in the strains of *S. litura* collected from both north and south India.

Thus, it is evident from the investigations that the third instar larvae of S. litura obtained from Punjab were resistant to all the five synthetic pyrethroids. The LC_{50} values obtained would serve as ready reckoner for the selection of insecticides for field strains. Also, such base line data could be used as critical inputs in deployment of synthetic pyrethroids and insecticide resistance management programmes.

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Cytochrome P450 Confers Single or Multiple Factorial Resistance?

Most insecticide resistance phenomenon are genetically determined and mediated by a group of microsomal enzymes called Cytochrome monooxygenases or *Cyps*. The cytochrome P450 monooxygenases are found from bacteria to mammals. In insects, these enzymes play key roles in process ranging from host plant utilization to xenobiotic resistance[1]. The P450 monooxygenases are involved in the metabolism of all class of insecticides, leading to an activation of the molecules or more generally to detoxification[2, 3]. The principle of P450 enzyme activation is oxidation of substrate with release of H_2O molecule in presence of a reductase (NADPH). The reaction occurs as follows:

$$\text{Substrate(S)} + (\text{NADPH} + H^+) + O_2 \rightarrow S(O) + \text{NADP}^+ + H_2O$$

Cytochrome b_5 can also stimulate this reaction. The key protein of this enzymatic system responsible for the specificity of the reaction is cytochrome P450. P450 stands for the absorption peak of this protein at 450 nm when reduced and saturated with CO. The genes for the cytochrome P450s are large families involved in a wide variety of metabolic functions. All gene members of the P450 super family are designated with a *Cyp* prefix, followed by a numeral for the family, a letter for the subfamily, and a numeral for the individual gene. There are eighty-three genes in *D. melanogaster* that putatively encode functional P450s. These have been classified into twenty-five different families, but more than 50% belong to either the CYP4 or CYP6 families[4].

The involvement of P450 enzymes in metabolic resistance by insects to insecticides has been worked out[2, 3]. A direct way to show the intervention of P450 in resistance to insecticides is to compare the NADPH-dependent metabolism of insecticide in resistant and susceptible strains. In the case of DDT-resistant strain $RDDT^R$ of *Drosophila*, the NADPH-dependent metabolism of DDT is ten times higher than that of a susceptible strain[5]. Using various PCR methods, P450's were found to be constitutively overproduced in resistant strains: *Cyp6A1* in Rutgers strain of housefly[6], *Cyp6D1* in Learn PyrR strain of housefly7, *Cyp6B2* in *Helicoverpa*[8] and *Cyp9A1* in *Heliothis virescens*[9]. Although the involvement of P450 in insect resistance is documented, the upregulation of this metabolic enzyme associated with resistance remains less understood[1, 10, 11].

A group led by ffrench-Constant, have employed *D. melanogaster* as a model insect to dissect the genetic basis of metabolic insecticide resistance[11, 12]. Employing microarray analysis of all P450's in *Drosophila melanogaster*, Daborn *et al*., (2002) showed that DDT-R, a gene conferring resistance to DDT, to be associated with over transcription of a single cytochrome P450 gene, Cyp6g1. They have employed Hikone-R (a resistance strain established from field collection in the early 1960s) and WC2 (a field collected DDT-resistant strain) relative to Canton-S (a susceptible reference strain). DDT-R locus maps to right arm of chromosome II at 64.5cM and over transcription of this gene alone confers resistance to DDT (used for vector control), neonicotinoid nicotinic acetylcholine receptor agonist (imidacloprid and nitenpyram), and a novel insect growth regulator (lufenuron). They also sequenced the first intron of Cyp6g1 in the same resistant and susceptible strains to determine the relatedness of the DDT-R alleles using PCR, which detect the presence of the transposon and based on the length of the product generated, showed insertion to present in all 20 resistant alleles examined. They demonstrated by transgenic experiments that over transcription of Cyp6g1 alone is both necessary and sufficient for P450 - mediated DDT resistance.

Recently Pedra *et al*., [13] by Genome-wide micro array analysis (Affymetrix array) have demonstrated that multiple cytochromes P450 are over expressed and potentially contribute to the DDT resistance phenotype. They employed (1) the laboratory DDT - selected Rst(2)DDT$^{91\text{-}R}$, (2) the isochromosomal DDT - resistant field isolate RST(2)DDT^{Wisconsin} and (3) the DDT - susceptible Cantons-S lines of *Drosophila melanogaster*, there are four detoxification enzyme gene constitutively over transcribed in both RST(2)DDT^{Wisconsin} and Rst(2)DDT$^{91\text{-}R}$ (Cyp6g1, Cyp12d1, Cyp6a2, Cyp6w1). The relative transcript expression of Cyp6a2 in Rst(2)DDT$^{91\text{-}R}$ was 255- and 9.1- fold greater than Canton-S and Rst(2)DDT^{Wisconsin}. To examine whether the position of genes over expressed in DDT-resistant *Drosophila* were random or clustered together in a region. The cytological position of each differentially transcribed probe set revealed that the transcripts were widely distributed across all chromosomes except for its moderate representation in the right arm of the second chromosome. Thus reported that, multiple cytochromes P450 are over expressed and potentially contribute to the DDT resistance phenotype. For the discrepancies found between their findings and those of Daborn *et al*., (2002); Pedra *et al*., (2004) listed the differences to be underlying at statistical methodologies, array technology employed, age or gender employed.

As far as now, the debate of single or multiple factorial influences in resistance development is still open. P450 appear to be a prime candidate to work on far sorting this issue. Answer to this question will not only unravel a chapter on evolution of insecticidal resistance but it will also help in designing accurate methodologies for monitoring resistance alleles of P450 and their spread in wild population of agricultural pests or vectors of diseases.

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Susceptibility of *Bactericera cockerelli* (Sulc) (Hemipterous: Psyllidae) to Insecticides in Tomato (*Physalis philadelphica* Lam.) in Zapotlán, Jalisco

KEY WORDS LC_{50}, Probit Analysis

INTRODUCTION The man and insects compete for agricultural products, that is the reason why numerous methods were developed and are applied for their control (1). The insecticides are the control method most used in the world due to their quick action, what derives in an irrational handling and causes problems like residuallity, contamination and resistance (2). The resistance is present in each one of the toxicological groups that include microbial and growth regulators (3). In Mexico the sucking insects among them *B. cockerelli* (Sulc), constitute one of the more economic important plagues for Solanáceas and cucurbitaceous (4); it is not known if there are differences in the capacity of this insect to affect their lodging plants or its answer to climatic factors (5), for what it is necessary to generate regional information about the biological effectiveness of the commercial and experimental pesticides as the ones in development, with the purpose of elaborating a basic table of products for its control (6).

The hypothesis of the work is that the control measures for *B. cockerelli* are not carried out correctly, for what is ignored which insecticides and doses are appropriate. The objective of this study was to determine the nymph susceptibility of *B. cockerelli* in bioassays, to different control agents in *P. philadelphica* tomato cultivation in Zapotlán, Jalisco.

MATERIALS AND METHODS The collection of leaves was carried out in the month of July, in the Municipality of Zapotlán, Jalisco, 200 leaves with presence of nymphs were randomly taken, and kept in paper bags; the bioassays were carried out in the Laboratory of pest control of the CUCBA-U de G., in which intersected 5 cm diameter foliar disks, until completing groups of 100 nymphs and they were placed in Petri glass boxes with humid filter paper; the LC_{50} of the following active ingredients was determined: Mineral Oil, Nicothiadine, Lambda-Cyhalothrin, Carbofuran, Carbosulfan and Diazinon, plus a witness without product. The technique used was that of the residual movie for the following concentrations: 1, 10, 100, 1,000 and 10,000 ppm, of A.I.; the reading of mortality was taken at 24 and 48 hours, by observation in stereoscope microscope and the nymph without coordinated mobility was considered dead; a Probit Analysis of Maximum Verisimilitude was carried out with the data of mortality that were obtained, by means of the statistical package SPSS (2001) version 10.0, to determine the LC_{50}.

RESULTS AND DISCUSSION The results of mortality of the active ingredients coincide that any insecticide works against *B. cockerelli* (6) (Table 1).

Table 1. - Insecticides and their doses (ppm), evaluated in tomato, with their percentage of mortality for nymphs of *B. cockerelli* .

Insecticide	Dose	Mortality %	LC50 in ppm
1. Diazinon	625 ppm	95	30.17
2. Carbosulfan	625 ppm	95	23.96
3. Nicothiadine	625 ppm	83.33	124.33
4.Lambda-Cyhalothrin	1,000 ppm	100	25.88
5. Carbufuran	3,125 ppm	88.88	130.06
6. Mineral Oil	3,125 ppm	84	52.78

Diazinon, carbosulfan and Lambda-Cyhalothrin coincide in that they are the insecticides that have bigger mortality in nymphs of *B. cockerelli* like it is reported in hot pepper (5, 7). As for LC_{50} the lower doses correspond to: carbosulfan, Lambda-Cyhalothrin and diazinon, the mineral oil had a medium LC_{50}, nicothiadine and carbofuran presented the highest LC_{50} (Figure 1).

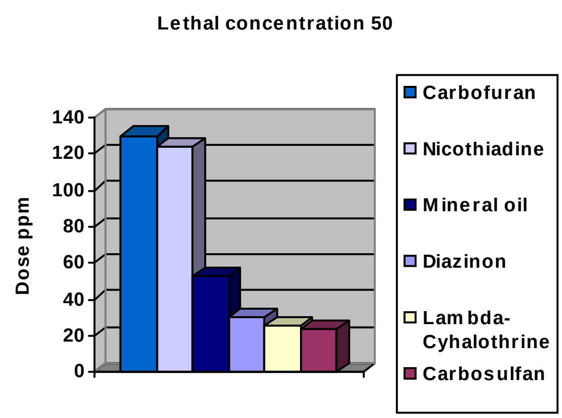

Figure 1. LC50 of the ingredients that were applied to nymphs from *B. cockerelli* at laboratory level.

CONCLUSIONS The diazinon and carbosulfan insecticides were those that presented higher death rank to smaller dose (625 ppm-95%), on the other hand nicothiadine controlled alone 83% (625 ppm), 12% less than the previous products, as for the mineral oil and carbofuran have control, but at high dose (3,125 ppm) and lastly Lambda-Cyhalothrin works but at the highest dose. The insecticides with smaller LC_{50} (more efficient) were: carbosulfan, diazinon and Lambda-Cyhalothrin. It is derived that all the current toxic groups have a satisfactory control at laboratory level, for what covers the hypothesis, it only remains to carry out field rehearsals to verify if the environmental conditions, those of *P. philadelphica* and *B. cockerelli* influence in the behavior of the insecticides.

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Project financed by: UDG/PROMEP-SEP

Development and Validation of Insecticide Resistance Management (IRM) based Integrated Pest Management (IPM) module for cotton pests

Cotton is one of the important commercial crops of India in general and Andhra Pradesh in particular. Damage caused by different insect pests, apart from seasonal factors, has been identified as the main reason for the fluctuating kapas yields and farmers fortunes. Farmers of Kurnool dist. are not exception to it. The farmers are mostly depending on insecticides for management of these insect pests. Use of several insecticide sprays at short interval, insecticide mixtures, and higher doses are not uncommon. Due to excess and indiscriminate use of synthetic chemicals resulted in the development of many fold resistance in *Helicoverpa armigera* to these chemicals. Dingra *et al*., 1988 were the first to report the insecticide resistance development capabilities of *H. armigera* in India. Studies conducted by Armes *et al*., 1992, 1996; Kranthi *et al*., 2001 revealed that resistance to pyrethroids and conventional insecticides in India were increasing. As a result the research was intensified to develop resistance monitoring and management strategies for cotton pests. Eventually Resistance monitoring centers were established throughout India in 26 districts of 10 cotton-growing states and for Kurnool district at RARS, Nandyal under Technology Mission on Cotton-Mini Mission II (TMC-MM II). The farmers participatory IRM based IPM trials were conducted in intensive cotton growing block of Kurnool dist, from 2002-2003 to 2005-06 with an aim to enhance the production and productivity of the cotton ecosystem while reducing the use of insecticides and increasing the farmers confidence in cotton pests management.

Insecticide Resistance studies conducted on Kurnool population of cotton bollworm *H. armigera* at Resistance Monitoring Centre (RMC), RARS, Nandyal, from 2003 season onwards indicated high resistance against synthetic pyrethroids (resistance frequencies >80% resistance factors >1000) and low to moderate levels against organophosphates and carbamate compounds. Based on our studies, available literature on new chemistries and taking clues from supporting additional information the location specific IRM based IPM module was formulated and tested; 1. No Spray up to first sixty days; Seed tretamnet with imidacloprid 70 WS or thiamethoxam 70 WS 5 g/kg seed, Inter cropping with cowpea, Stem application at 40 DAS, Avoidance of broad spectrum OP chemicals, in case of emergency spray diafenthiuron (Pegasus) 1 g /l 2. Window II. 60-75 days; release *Trichogramma* during peak egg load period, spray soft biopesticides such as *Bt*, NPV or neem based formulations, Incase of non availability of these, spray endosunfan 35% EC 2ml/l 3. Window III. 75-90 days. Spray bioselective and least resisted insect growth regulators like novaluron or lufenuron 1ml/l 4. Window IV. Bollworm infestation 90-110 days; Spray new chemistries like spinosad or emamectin benzoate or indoxacarb 5. Peak bollworm-110-140 days; Spary quinalphos or chlorpyriphos or thiodicarb, rotate them and two to three days after spray left over population should be hand picked and destroyed 6. Window V. Pink bollworm; ETL based spray of synthetic pyrethroids.

The location specific IRM based IPM strategies were validated through farmers participatory filed trials from 2002-03 to 2005-06 in 3307 ha in 79 intensive cotton growing villages of Kurnool dist. of Andhra Pradesh. Cluster of villages were selected mainly based on their socio economic status, crop history and pesticide use pattern and convenience for supervision and implementation of the strategies. These strategies were disseminated using indigenous techniques like street plays, dramas, and folk songs because this form of massage dissemination was recognised as more appealing with dramatic impact, and has been found close to the hearts of rural folk. Besides folk arts, keeping the importance of electronic media in dissemination of technologies an innovative Hello-IRM an interactive live phone in programme was devised and broadcasted through All India Radio, Kurnool for the benefit of farmers. Team of resource persons from RARS, Nandyal was involved to respond the queries posed by the farmers immediately. Further, one field worker per village was employed for effective dissemination of strategies. This project staff, staying in the project villages, has educated the farmers through out the season about latest crop production and pest management technologies.

Data on pest incidence, natural enemy activity, No. of insecticides sprayed, quantity of insecticides used in project fields and non-project fields were collected. Yield parameters and economics of project farmers and non-project farmers were recorded and impact of IRM strategies on yield and economics has been worked out (Table 1). Marginal differences were recorded in the incidence of sucking pests and bollworms. Natural enemy activity was comparatively more in project operated fields, on an average around 35% more natural enemy activity was recorded in IRM fields. The farmers of project operating villages saved on an average 5.8 sprays over non-project farmers. No major differences were recorded in terms yield and gross returns. However, with reference to total expenditure and net returns, project farmers spent 36% less expenditure and realized 30% more net profit than non-project farmer/acre. The overall monetary benefit accrued to the farmers by adoption of IRM based IPM module was estimated at 209 lakhs.

Table 1: Impact of IRM based IPM module on pesticide usage, yield and economics

Year	Reduced no. of sprays	Increase in yield kgs/acre	Gross returns Rs/acre	Net returns Rs./acre
2002-03	5.77	-13	-213	1227
2003-04	6.91	116	1987	3451
2004-05	7.64	10	145	1171
2005-06	3.18	41	902	4191
Average	5.87	61.6	705	2510

ACKNOWLEDGMENTS Thanks are due to Dr. T. Yellamanda Reddy, Associate Director of Research, Regional Agricultural Research Station, Nandyal, for providing necessary facilities and Dr. K. R. Kranthi, Principal Investigator, TMC-MM II, Central Institute for Cotton Research, Nagapur, for extending necessary technical support. Financial support from TMC-MM II is gratefully acknowledged.

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Bioefficacy of Bifenthrin as a Prophylactic Against Four Major Stored Grain Pests

ABSTRACT Prophylactic spraying of storage surfaces with insecticides is the most safe and efficient method to protect grain. The studies were conducted to know the efficacy of bifenthrin when used for disinfecting ware houses and jute sacks against four different stored grain pests i.e., *Sitophilus oryzae*, *Tribolium castaneum*, *Trogoderma granarium*, and *Rhizopertha dominica* at Indian Grain Storage Management and Research Institute, Hyderabad during 2004. The toxicology studies under laboratory conditions revealed that the LD50 values of bifenthrin against *S. oryzae, T. castaneum, T. granarium* and *R. dominica* were 3.389, 46.65, 4.509 and 5.573 respectively when treated on concrete slabs and 4.629, 48.0, 5.056 and 6.042 respectively when treated on jute strips. The investigations revealed that bifenthrin when used for

prophylactic control is effective only against *S. oryzae, T. granarium and R. dominica* but not against *T. castaneum*.

KEYWORDS Bifenthrin, bioefficacy, stored grain pests.

INTRODUCTION Insects cause considerable losses both in quality and quantity of stored food grains. For controlling these storage pests, malathion, DDVP, pirimiphosmethyl, chlorpyriphos methyl, fenitrothion, alluminium phosphide, bioresmethrin, fenvalarate, deltamethrin, ethylene dibromide, methoprene etc are being employed as prophylactic and curative measures. However, prophylactic spraying of storage surfaces with insecticides is the most safest and efficient method to protect grain. Several insect species of stored grain have already developed significant levels of resistance to most of the available grain protectants (Zettler and Cuperus, 1990; Collins *et al*., 1993; Guedes *et al*., 1996; Lorini and Galley, 1999) and there are also concerns over organophosphate residues in grain even after several months of storage. Hence, a grain protectant that gives adequate control of a range of stored grain insects, while resulting in lower levels of residues and minimal effect on non target organisms can solve much of the storage problems. Recently bifenthrin, a third generation pyrethroid with high insecticidal activity was developed and demonstrated as great promise in grain protection against several stored pests (Daglish and Wallbank, 2003; Wilkin *et al*., 1994). The present studies were conducted to know the efficacy of bifenthrin when used for disinfecting ware houses and jute sacks against four different stored grain pests *i.e*., rice weevil *Sitophilus oryzae* (L.), rust red flour beetle *Tribolium castaneum* (Herbst), khapra beetle *Trogoderma granarium* (Everts), and lesser grain borer Rhizopertha dominica (F.) at Indian Grain Storage Management and Research Institute (IGMRI), Hyderabad during 2004.

MATERIALS AND METHODS The present studies were conducted at Indian Grain Storage Management and Research Institute, Hyderabad during 2004. Technical grade bifenthrin was supplied by M/s FMC Corporation Pvt Ltd, Australia.

Maintenance of insect culture: Except *T. granarium*, adult stages of insects were taken for the study. Since *T. granarium* adult longevity is only few days and harmless, larvae were taken for the study. The test insects i.e *S. oryzae*, *T. castaneum*, *T granarium*, and *R. dominica* were reared in separate plastic containers on sterilized whole sorghum grain in an environmental chamber at 30±1 ºC and 65±5% RH. For this, each individual container was infested with 50 full grown larvae of test insects separately to get adults of same age. To obtain *T. granarium* larvae, 50 pupae were kept in a container and after adult emergence, they were separated and released into another container for egg laying. After one week eggs were collected by sieving through a 1 mm sieve and transferred to fresh grain. Seven day old larvae were used for the study.

Treatments: The experimental procedure followed the general guidelines standardized by IGMRI, Hapur, India. The experimental treatments were imposed on concrete slabs and jute strips simulating the warehouse storage conditions. There were six treatments in each, replicated thrice in a randomized block design. To conduct the experiment, raised concrete slabs (0.25 m²) were disinfected with formaldehyde and later cleaned with distilled water. The slabs were secluded with acrylic sheets. The technical grade was diluted with distilled water so that the experimental area will receive 20, 30, 40, 50 and 60 mg a.i/m² (doses within the range recommended by the manufacturers). The automizer was calibrated in a preliminary test, and found that 7.5 ml of spray fluid is required to cover 0.25 m² surface area of concrete slab/jute strip. After ensuring complete drying of concrete slabs, 7.5 ml of distilled water was sprayed on control slabs from a height of 20 cm on the surface using an automizer with the spray nozzle turned downwards. Starting from the least concentration, all the treatments were imposed on the respective concrete slabs and allowed for 30 minutes drying. Then 20 insects of each test species were released on to the respective treated slabs using camel hair brush and the surface was firmly covered with PVC plastic film to prevent insect escape and entry of outside insects into treated area. The insects were allowed for half an hour to get insecticidal exposure. Then the treated insects were transferred into plastic vials with sterilized whole sorghum grain. The insects were maintained in an environmental chamber and mortality was assessed, 72 hours after treatment. Similar experiment was done separately on jute strips of 0.25 m² with above concentrations. The data was subjected to probit analysis after correcting the percent mortalities using Abbott's formula:

$$\frac{T - C}{100 - C} \times 100$$

where,
T= Percent mortality in treatment,
C= Percent mortality in control.

The percent mortalities were plotted against log concentration of insecticide and LD_{50} values of bifenthrin for each insect were obtained.

Table 1. Toxicity of bifenthrin treated on concrete slab against four stored grain insects

Insect pest	LD_{50}	Regression	Chi-square
S. oryzae	3.389	Y = 4.316 + 1.289 x	0.288
T. castaneum	46.65	Y = 1.296 + 2.219 x	2.111
T. granarium	4.509	Y = 4.570 + 0.657 x	0.092
R. dominica	5.573	Y = 3.957 + 1.397 x	0.289

RESULTS AND DISCUSSION The toxicology studies under laboratory conditions revealed that the LD_{50} values of bifenthrin against *S. oryzae, T. castaneum, T.*

granarium and *R. dominica* were 3.389, 46.65, 4.509 and 5.573 respectively when treated on concrete slabs (Table 1). Where as, the LD_{50} values of bifenthrin for the same insects when treated on jute strips were 4.629, 48.0, 5.056 and 6.042 respectively (Table 2). Chi-square values also indicated that there was no heterogeneity between observed and expected mortalities of all the four insect pests. In each case, the LD_{50} values recorded on concrete slabs were lower than the values recorded on jute strips indicating that the concrete slabs offered uniform coverage of insecticide and more contact surface to the insects. In both the experiments, bifenthrin was found to be highly effective against S. oryzae even at low concentrations followed by *T. granarium* and *R. dominica* in sequence. However, *T. castaneum* adults were not affected much by bifenthrin which required 46.65 and 48.0 mg a.i./m ^{2} on concrete slabs and jute strips respectively, to cause 50 percent mortality. From this it is evident that *T. castaneum* has already developed tolerance to this product. The results observed here are consistent with the earlier findings of Wilkin *et al*, (1994) who found bifenthrin was very active against *R. dominica* and *S. oryzae*, but not against *T. castaneum*. In addition, Collins (1990) detected high levels of pyrethroid resistance in *T. castaneum.* Further, Reddy and Srivastava (2003) showed better persistent toxicity of deltamethrin WP than bifenthrin WP on jute against *T. castaneum.* Though *S. oryzae* and *R. dominica* were found susceptible to bifenthrin, reports of high level resistance to pyrethroids by Heather (1986) and Collins *et al*, (1993) suggest that these insects also have the potential to evolve resistance to bifenthrin. Since there is no single protectant that can control all species that attack stored grain, synergized combination of two products is inevitable. Successful control of R. dominica (Ali *et al*., 2003); *Sitophilus granarius* (L.) and *T. granarium* (Sokolov, 2004) was achieved with a combination of bifenthrin and malathion. While, piperonyl butoxide synergised bifenthrin plus chlorpyriphos methyl prevented *R. dominica*, *T. castaneum, Oryzaephilus surinamensis* (L.) and *Cryptolestes ferrugineus* (Steph.) from producing live progeny for upto 7 months (Daglish et al., 2003). From the investigations it is clear that bifenthrin when used for prophylactic control is effective only against *S. oryzae*, *T. granarium* and *R. dominica*. Insensitivity of *T. castaneum* to bifenthrin suggests that a combination with an organophoshate compound would be a better option to control a range of stored pests.

Table 2. Toxicity of bifenthrin treated on jute surface against four stored grain insects

Insect pest	LD_{50}	Regression	Chi-square
S. oryzae	4.629	Y = 4.109 + 1.338 x	0.377
T. castaneum	48	Y = 0.907 + 2.434 x	1.357
T. granarium	5.056	Y = 4.502 + 0.707 x	0.147
R. dominica	6.042	Y = 3.773 + 1.570 x	0.421

ACKNOWLEDGEMENTS Authors are thankful to Sri. K.M. Nimje, Director Officer- in-charge, IGMRI and Sri U.C.Gupta, Technical Officer, IGMRI for providing technical assistance and facilities for successful completion of the study.

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Forming of Cross-Resistance in Housefly (*Musica domestica* L.) Larvae to biotic and abiotic stressors under selection

The problem of insect resistance to insecticides is old phenomen, which in time attract all greater attention to itself. In literature for the first time this problem was reflected at the beginning XX age, but attracted to itself significant attention by entomologists, chemists, biochemists and the other scientists only with appearance of DDT-resistance in housefly *Musca domestica* L in 1946 y. (Perry, 1974). If the resistance was uncommon in beginning 50th years, now presence of sensitive populations is uncommon. Besides of that, if firstly observed resistance of one species to one insecticide, presently often one species possesses the resistance to several insecticideses immediately. Additionally insecticide selection can form resistance to such different factors, as heating, cooling, starvation and drying (Tewari, Pandey, 1978), as well as to treatment of formaldehyde, and raised gravitation (Chernysh, Luhtanov, 1985). Resistant to raised gravitation fruit flyes also have the long-time resistance to heating (Minois et al., 1999). Thereby, under influence of insecticides and the other stressors specific and nonspecific, the cross-resistance can be formed in insects.

The housefly is a very convenient object for study for the questions of the resistance forming in laboratory condition. This species has a short cycle of the development (about month), which allows work during the whole year, since well reproducing independently of season of the year. Exactly so we studied the processes of the resistance and cross-resistance forming to different factors used this object.

The studies were conducted on III age larvae of the housefly *Musca domestica* L. For study of the resistance forming the housefly larvae of the sensitive (S) strain Cooper were divided into groups and each group was selected by the corresponding stress-factor: bithoxybacillin (in concentration from 0,01% in F_1 up to 0,19% in F_{30},the strain R_{BTB}), malathione (in concentration from 0,03% in F_1 up to 0,47% in F_{30},the strain R_M), high (from 35°C in F_1 up to 64°C in F_{30}, the strain R_H) and low (from 15°C in F_1 up to 7°C in F_{30}, the strain R_C) , temperatures.

The criterion of housefly larvae sensitivity to the preparations was the efficient concentration, led to 50% mortality of individuals (EC_{50},%), which was defined by the method of probit-analysis (Popov, 1965). The degree of resistance reached in housefly larvae was characterized by the resistance index (RI), which presents itself ratio of resistant strain EC_{50} to EC_{50} of sensitive strain (Methodical instructions, 1990).

Results of the evaluating of cross-resistance level in selected strains in 10th, 20th and 30th generations to selectants with toxic action are presented in table 1

Table 1. Cross-resistance in selected strains of house flies to toxicants

Generation	Index	Strain R_{BTB}	Strain R_M	Strain R_C	Strain R_H
Bithoxybacillin					
F_0 (Strain S)	EC_{50}(%)	0.0047±0.0008			
F_{10}	EC_{50}(%)	0.0171±	0.01± 0.001	0.014 ±	0.064± 0.007
	RI	3.64	3.2	2.98	13.62
F_{20}	EC_{50}(%)	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
F_{30}	EC_{50}(%)	0.059±0.0097	0.041±0.002	0.025±0.002	0.037±0.0023
	RI	12.55	8.72	5.32	7.87
Malathion					
F_0 (Strain S)	EC_{50}(%)	0.0168±0.0017			
F_{10}	EC_{50}(%)	0.012± 0.001	0.031± 0.002	0.026 ±	0.0041± 0.0016
	RI	0.71	1.85	1.55	0.24
F_{20}	EC_{50}(%)	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
F_{30}	EC_{50}(%)	0.034±0.0038	0.57±0.087	0.023±0.001	0.021±0.0025
	RI	2	33.53	1.35	1.25

Practically all selected strains of the house fly in 10th generation show at least small cross-resistance to BTB, and strain, selected by high temperature - more considerable resistance (RI=13,6). At the same time the cross-resistance level to malathion is small (RI=1,55 and 1,85) or the resistance is absent (RI=0,71 in larvae of the strain R_{BTB} and RI=0,24 in larvae of the strain R_H). To 20th generation the cross-resistance increases to this preparation in house flies, selected by BTB and malathion. In flies, selected high temperature the cross-resistance to malathion is increased, but in flies, selected low temperature, the cross-resistance to BTB is increased while cross-resistance to malathion does not change. In 30th generation increase of cross-resistance to BTB observed only in the strain, selected by malathion, while in strains, selected by high or low temperature, occurs certain reduction of cross-resistance to this preparation. Cross-resistance level to malathion does not increase. in all strains in 30th generation.

Direct applying of the probit-analysis method for temperature sensitivity estimation turned out to be difficult. If at treatment by insecticides from different classes (pyrethroids, inhibitors of the chitin syntheses, organophosphorus or organochlorus compounds) or by high temperature it is revealed the proportional dependency of the mortality percent from concentration substance then in the case of the low temperature influence this curve looks like sinusoid. However the certain diapason exists, within of which all explored strains have proportional acute increase of mortality. Under the influence of the high temperatures in our experiment the most rectilinear increase of mortality is registered within from +40ºC to +50ºC. In the variant with low temperatures similar increase of mortality is noted within from +10º before +5ºC.

We offer to use for comparison of different groups of insect, in our case - high and low temperature selected house fly strains, the coefficient of temperature sensitivity (C_T), definied as ratio of mortality values in extreme points of the critical temperature rainge for selected strains to the same differences for sensitive, or "basic" strain (Sokolyanskaya *et al.,* 2005).

For such index value 1.0 means absence of some difference in sensitivity to the change of the temperature. If C_T value exceeding 1.0, it evidens the decrease of sensitivity (increasing of resistance) at comparing witn basic group of insects; value C_T smaller than 1.0 indicates the increase the increase of sensitivity (the reduction resistance). Thereby, it is possible to estimate and compare the results of selection in R_{-H} and R_{-C} strains there (Table 2).

Table 2. Cross-resistance in selected strains of house flies to temperature

Parameter	Generation	Index	Strain R_{BTB}	Strain R_M	Strain R_C	Strain R_H
High temperature	F_0(Strain S)	D*	30,7			
	F_{10}	D	29.6	32	37.3	6.8
		K_T	1.04	0.96	0.82	4.51
	F_{20}	D	44.7	5	34	31.1
		K_T	0.69	6.14	0.9	0.99
	F_{30}	D	23.9	6.4	23.8	31.92
		K_T	1.28	4.8	1.29	0.96
Low temperature	F_0(Strain S)	D	44,2			
	F_{10}	D	43	16	34.7	34.8
		K_T	1.03	2.76	1.27	1.27
	F_{20}	D	18.8	13	42	20
		K_T	2.35	3.4	1.05	2.21
	F_{30}	D	20	6.4	9.01	12.77
		K_T	2.21	6.91	4.91	3.46

*D- difference of mortality in extreme points of the critical temperature range.

The selected BTB and low temperature strains show similar with sensitive strain attitude to high temperature i.e resistance does not exist. In malathion selected strain the cross-resistance to high temperature developed to 20th generation, which is reducing to very small values in 30th 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 +10ºC. Thereby the exposure by temperature +10ºC is adaptogenic stress-factor for all selected strains. In 20th generation of all selected strains was observed the low temperature sensitivity lower than in S strain (C_T>1) i.e. cross-resistance is formed to low temperature, which is increases to 30th generation, particularly in malathion selected strain. Thereby, malathion selected strain in process of the selection develops resistance not only to selectant, but also cross-resistance to others nonspesific factor.

Proceed from foregoing, we consider that forming of cross-resistance to sharp reduction of the temperature in all strains is indicate of adaptive nature of cold stress that also can be connected with specifics of the influence of the low temperature. Besides, achievement of significant cross-resistance level (5-8-times) in all strains to action of the bacterial preparation BTB is indicative of immune systems activations in strains selected by other factors; spare that proof - an absence of similar level cross-resistance to malathion in these strains i.e. forming to cross-resistance develops by the other mechanism.

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Wet land weed *Sphaeranthus indicus* Asteraceae (Linn.) a potential green pesticide for managing Angoumois grain moth *Sitotroga cerealella* (Oliv.)

ABSTRACT Screening extracts of nine different plants for the insecticidal activity to the Angoumois grain moth, *Sitotroga cerealella* (Olivier) was carried out. The plants used are Negundo (*Vitex negundo*) (Linn.)., East Indian Globe Thistle (*Sphaeranthus indicus*) (Linn.), Jamaica mountain sage (*Lantana camara*) (Linn.)., Dry chillies (*Capsicum annum*) (Linn.)., Soap berry (*Sapindus emarginatus*) (Vahl.)., Curry leaf (*Murraya koenigii*) (Linn.)., Indigo (*Tephrosia purpurea*) (Linn.)., Crotons (*Crotons sparsiflorus*) (Linn.). and candle stick (*Cassia alata*) (Linn.). Among them *V. negundo* has proved to have high efficacy for reducing the percent infestation and weight loss without affecting the germination. The adult longevity of *S. cerealella* was also reduced. Next comes the wet land weed *S. indicus* which was equal to that of negundo in efficacy. As *S. indicus* was having ethnopharmacological properties and not studied extensively for their toxicological character, further fractionation was carried out by using column chromatography techniques. Fractions from acetone and acetonitrile extraction showed their efficacy in reducing the percent infestation and weight loss due to their insecticidal activity effect on this pest.

KEYWORDS *Vitex negundo, Sphaeranthus indicus, Sitotroga cerealella*, Insecticidal activity, Fractionation.

INTRODUCTION Rice is stored either as paddy or as rice. But considerable loss to a tune of 6.58 percent is bound to occur during storage due to various infections (Dutta, 1984).

Among the insects, the Angoumois grain moth, *Sitotroga cerealella* (Olivier) (O: Lepidoptera: F: Gelechiidae) is a serious pest damaging the whole grains of paddy (Champ and Dyte, 1977). However, the use of chemicals against storage pests not only increased the cost of production but also resulted in insecticide residues and insecticide resistance.

Use of biopesticides in pest management is gaining importance recently. Hence studies were conducted in screening nine different plants for toxicity to the *S. cerealella*. Among them Negundo, *Vitex negundo* (Linn.) (F: Verbanaceae) is known for insecticidal property and studied in detail. But East Indian Globe Thistle, *Sphaeranthus indicus* (Linn.) (F: Asteraceae) possess some ethnopharmacological and antimicrobial properties in curing some skin diseases (Ram *et al.*, 2004). It also acts as blood purifier. This is a common wet land weed especially after harvest and comes to flowering and fruiting from March to June (Plate 1) and not studied in detail. Hence fractionation of *S. indicus* is aimed to identify which fraction has the insecticidal property.

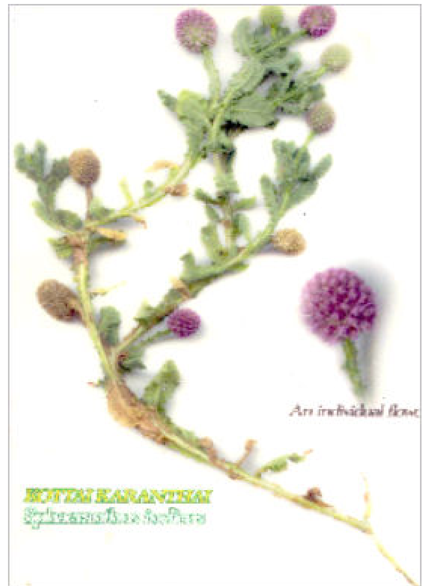

Plate 1: East Indian Globe Thistle *Sphaeranthus indicus* (L.)

MATERIALS AND METHODS Mass culturing of Angoumois Grain Moth: Paddy variety CR 1009 was used for mass culturing and for conducting various experiments. The mass culturing of S. cerealella was carried out by confining 10 - 20 freshly emerged moths of both sexes in plastic containers of 59 x 21 x 18 cm with 500 g of paddy grains maintained at 28 ± 2° C (Heinrichs *et al.*, 1985). The freshly emerged moths were used for various experiments.

Extraction Procedure, Percentage Infestation and Percentage weight loss: All plant materials were shade dried and extracted with hot water @ 50 g / 1 lit so as to get five percent extract (Sighamony *et al.,* 1990). After cooling, 75 ml of extract is mixed with 100 g of paddy grains and shade dried. Such treated grains were exposed to *S. cerealella*. adults 1:3 for male and female. In each replication two males and four female moths were confined. Further adult longevity, percent infestation (Mohan and Sundara Babu, 1999), percent weight loss (BIS, 1989) and germination rate (ISTA, 1985) were recorded at 10, 30 and 50 days after treatment.

Fractionation and bioefficacy studies: *S. indicus* was found to be effective among the different plants and on par with negundo plant. The whole extract was split into several fractions by column chromatography (Dhaliwal *et al*., 1990).

Two kg of powdered sample was extracted with acetone in various batches, pooled and kept for 72h, and the sample was condensed with rotary vaccum evaporator to expel all the solvent. Then the collected sample devoid of acetone was eluted in silica gel column. The column was first packed with anhydrous sodium sulphate and the silica gel was mixed with charcoal in 1:1 ratio and again filled at the top with anhydrous sodium sulphate in layers. To this the extracted sample was poured and eluted serially with solvents *viz.*, hexane, benzene, chloroform, ethyl acetate, acetonitrile and acetone in the same order. Then each eluted material was again condensed by rotary vaccum evaporator to remove the excess solvent and it was kept in deep freezer for further use in bioefficacy studies.

Each fraction was mixed with grains and the same procedure as described earlier for evaluation of bioefficacy and the data regarding the percentage infestation and percentage loss of weight were observed. Data on percent values were converted into corresponding angular transformation for statistical analysis (Snedecor and Cochran, 1967).

RESULTS Adult mortality: A fifty percent mortality of both the sexes were seen in the extracts of soap berry, negundo, east indian globe thistle, jamaica mountain sage, dry chillies and curry leaf on the second day. In candle stick extracts the fifty percent mortality of both sexes was recorded on third day. In indigo and crotons the females also showed 50 percent mortality on third day. But in all the extracts the 100 percent mortality of both the sexes were observed on fourth day (Fig. 1&2).

Fig 1. Mortality of adults of (female moths) Angoumois grain moth *Sitotroga cerealella* (Olivier) in paddy grains treated with different botanicals

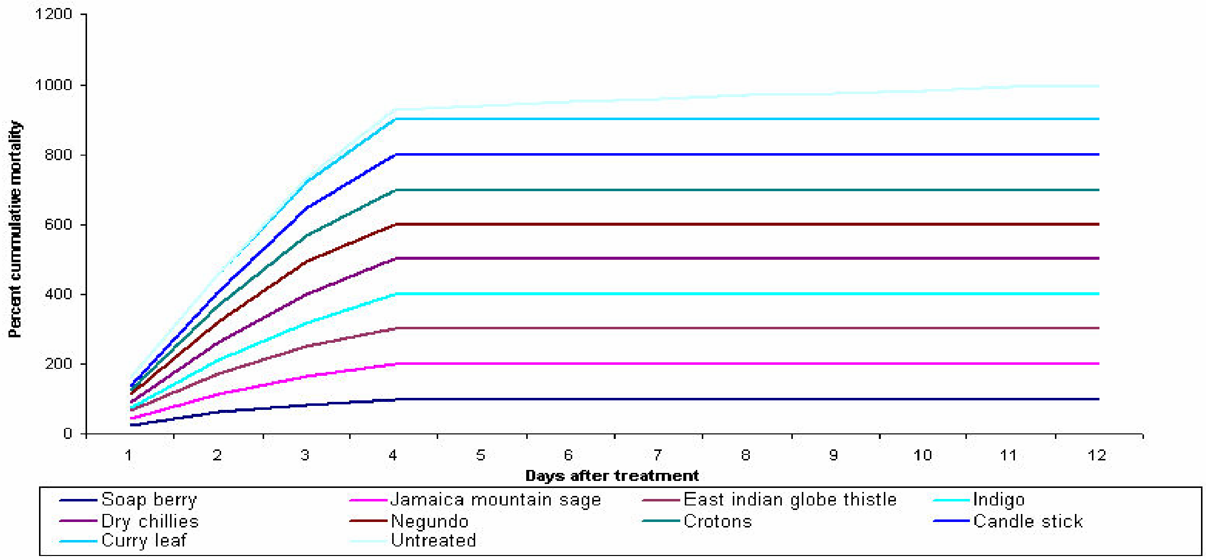

Fig 2. Mortality of adults of (male moths) Angoumois grain moth*Sitotroga cerealella* (Olivier) in paddy grains treated with different botanicals

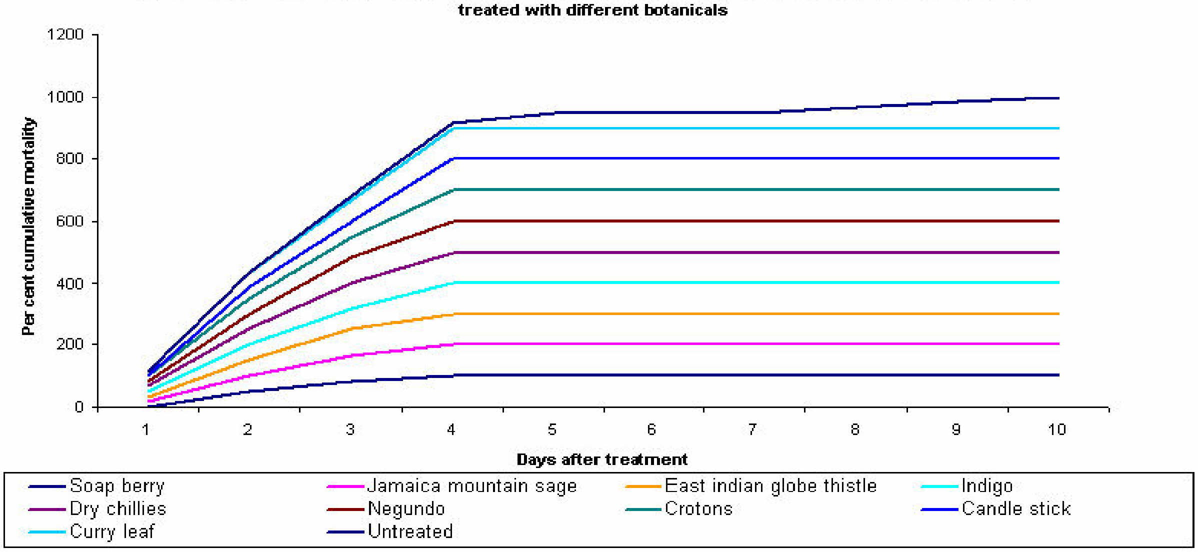

Table 1. Effect of extracts of botanicals on the infestation level, weight loss and germination loss in paddy by Angoumois grain moth *Sitotroga cerealella.*

Sl. No.	Treatments	Per cent infestation (Mean of three replications)			Per cent weight loss (Mean of three replications)			Germination per cent (Mean of four replications)
		10 DAT	30 DAT	50 DAT	10 DAT	30 DAT	50 DAT	90 DAT
1	*Sapindus emarginatus* (Soap berry)	28 *(31.93)* [a]	32.3 (34.64) [a]	33.3 (35.26) [a]	19.11 (25.89) [c]	30.55 (33.54) [abc]	42.11 (40.43) [ab]	96 (80.12) [ab]
2	*Lantana camara* (Jamaica mountain sage)	27.6 (31.73) [a]	31.6 (34.24) [a]	32.6 (34.86) [a]	18.41 (25.38) [abc]	30.53 (33.54) [abc]	42.11 (40.44) [ab]	94 (76.01) [abc]
3	*Sphaeranthus indicus* (East Indian Globe Thistle)	27.6 (31.73) [a]	31.3 (34.03) [a]	32 (34.44) [a]	17.18 (24.45) [a]	29.98 (33.17) [ab]	40.89 (39.74) [ab]	96 (80.12) [ab]
4	*Tephrosia purpurea* (Indigo)	28.6 (32.37) [a]	37.6 (37.86) [b]	38 (38.06) [b]	22.27 (28.13) [d]	32.1 (34.47) [bc]	43.54 (41.24) [b]	91 (72.87) [c]
5	*Capsicum annum* (Dry chillies)	28 (31.95) [a]	32.3 (34.64) [a]	33.3 (35.26) [a]	18.6 (25.50) [bc]	30.73 (33.66) [abc]	42.24 (40.51) [ab]	94 (76.01) [abc]
6	*Vitex negundo* (Negundo)	27.6 (31.72) [a]	31 (33.83) [a]	31.6 (34.24) [a]	17.32 (24.58) [ab]	28.36 (32.14) [a]	40.15 (39.29) [a]	97 (81.35) [a]
7	*Crotons sparsiflorus* (Crotons)	29 (32.58) [a]	37.6 (37.86) [b]	38 (38.05) [b]	21.18 (27.37) [d]	32.6 (34.78) [c]	43.66 (41.34) [b]	92 (73.83) [bc]
8	*Cassia alata* (Candle stick)	29.6 (32.99) [a]	39.6 (39.04) [b]	39.6 (39.04) [b]	22.22 (28.09) [d]	32.57 (34.78) [c]	43.93 (41.50) [b]	91 (72.87) [c]
9	*Murraya koenigii* (Curry leaf)	28 (31.93) [a]	32.3 (34.64) [a]	33.6 (35.46) [a]	18.89 (25.73) [c]	30.8 (33.68) [bc]	42.34 (40.55) [ab]	93 (75.06) [abc]
10	Untreated	50 (45.00) [b]	65 (53.76) [c]	65.3 (53.97) [c]	24.14 (29.40) [e]	50.25 (45.13) [d]	60.98 (51.35) [c]	75 (60.05) [d]
	CD (p = 0.05)	(2.59) **	(2.56) **	(2.37) **	(0.98) **	(1.53) **	(1.94) **	(6.74) **

Figures in the ( ) are arc sin transformed values / Means in a column followed by same letter are significantly not different DAT – Days after treatment / ** Significant at 0.01 per cent level

The adult mortality distinctly varied in untreated grains where the males and females showed 50 percent mortality on fifth and sixth day. In untreated grains all male and female were alive on 10th and 12th day also.

Percent Infestation: The percentage infestation at 10 DAT showed a significant difference between the plant extract treated (27.6 - 29.6 percent) and untreated paddy grains (50.0 percent) (Table 1).

Soap berry, negundo, east indian globe thistle, jamaica mountain sage, dry chillies and curry leaf were better than indigo, crotons and candle stick both at 30 and 50 DAT. Among them negundo and east indian globe thistle showed a minimum level of infestation (Table 1).

Percent weight loss: The percent weight loss due to damage caused by *S. cerealella* at 10 DAT indicated that east indian globe thistle showed a minimum of 17.18 percent weight loss which was on par with negundo. Soap berry, jamaica mountain sage, dry chillies and curry leaf were moderately effective. Indigo, crotons and candle stick though showed higher weight loss than other treatments but statistically less than untreated grains (24.14 percent). A similar trend was seen at 30 and 50 DAT. But negundo was superior to other treatments followed by *S. indicus* (Table 1).

Germination tests: In plant extract treated grains, the germination was between 91.0 - 97.0 percent higher than untreated grains (75 percent) (Table 1) indicating that application of botanicals protected the paddy grains from insect damage.

All the treatments were effective in checking the pest. Among them S. indicus is as effective as that of *V. negundo.*

Fractionation of the whole extract of S. indicus: Six fractions of global extract of *S. indicus* were tested against *S. cerealella*. The percent infestation at 10 DAT was lowest (10.6 percent) in acetone and acetonitrile fractions, but other fractions were superior to untreated grains showing 27.0 percent infestation. Similar observation at 30 DAT also indicated that acetone and acetonitrile fractions showing lowest (12.6 and 13.0%) percent of infestation, whereas the untreated check showed 29.6 percent damage (Table 2).

The percentage weight loss in paddy grains also had a corroborative out come of minimum percent weight loss both at 10 (6.87%) and 30 DAT (7.33%) in acetone and 7.00 and 7.94 percent in acetonitrile respectively, as against 19.61 percent in untreated check (Table 2).

Table 2. Percentage infestation and weight loss in paddy grains treated with fractions of *Sphaeranthus indicus*

Sl.No.	Treatments Fractions of	Per cent infestation (Mean of three		Per cent weight loss (Mean of three	
		10 DAT	30 DAT	10 DAT	30 DAT
1	Hexane	14 (21.91) [b]	15 (22.73) [ab]	7.95 (16.32) [bc]	9.16 (17.59) [c]
2	Benzene	15.3 (23.04) [b]	16.6 (24.08) [b]	8.92 (17.31) [c]	11.1 (19.42) [d]
3	Chloroform	12.6 (20.84) [ab]	13.3 (21.40) [ab]	7.2 (15.53) [ab]	8.7 (17.12) [bc]
4	Acetonitrile	10.6 (19.03) [a]	13 (21.12) [ab]	7 (15.30) [a]	7.94 (16.32) [ab]
5	Ethyl acetate	12.6 (20.84) [ab]	13.6 (21.68) [ab]	8.01 (16.39) [bc]	8.19 (16.57) [b]
6	Acetone	10.6 (19.05) [a]	12.6 (20.84) [a]	6.87 (15.15) [a]	7.33 (15.68) [a]
7	Untreated	27 (31.30) [c]	29.6 (32.99) [c]	17.49 (24.70) [d]	19.61 (26.25) [e]
	CD (p = 0.05)	(2.28) **	(2.43) **	(0.99) **	(0.81) **

Figures in the ( ) are arc sin transformed values
Means in a column followed by same letter are significantly not different
DAT – Days after treatment / ** Significant at 0.01 per cent level

DISCUSSION The effect of plant extracts on *S. cerealella* clearly indicated that *V. negundo* and *S. indicus* having insecticidal property. Similar studies on the insecticidal activity of botanicals against rice weevil *Sitophilus oryzae* (Linn.) indicated that custard seed and neem seed powder protected the wheat grains (Mishra *et al.,* 1992).

The essential oils of curry leaf, *Murraya koenigii* against pulse beetle *Callosobruchus chinensis* (Linn.) showed a contact, ovicidal and fumigant action with a diffusible property at 340 ppm level (Pathak *et al*., 1997).

In stored maize and paddy the percent grain damage by *S. cerealella* was reduced using neem leaf and neem kernel (Yadu *et al.,* 2000). The insecticidal property of leaves of *V. negundo* against storage pests of paddy was reported earlier (Baskaran and Narayanasamy, 1995).

The reduction in percent weight loss by using neem, castor and mauha oil was reported in pulse beetle *C. chinensis* (Singh *et al*., 2001). In stored maize and paddy with neem leaves afford protection against *S. cerealella* (Yadu *et al.*, 2000). At 50 DAT damage in untreated was maximum (60.98 percent) as in similar studies 50.5 percent (Shazali, 1987) and 65 percent (Dakshinamurthy, 1986) and in all the other treatments it ranged from 40 to 43.93 percent.

Use of neem products, leaves of eucalyptus, sarifa and *Lantana* reduced the percent germination to 71% and 92.02% in maize and paddy against *S. cerealella* respectively (Yadu *et al.*, 2000). The germination was higher in wheat than in *Melia azadirach* treated grains for *S. oryzae* as reported by Singh and Mall (1991).

Acetone and acetonitrile fractions were showed insecticidal activity to *S. cerealella*. Similarly fractionation of leaves of *V. negundo* by using ethanol solvent in silica gel column and thin layer column chromatographic technique lead to the isolation of a ketone compound namely "2 - heptatriacontanone". They acted as oviposition inhibitor against the *S. cerealella*, lesser grain borer *Rhizopertha dominica* (Fab.) and rice weevil *S. oryzae* as reported by Prakash *et al.* (1990). Hence, *S. indicus* may be a potential green pesticide in future and isolation and identification of the active principle may lead to identify some compounds having insecticidal activity for storage pests.

ACKNOWLEDGEMENTS The author wishes thanks to Professor & Head, Department of Agricultural Entomology and the DEAN, PAJANCOA & RI, Karaikal for providing facilities.

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Population structure of Colorado Potato Beetle Leptinotarsa decemlineata Say in the South Urals

INTRODUCTION The Colorado potato beetle (CPB) *Leptinotarsa decemlineata* Say (Coleoptera, Chrysomelidae) is interesting and new object from the point of view of population studies. This species, being evolutionarily comparatively young, with the continuous processes of speciation (Ushatinskaya, 1981; Fasulati, 2000), is distinguishing by significant intraspecific polymorphism and, as a result, is characterized by striking ecological plasticity and adaptability. All this makes possible for it to enlarge successfully its area (Fasulati, 2002), being adapted to the new climatic conditions and the anthropogenic influence. In particular, in the territory of Russia this phytophagous insect already exceeded the boundaries of the assumed northern propagation on 200-300 km (Figure 1). Adaptation to the anthropogenic influence realizes as resistance to almost all existing at the present moment insecticides (The database of arthropods…). It becomes obvious, that for control and regulation of the number of this pest not enough only to develop new insecticides or to select the resistant strains of potatoes (including transgenic strains).

Fig 1. Basic area of CPB in Russia. Solid blue line – boundary of area. By filling is shown the region of the propagation of the resistant to the insecticides populations (Pavlyushin et al., 2005).

It is known that the population is the unit of control both of economically "useful" species and "pests" (Yablokov, 1987; Altukhov, 2003). Consequently, it is necessary to study CPB at the population level, in the first place, for the purpose of monitoring the processes of adaptations forming (in particular - the development of resistance to the insecticides), and in the second place, for search and optimization of the methods of microevolutionary processes analysis in the concrete places of CPB inhabiting - under conditions of the South Urals in our case.

For evaluating the degree of polymorphism and study of the microevolutionary processes in the populations of CPB it is possible to use the phenetic methods, based at the analysis of the morphs frequency of imago (cover spots of pronotum, elytra, head, the abdomen), nervation of wings, pigmentation and the phenes of the cover spots of larva, pigmentation of eggs. Is assumed that the given signs are hereditary caused, in particular - cover spots of pronotum (Gritsenko *et al.*, 1998) and elytra (Ovtchinnikova *et al.*, 1982). This approach makes it possible to differentiate clearly the structure of the CPB populations in its area. In particular, in the territory of Eurasia S.R. Fasulati demonstrated (analyzing the morphs frequency of pronotum) seven population complexes associated to the basic agroclimatic zones (Methodical recommendations…, 1993; Vilkova *et al.*, 2005), which are biotaksonomically "... apparently, are close to the geographical races in K.M.Zavadsky's classification..." (Zavadskiy, 1967; Fasulati, 2002).

Thus, the purpose of this work was the analysis of the polymorphism of CPB in the South Urals (Republic Bashkortostan) with the applying of of phenetic analysis methods and the emerging of its formed at present population structure.

MATERIALS AND METHODS In the work were used the imago of CPB, collected in the territory Bashkortostan in 2002 - 2005 years. The places of collections in local populations were indicated in Table 1. For the phenetic analysis were used the phenes of vortex (Klimets, 1988; Benkovskaya *et al.*, 2006), back of the head (Benkovskaya *et al.*, 2006), pronotum (Fasulati, 1985) and elytra (Klimets, 1988; Benkovskaya *et al.*, 2006), in sum 22 variations.

The analysis of the intra-population variety, based on the phenetical structure of local populations, was conducted with the applying of two indices, proposed by L.A. Zhivotovskiy - average number of variations μ and the portion of the rare variations h:

$$\mu = \left(\sum_{1}^{m} \sqrt{p_m}\right)^2 \qquad h = 1 - \frac{\mu}{m}$$

where m is quantity of variations in the population, p - frequency of variation (Zhivotovskiy, 1982).

Estimation of the interpopulation differences. The index of the similarity among populations r was used for calculating the genetic interrelations between the local populations of CPB:

$$r = \left(\sum_{1}^{m} \sqrt{p_m q_m}\right)^2$$

where m - quantity of variations in population; pm, qm - frequency of variations in the compared populations (Zhivotovskiy, 1982).

The dendrograms of phenetical interrelations between the local populations of CPB were constructed by the values of index r with using the STATISTICA for Windows 5.0 (1984-1985, Statsoft, Inc.) software package. The clustering was performed by Ward's method, as a distance measure were used Euclidean distances.

Table 1. Collection of the samples of the CPB imago.

Region	The local population	N	Region	The local population	N
Tatyshlinskiy	1. Shulganovo	167	Beloretskiy	12. Mezhgorie	56
				13. Beloretsk	68
Askinskiy	2. Klyuchi	100	Burzyan-skiy	14. Starosub-khangulovo	100
Baltachevskiy	3. Staroyaksheyevo	200	Gafuriy-skiy	15.Tabynskoe	90
Salavatskiy	4. Maloyaz	100	Fedorov-skiy	16. Dedovo	60
	5. Nasibash	100			
Ilishevskiy	6. Yunny	88	Sterliba-shevskiy	17. Sterliba-shevo	98
				18. Aydarali	60
				19. Buzat	100
Kushnarenkovskiy	7. Sharipovo	60	Tuyma-zinskiy	20. Tuymazy	150
				21.Subkhan-kulovo	100
Ufimskiy	8. Dmitriyevka	70	Miyakin-skiy	22. Kirgiz-Miyaki	150
	9. Dema	65		23. Novy Mir	70
	10. Shemyak	100		24. Miyaki-Tamak	100
Karmaskalinskiy	11. Staromu-sino	70	Meleuzov-skiy	25. Zirgan	100

RESULTS AND DISCUSSION During the first stage of our investigation were analyzed the frequencies of the phenes of the cover spot patterns of four body counterparts - vortex, back of the head, pronotum and elytra. In this case, according to the results of phenetical analysis were calculated the matrices of the phenetic distances r between the samples from the local populations. Constructed according to these distances the dendrogram of phenetic interrelations is given in Fig. 2, to the left.

As a whole, according to the results of cluster analysis, in the Bashkortostan it is possible to isolate two large intra-population groups, which include: (a) local populations from the center of the examined territory and (b) local populations from the periphery of territory (Fig.2, to the right). These group of populations besides their geographical localization are differing by the level of intrapopulation variability. For the cluster (a) μ=3.628, h=0.347, for the cluster (b) μ=3.934, h=0.286.

It is possible that in the initial stage of the formation of the CPB population as basis for its structure differentiations served the first hotbeds of its appearance (Alsheevskiy, Al; Kumertauskiy, K, and Arkhangelskiy, Ar, regions), i.e., was exhibited the kind of the principle of founder. As the indirect confirmation of the influence of the principle of founder on further formation of population structure in South Urals can serve the fact that on the basis of the phenes frequencies comparison of cover spots of the samples number in our laboratory was already assumed about the settling of the larger part of the Bashkortostan territory by CPB from the southern (steppe) center of settling (Fig 2, to the right)

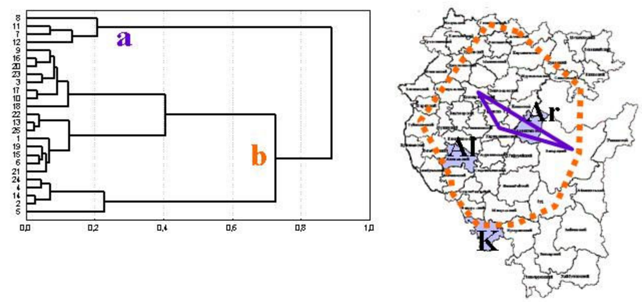

Fig.2. To the left - the dendrogram of the relations of local populations constructed by index *r* on the average value for four types of phenes. The numeration of local populations corresponds to the same in tabl.1. To the right - localization of the distinguished clusters in the Bashkortostan territory. Solid blue line - group (a), orange dotted line - group (b). Ar - Arkhangelskiy region, Al - Alsheevskiy, K - Kumertauskiy.

(Benkovskaya *et al*., 2000).

The effect of founder proved to be so strong that it not was levelled by the subsequent settling of CPB from the West and, possibly, it was intensified by the selective action of insecticidal press (unpublished data).

At the same time, according to the evolutionary concept of Wright (1951), the subdividing of population to a number of the local populations contributes to the maintenance of considerably larger genetic variability in comparison with the united panmictic population. It is considered that this variety makes possible for the subdivided population to react more effectively to the environmental changes and, being adapted to them, to change its genotypic structure (Kokhmanyuk, 1982).

From the onset of a CPB to the territory Bashkortostan it passed about 30 years. During this time in its population was formed the subdividing (on the basis of the adaptations of gene pool to the determined environmental conditions), that contributing to more effective reaction to environmental changes, in particular - to the anthropogenic influences in the form of insecticides applying.

Possibly, this was one of the mechanisms, which brought to the kind of "half-isolation" (in Wright - Altukhov terms), which is been one of the fundamental characteristics of the subdivided natural populations (Altukhov, 2003). Thus, data of the analysis of the phenetical structure of the examined samples of CPB allow to isolate in the Bashkortostan territory at least two forming intrapopulation groups. These groups we have named conditionally "central" (a) and "peripheric" (b). The presence of two groups of CPB determines the necessarity of the differentiation for plant protection schemes. The basis of this differentiation must be the requisite yearly monitoring of resistance and dynamics of phenetic composition in the populations of Colorado potato beetle.

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Resistance of Pollen Beetle (*Meligehes aeneus* F.) in Poland

ABSTRACT Chemical plant protection is still an indispensable method in effective oilseed rape protection against the Pollen beetle -(PB) (*Meligethes aeneus* F) in Poland. This species had developed strong resistance in this country against active substances of pyrethroid insecticides used to control it. Therefore the objective of the study was to determine the present susceptibility level of Polish populations of PB to main groups of insecticides recommended in Poland for PB control. Another aim was to determine mechanisms that can be responsible for PB resistance in Poland.

KEYWORDS *Meligethes aeneus*, Pollen beetle, insecticide resistance, pyrethroids, organophosphorous, neonicotinoids, oilseed rape, resistance mechanisms.

Resistance of PB has been a severe problem in Poland for a number of years (Lakocy 1967, 1977). The dynamic and widespread of PB resistance in Poland to pyrethroids insecticides and increase in tolerance to neonicotinoids and organophosphorous has created a need for resistance monitoring and elaborating the strategies for the management of PB resistance to all synthetic insecticides recommended in Poland. Nowadays a lot of consideration is taken to neonicotinoids because the importance of these class of insecticides for PB control in Poland systematically increases. Researches concerning actual susceptibility level of PB to selected active substances were performed in the Institute of Plant Protection in Poznan and provided important information on PB resistance to insecticides applied currently in Poland.

In our research, populations from all areas demonstrated high level of resistance to pyrethroid group and some level of resistance to neonicotinoid insecticide - acetamiprid. Survival at recommended concentrations in case of pyrethroids and neonicotinoids indicated occurrence of resistance to these active substances in examined populations. In laboratory studies (2003 - 2006) pyrethroid insecticides examinated in recommended doses were less effective in controlling PB. (Wegorek 2004, Wegorek and Zamoyska 2005). The results of insecticide susceptibility monitoring indicated that PB beetles do not show resistance to chlorpyrifos. The results concerning chlorpyrifos indicated that PB populations tolerant to pyrethroids and neonicotinoids were positive cross-resistant to this active substance. Resistance mechanisms were detected using piperonyl butoxid, tributylinacetate and carbaryl as synergists. High synergism of pyrethroid insecticides with piperonyl butoxid (oxidative metabolism) and low synergism with carbaryl (hydrolytic mechanism) and tributylinacetate (penetration factor) showed that within these three - the main mechanism of PB resistance can be oxidative metabolism. The analise of mitochondrial cytochrome oxidase (I and II subunits) nucleotide sequence by using PCR and sequencing, as an example of an oxidative enzyme indicate occurrence of many substitution in mtCOI and mtCOII sequences when compared with sequences alredy deposited in GenBank. The existing substitutions in both subunits DNA sequences entail amino acid substitution that can change secondary structure of those peptides. However, those substitutions can result from variability in populations that are geographically isolated.

CONCLUSIONS PB is an example of insect species that can develop strong resistance mechanisms to most active substances used to control it in Europe (Hansen 2003, Heimbach at all.2006, Lakocy 1967, 1973, Wegorek 2005). In the case of studied pyrethroids and neonicotinoids, the survival of PB beetles in laboratory tests achieved very high level at doses higher than those commercially recommended. The decrease of PB susceptibility to examined active substances indicates the decline of chemical treatments efficiency under field conditions and points at selection of populations that metabolise high doses of insecticides faster and more effectively. Thus, continuous and widespread use of pyrethroids and neonicotinoids in Poland can lead to control failure. PB strains were still excellently controlled with organophosphorous (chloropirifos) insecticides. The resistance mechanism of surviving beetles using pyrethroids active substance and acetamiprid is caused mainly by enhanced oxidative metabolism (high synergism of pyrethroid insecticides with piperonyl butoxid and low with carbaryl and tributylin acetate - although some symptoms point at insensibility of nervous tissue in the place of biological activity of active substance. Positive cross resistance to chlorpyrifos is probably due to the creation of the very toxic metabolite of this active substance under the influence of oxidative enzyme action. The metabolite (oxidative analogue of chlorpyrifos) is very strong toxin for PB and other insects species (Rozanski 1992). However this conclusion requires further research.

Considering the possibility of high occurring PB resistance, it is necessary and advisable to control application of insecticides rationally, and to take into account their association with different chemical groups and mechanisms of resistance.

Only systematic monitoring of susceptibility of PB strains might ensure more effective protection of oilseed rape crops in Poland. Therefore one should take into consideration the necessity of PB susceptibility monitoring by regional plant protection services according to the method developed by the Institute of Plant Protection in Poznan in the year 2003.

Studies on mechanisms of PB resistance to active substances are very useful because they will allow to enterprise a better strategy for delaying PB resistance. Problems with PB resistance occur also in Germany, Sweden, Denmark and Estonia and France.

Presently the registration authorities must take into consideration the resistance risk analysis based on EPPO standards and recommendations (EPPO 2001). So during the registrative investigations, resistance phenomenon of agrophages must be seriously taken into consideration. Also chemical companies should take responsibility for constant control of efficacy of their products and update them if needed in accordance with new results from susceptibility monitoring. The future of chemical protection of rape plants against PB in Poland will be dependent on many factors, among which the resistance of this species will be one of the most important. The results suggest that at present the situation of PB resistance limitations in use of pyrethroid and neonicotinoid group is indispensable in Poland. Also, rational application of all recommended insecticides and their rotation including different modes of their molecular action is necessary. Oversensitiveness of PB to chlorpyrifos (Table 1.) should be used in recommendations and future practical strategies of rape protection in Poland.

Table 1. Susceptibility level of pyrethroid resistant Pollen beetle population (Winna Gora - Poland) to some active substances of pyrethroids, noenicotinoids and organophosphorous insecticides (2006).

Chemical group	Active substance	Recommended concentration (%)	LC95 concentration (%)
Pyrethroids	zeta-cypermethrin	0,005	0,021
	esfenvalerate	0,012	0,035
	bifenthrin	0,005	0,008
Neonicotinoids	acetamiprid	0,012	0,019
Organophosphorous	chlorpyrifos	0,144	0,00017
	fenitrothion	-	0,00064
	dimethoate	-	78,412
	phosalone	0,35	0,907
	malathion	-	0,079

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Estimation of resistance in *Helicoverpa armigera* (Hubner) to carbaryl and its effect on biology

ABSTRACT Application of sub-lethal concentrations of carbaryl to the 3rd instar larvae of *Helicoverpa armigera* up to five generations registered survival at the highest concentration in each successive generation after exposure. The resistance ratio increased up to 4.44-fold as compared to first generation. Out of seven biological parameters studied for carbaryl-selected, parental and susceptible strains, three parameters viz. oviposition period, fecundity and female adult longevity were significantly affected.

KEYWORDS Resistance, Carbaryl, *Helicoverpa armigera*, Biology

Helicoverpa armigera (Hubner) (Lepidoptera: Noctuidae) is charismatic and one of the most dominant insect pests in agriculture, accounting for the consumption of over 55 percent of total insecticides used in India (Puri,1995).The problem of this pest is magnified due to its direct attack on fruiting structures, voracious feeding habits, high mobility and fecundity, multivoltine, overlapping generations with facultative diapause, nocturnal behaviour, migration, host selection by learning and propensity for acquiring resistance against wide range of insecticides (Satpute and Sarode,1995; Sarode,1999). Loses solely due to this pest up to Rs. 10,000 million have been reported in crops like cotton, pigeon pea, groundnut, sorghum, pearl millet, tomato and other crops of economic importance (Raheja, 1996).

H. armigera has assumed such proportions in India that for the past decade, farmers and plant protection agencies of Central and State governments have virtually become perplexed regarding its control which ultimately has lead to an array of social, economical and political problems. Of these, the major concern is the development of resistance in this pest to almost all known insecticides. *H. armigera* has, thus, been documented as the best example holding multiple resistant mechanism as well as cross resistance characters attributed to repeated exposures for longer periods to a succession of insecticide groups (Horowitz and Denholm, 2000).Therefore, it became necessary to assess the potential of development of resistance to a widely recommended carbamate insecticide i.e. carbaryl, besides evaluating the comparative fitness costs of this pest.

MATERIALS AND METHODS **Rearing of test insect:** A mass culture of *H. armigera* was initiated by collecting about 200 larvae from the farmer's fields in and around Jammu in scintillation vials and brought to the laboratory and maintained at 26± 2º C in a BOD. The larvae were provided with the leaves of *Rumex* sp. to feed upon till they attained pre-pupal stage. After the emergence of adults, one generation of the pest was reared on artificial diet (Singh and Rembold, 1992) prior to segregation. After multiplying the culture in the laboratory for two successive generations, the whole stock was divided into two lots. One lot was marked as parental stock where as the other was used for exposure to carbaryl. Hundred pupae of susceptible strain of *H. armigera* were also procured from the Division of Entomology, IARI, New Delhi. These pupae were also reared till the emergence of adults and thus the culture was further multiplied to be used in the bioassay studies with the same method as that of field collected *H. armigera*.

Preparation of insecticidal concentrations: The proprietary product of carbaryl was used to prepare one percent stock solution in acetone from which further dilutions were prepared subsequently.

Bioassay and Laboratory Selection: Sub-lethal concentrations of insecticide prepared based on the recommended concentration of insecticide as per package and practices of SKUAST-J for the control of *H.armigera* (Simwat, 1994) were applied to the thoracic dorsum of each 3rd instar larva of susceptible as well as field collected strain of the test insect separately with the help of a Merck micropipette @ 1.0µl per larva. Six to seven concentrations of carbaryl were utilized for exposure in each generation. Besides, a set of control (with acetone only) was also maintained with each exposure to work out the correct mortalities. Ten larvae per replicate were treated with one concentration and three replications were maintained for each concentration. The treated larvae were housed in the Petri plates (9 cm diameter) individually and were kept in B.O.D. incubator (26 ± 2°C; 12: 12 L/D period). The mortality data was recorded 24 hours after the treatment. The survivals obtained at higher concentrations were shifted to clean rearing trays consisting of hundred cells each and provided with fresh artificial diet until pupation. The progeny of the first surviving lot was termed as F_1 generation. The exposures and the selections were conducted subsequently up to 5 generations. The parental and susceptible strains were also maintained through out the period of study without any exposure to insecticide to observe the biological parameters.

Quantification of insecticidal resistance: The degree of development of resistance through different generations was determined by working out LC_{50} values in each generation by computer aided statistical programme SPSS 10.0 and computing the resistance ratio. The resistance ratio for any generation was worked out by dividing LC_{50} for that generation with LC_{50} value of the parental strain. The observations on larval mortality were recorded by considering the larvae as moribund when prodded with a fine camel hair brush.

Biological studies: The newly emerged moths from the carbaryl-selected, parental, and susceptible strains were desexed so as to get at least 10 adults of both the sexes. The desexing was done on the basis of morphological characters. These moths were kept in separate glass jars (15 cm diameter) covered with muslin cloth and provided with cotton swab soaked in 10 percent sucrose solution to serve as food to initiate the studies on the various biological and developmental attributes of carbaryl-selected, parental and susceptible strains. Statistical analysis of the data: The mortality data was analysed to work out LC_{50} value at 95 percent confidence interval by SPSS 10.0. The data on the various parameters of the biology of the insecticide selected, parental and susceptible strains were subjected to analysis of variance with Tukey's HSD test.

RESULTS AND DISCUSSION Third instar larvae of *H. armigera* were selected for five generations to various discriminating concentrations to obtain maximum mortality at the highest concentration in each generation. The minimum survival of *H. armigera* larvae obtained in the 1st generation at 0.35 percent concentration (Table 1) was 16.67 percent among all the concentrations used. Similarly, the lowest survival of 16.67, 12.00, 12.00 and 10.00 percent were obtained in the subsequent bioassays up to 5th generation, respectively.

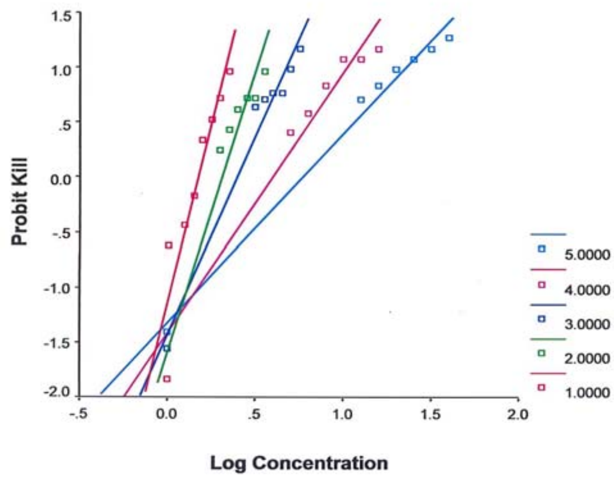
Table 1. Selection of residual population of *Helicoverpa armigera* in various generations exposed to carbaryl.

Generation	Concentrations used %	Percent mortality	Residual population
F1	0.005	26.66	X
	0.1	33.33	X
	0.15	43.33	X
	0.2	63.33	X
	0.25	70	X
	0.3	76.66	X
	0.35	83.33	**(v)**
F2	0.3	60	X
	0.35	66.67	X
	0.4	73.33	X
	0.45	76.67	X
	0.5	76067	X
	0.55	83.33	**(v)**
F3	0.5	74	X
	0.55	76	X
	0.6	78	X
	0.65	78	X
	0.7	84	X
	0.75	88	**(v)**
F4	0.7	66	X
	0.8	72	X
	0.9	80	X
	1	86	X
	1.1	86	X
	1.2	88	**(v)**
F5	1.1	76	X
	1.2	80	X
	1.3	84	X
	1.4	86	X
	1.5	88	X
	1.6	90	**(v)**

Rejected (X) Selected (v)

After subjecting the data to probit analysis, the results obtained with the Pearson Goodness of Fit, chi square values revealed that heterogeneity in the population of the test insect was significant ($p > 0.05$) in the 1st generation which on the other hand, gave homogenous response of the population of H. armigera in the successive generations ($p < 0.05$) as was evident from the observed and expected responses. The regression lines in the 1st generation (slope 5.99±0.16) were significantly different from the other log concentration - mortality lines obtained in the 2nd to 5th generation (Fig. 1). The percent LC_{50} value (Table 2) recorded in the 1st generation was 0.17064 which subsequently increased to 0.75796 in the 5th generation and the insect developed 4.44 -fold resistance in the 5th generation as compared to 1st generation. It clearly indicated that the *H. armigera* has propensity to develop more resistance to carbaryl used on various vegetable crops in Jammu.

Figure 1. Dose-mortality regression lines of carbaryl against *Helicoverpa armigera* for five generations



Though several workers (Lal, 1998; Patel and Koshiya, 1999; Kapoor *et al.*, 2000) have come out with the report of development of resistance to carbaryl in *H. armigera*, yet the information is scanty on generation-wise study on the potential of development of resistance in this pest. However, it could be inferred that the potential of resistance development to carbaryl is extremely high in Jammu strain of *H. armigera* and any increase in the use of this insecticide for the control of this pest in field may lead to future control failures. Though the information in development of resistance to carbaryl in *H. armigera* is available from the field collected strains, yet no systematic study has been conducted so far to detect the resistance level in the laboratory bred generations. However, Ahmad and McCaffery (1988) observed 28-fold resistance in Thailand strain of H. armigera to carbaryl. Armes *et al.* (1992) advocated that up to 80 percent carbaryl consumption on cotton was responsible for the multiple cross resistance in this pest to carbamates. 3.36 to 4.83-fold (Satyavani *et al.* 1991) and 4.83-fold (Mehta *et al.*, 1992) resistance to carbaryl corroborate the findings of the present study. An increase in the resistance level of *H. armigera* to carbaryl (9.10-fold) had been detected by Patel and Koshiya (1999) in Gujarat which increased to more than 10-fold as observed by Patel *et al.* (2000). The differential resistance reactions at geographically different places could be attributed to repeated applications of the same insecticides and/or

Table 2. Toxicity of Carbaryl to third instar larvae of *Helicoverpa armigera* in different generations.

Generation	Heterogeneity X2(n-1)	Regression equation	Slope± S.E.	Fiducial limits*	LC50 (%)	Resistance ratio
I	χ^2 (6) = 6.65	Y= -1.02254+5.99218x	5.99±0.16	0.14051	0.17064	1
II	χ^2 (5) = 4.343	Y= -1.34905+4.60579x	4.60±0.28	0.2929	0.2929	1.72
III	χ^2 (5) = 3.629	Y= -1.36230+3.52565x	3.52±0.24	0.3864	0.3864	2.26
IV	χ^2 (5) = 3.465	Y= -1.34070+2.32081x	2.32±0.22	0.57769	0.57769	3.38
V	χ^2 (5) = 1.472bb	Y= -1.30356+1.71982x	1.71±0.23	0.75796	0.75796	4.44

* at 95% confidence interval

mixing of the different groups of insecticides and/or the quality of the insecticides used (Fakrudin *et al*., 2003).

COMPARATIVE BIOLOGY Out of seven biological parameters, only three parameters, viz., oviposition period, fecundity and adult-female longevity (Fig.2) had been adversely affected in the carbaryl-selected *H. armigera*. On the other hand, no significant differences in other parameters were observed in all the three strains of test insect.

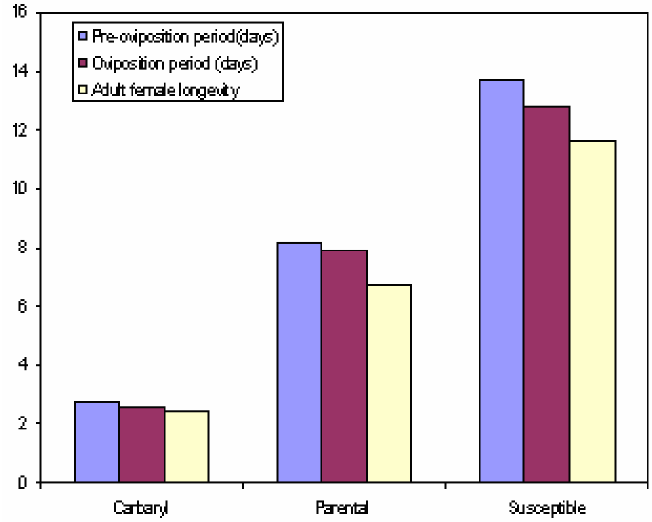


Figure 2. Differential biological attributes among various strains of *Helicoverpa armigera*

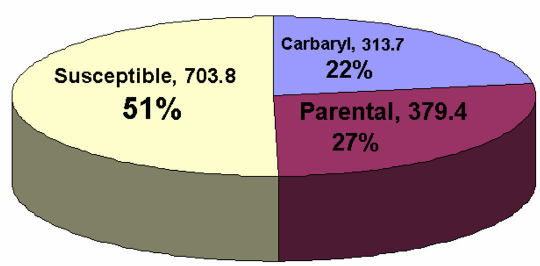
Oviposition period: The mean oviposition period of carbaryl-selected and parental strains was 8.15 ± 0.35 and 7.92 ± 0.36 days, respectively, whereas the corresponding figure for susceptible strain was 6.74 ± 0.35 (Table 3).Both the carbaryl-selected and parental strains were significantly different from susceptible strain of *Helicoverpa armigera*.

So far, the literature lacks any report regarding the effect of carbaryl selection on this parameter. However, the differences in the oviposition period of *H.armigera* reported by Dong *et al*. (1996) and Xia *et al*. (2001) have been attributed probably to the reproductive disadvantages in the resistant female moths caused due to pesticide selection.

Fecundity: The average fecundity of carbaryl selected and parental strains were 313.7 (305-320) and 379.4, respectively (Table 3), whereas, the corresponding values for the susceptible strain was 703.8 (670-719). It is evident from the data that the selection pressure of carbaryl resulted in the considerable reduction of egg laying capacity of *H. armigera* as compared to susceptible strain. The number of eggs laid by the females of carbaryl selected strain significantly different from that of the parental strain (Fig.3) which revealed that the selection with carbaryl for five generations had ostentatious effect on the fecundity of this insect. Campanhola (1988) recorded a significant reduction in the fecundity of resistant females of *H.armigera* who produced 1200 eggs each as compared to 2500 eggs produced by one susceptible female. It was reported that the reduced fecundity was the consequence of metabolic resistance to insecticides. Further, it was advocated by various workers (Forrester *et al*., 1993; Glenn *et al*., 1994) that the females of this pest have relatively higher tendency for the development of resistance to insecticides. Recently, these observations got further strengthening by striking differences observed on the effective fecundity of resistant females of this pest (Xia *et al*., 2001) which was found decreased significantly. The results obtained in the present findings are in accordance with the results of these workers.

Adult longevity: The average female longevity of the carbaryl-selected, parental and susceptible strains was 13.70 ± 0.76, 12.77± 0.78 and 11.61± 0.55 days, respectively (Table 3). The significant difference in the female longevity exhibited by carbaryl-selected, parental and susceptible strains of *H.armigera* might have occurred due to variations in the rearing conditions in the laboratory. Since no information is available in literature on this aspect, it is suggested that repetitive work is required to be undertaken to authenticate the data.

Figure 3. Differential Fecundity among various strains of *Helicoverpa armigera*



ACKNOWLEDGEMENT Authors are thankful to Dr.R.M.Bhagat, Professor & Head, Division of Entomology, SKUAST-Jammu for providing facilities to conduct the present work.

Table 3. Biological attributes of insecticides-selected, parental and susceptible strains of *Helicoverpa armigera.*

Strain Selected	Pre-oviposition Period (days)	Oviposition Period (days)	Post-oviposition Period (days)	Fecundity (no. of eggs)	Incubation Period (days)	Hatchability (% eggs hatched)	Adult Longevity (days) Male	Female
Carbaryl	2.75±0.52ab (2.4 -3.2)	8.15±0.35c (8.0-8.3)	2.80±0.70 (1.7- 3.4)	313.7±2.30c (305- 320)	4.01±0.80 (3.2- 4.9)	91.8±1.37 (89.0-95.0)	10.19±0.66 (9.1-12.0)	13.70±0.76cd (12.9-14.3)
Parental	2.54±0.50ab (2.0-2.8)	7.92±0.36ab (7.7-8.5)	2.31±0.68 (1.6-3.0)	379.4±2.85b (370-394)	4.16±0.75 (3.0-4.0)	93.8±1.18 (92.0-96.0)	10.68±0.88 (9.0-11.2)	12.77±0.78b (11.6-13.9)
Susceptible	2.43±0.43a (2.0-2.7)	6.74±0.35a (6.6-7.0)	2044±0.60 (1.6-2.8)	703.8±4.00 (670-719)	3.90±0.73 (3.1-4.7)	93.9±1.20 (92.0-96.0)	9.38±0.62 (8.9-10.0)	11.61±0.55a (10.9-12.1)
	NS		NS		NS	NS	NS	

Mean ± S.E.(m) of 10 pairs / individuals

Figures in parenthesis represent range

Mean followed by the same letter in a column are not significantly different (p = 0.05), Turkey multiple comparison test (HSDa)

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Research in Resistance Management

Susceptibility of Canadian Colorado potato beetle populations to imidacloprid and metaflumizone, 2006

INTRODUCTION The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say), is a major pest of potato and one of the most challenging agricultural insect pests in terms of insecticide resistance, having developed resistance to at least 49 different active ingredients and all major insecticide classes (Whalon *et al*. 2006). Since its registration in 1995, imidacloprid (Admire® 240F, Bayer CropScience) has been the insecticide of choice for CPB management throughout most of North America. Even today it continues to provide good control for the vast majority of growers. Nonetheless, imidacloprid resistance, or its onset, has been reported (Mota-Sanchez *et al.* 2000, Olson et al. 2000, Tolman *et al.* 2005, Mota-Sanchez *et al.* 2006).

A Canadian survey of CPB susceptibility to imidacloprid was last conducted in 2003 (Tolman *et al.* 2005). At that time, low levels of tolerance were found but control failures in the field were not reported by growers. Since 2003, however, some Canadian growers have reported reduced efficacy of imidacloprid and concerns of future imidacloprid resistance have risen. The present study updates the current status of imidacloprid resistance in selected Canadian CPB populations from 4 provinces. Several populations were included for study based on reports by growers of reduced imidacloprid efficacy. We also document susceptibility of some of these populations to metaflumizone (BAS 320I, BASF), a novel semicarbazone insecticide with activity against key coleopteran and lepidopteran pests. Metaflumizone has favorable toxicological and environmental profiles, has exhibited low impact on beneficial arthropods (natural enemies and pollinators), and has been designated a Reduced Risk Candidate by the US EPA.

MATERIALS AND METHODS **Insects:** An insecticide-susceptible CPB strain reared for over 80 generations on potato at the Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada (London, Ontario) was used as the reference strain. Fourteen field populations from four Canadian provinces - Prince Edward Island (3), Quebec (3), Ontario (6), and Manitoba (2) - were collected by research or extension personnel using standardized collection and shipping kits, forwarded to collectors several weeks before CPB adults emerged from diapause. Collections of CPB adults were sent via overnight courier to the University of Guelph. Upon receipt, 30-35 adults each were promptly transferred to oviposition cages containing potted potato plants, and held in a rearing room at 25º C and 16:8 [L:D]. Three times per week egg masses from each population were excised from plants, placed in labeled Petri dishes, and transferred to an incubator at 25º C, 16:8 [L:D], or to an incubator at 12ºC, 16:8 [L:D], if slowed development was required to synchronize hatch. Bioassays were conducted on 12-24 h old first instars from the laboratory F1 generation of each field collection.

Chemicals: Formulated imidacloprid (Admire® 240F, 240 g AI L^{-1}) was supplied by Bayer CropScience Canada Inc. (Calgary, Alberta, Canada). Formulated metaflumizone (BAS 320I, 220 g AI L^{-1}) was supplied by BASF Canada Inc. (Mississauga, Ontario, Canada).

Bioassays: Residual leaf-dip bioassays were conducted to determine the susceptibility of CPB populations to imidacloprid (14 field populations) and metaflumizone (3 field populations). Insecticides were each suspended in deionized water to give stock solutions of 1000 ppm. Serial dilutions were subsequently prepared to give concentrations ranging 0.1-300 ppm. As determined in preliminary tests, concentrations that caused 5-95% mortality were used in bioassays. Using a stainless steel cork borer, 1.5 cm diameter discs were cut from potato leaves harvested from potato plants grown in an insecticide-free greenhouse. Discs were dipped in insecticide solution for approximately 4 s and placed on a wire rack to air dry. Two dry discs were each placed on a Whatman No. 1 filter paper (1.5 cm diameter) in a well of a 32-well bioassay tray (Oliver Products Company, Grand Rapids, MI). Five first instars of a given population were transferred to each well using a clean fine-haired paintbrush. When all wells contained larvae, bioassay trays were sealed with a thin transparent polyester plastic using 3M Spray Mount™ (3M, London, Ontario, Canada), labeled and transferred to a holding room (25º C, 16:8 [L:D]). After 48 h the number of dead larvae per well was recorded.

For each insecticide and CPB population, at least 3 bioassays (one bioassay was one tray) were conducted, each on a separate day. Each bioassay consisted of at least 7 concentrations with 3 replicates per concentration. Concentration-mortality regression lines were generated for each population using PROC PROBIT (SAS Institute 2001). Concentrations lethal to 50 percent of the CPB larvae (LC_{50}), 95% confidence limits, regression line slopes and chi-squared goodness-of-fit test results are reported.

RESULTS AND DISCUSSION Limited utility of alternative control options for growers dictates heavy reliance on chemical insecticides to control CPB. For most of the past decade growers have effectively managed CPB with foliar or in-furrow/seed treatment applications of imidacloprid. And while imidacloprid continues to provide good CPB control for most growers, imidacloprid resistant CPB populations have developed in the United States (Zhao *et al.* 2000, Mota-Sanchez *et al*. 2006) and grower accounts of reduced efficacy have been reported in Ontario and Quebec (A. Dornan, personal communication)Field Development Representative, Bayer CropScience, Rockwood, Ontario, Canada. The present study therefore sought to determine the current status of imidacloprid susceptibility in selected Canadian CPB populations. We also assessed the susceptibility of several of these populations to metaflumizone to determine cross-resistance potential with imidacloprid-resistant beetles.

CPB populations were highly variable in their susceptibility to imidacloprid (Table 1). Whereas the LC_{50} of several populations was less than 4-fold that of the insecticide-susceptible strain, other populations displayed decreased susceptibility to imidacloprid with LC_{50} values up to 22-fold that of the insecticide-susceptible strain, indicating hotspots where future control failures are foreseeable. Most populations from Prince Edward Island and Ontario were quite susceptible to imidacloprid, although some were tolerant of lower concentrations. Ontario populations ON4 and ON5 were from farms where growers reported decreased CPB sensitivity to imidacloprid or shorter-lived residual activity compared to previous years. As expected, these populations had LC_{50} values significantly higher than the insecticide-susceptible population and the other Ontario populations tested. All tested populations from Quebec exhibited greatly reduced sensitivity to imidacloprid compared to the laboratory-susceptible strain. Conversely, both populations from Manitoba were highly susceptible (Table 1). These types of variable responses are not unusual since rather than resistance evolving in isolated areas and spreading geographically, discrete CPB populations respond to local selection pressures (Bishop and Grafius 1996). While resistance in CPB is most strongly correlated with history of insecticide application (Roush *et al.* 1990, Tisler and Zehnder 1990, Dively *et al.* 1992, Huang *et al.* 1995), crop rotation is also thought to be crucial for effective, long-term management (Wright 1984, Bishop and Grafius 1996). Indeed, populations with the lowest susceptibilities to imidacloprid in the present study were generally from farms practicing inadequate crop rotation (A. Dornan, personal communication)Manager, Research & Commercial Development, BASF Canada, Inc., Mississauga, Ontario, Canada.

CPB field populations displaying up to 13-fold reduced susceptibility to imidacloprid were very susceptible to metaflumizone (Table 2). This was not unexpected given the unique mode of action of metaflumizone. Unfortunately bioassays with metaflumizone were possible only near the end of the summer, limiting the number of populations tested. Nonetheless, along with field trials confirming good efficacy against CPB (W. Barton, personal communication), the lack of cross-resistance with imidacloprid suggests metaflumizone could be an effective control and resistance management option for potato growers. The biocide spinosad and chitin synthesis inhibitor novaluron are also recently developed compounds with insecticidal activity against CPB and modes of action different from that of neonicotinoids. While cross-resistance and future resistance development to these compounds is of some concern (Cutler *et al.* 2005, Tolman *et al.* 2005, Mota-Sanchez *et al.* 2006), along with metaflumizone they constitute a suite of reduced-risk alternatives that should be very useful in CPB resistance management programs.

CPB continues to be a formidable challenge for insecticide resistance management researchers. Work in the United States indicates that CPB tolerance or resistance to imidacloprid is becoming widespread and that alternation with more recently developed neonicotinoids, such as thiamethoxam, is likely to be a inadequate long-term pest and resistance management strategy (Mota-Sanchez et al. 2006). Although imidacloprid continues to provide good CPB control for most Canadian potato growers, the present study highlights the potential for future control failures if they rely solely on imidacloprid for CPB management. Our work also demonstrates that metaflumizone may be an effective option for growers encountering imidacloprid-resistant beetles.

Table 1. Susceptibility of Colorado potato beetle (CPB) first instar larvae from populations originating from several Canadian provinces (PE=Prince Edward Island; NB=New Brunswick; QC=Quebec; ON=Ontario; MB=Manitoba) to imidacloprid (Admire 240F).

Population	*n*	Region (County)	LC50 (95% C.L.)	Slope	c^2	Tolerance Ratio[a]
Lab Susceptible	558	--	1.40 (1.15-1.70)	1.66	11.28	1
PE1	424	Queens	2.55 (2.06-3.09)	1.82	3.4	1.82
PE2	526	Prince/Tryon	2.10 (1.62-2.60)	1.31	5.89	1.5
PE3	393	Prince/Brae	7.19 (5.89-8.57)	2	9.13	5.13
QC1	584	Pont-Rouge	18.47 (12.64-28.25)	1.79	15.49	13.19
QC2	345	St-Thomas-de-Joliette	20.39 (17.04-25.74)	2.45	2.04	14.56
QC3	388	St-Thomas-de-Joliette	30.38 (20.43-50.16)	1.4	14.68	21.7
ON1a[b]	709	Lambton Shores	4.61 (3.74-5.83)	1.25	7.95	3.3
ON1b[b]	685	Lambton Shores	4.41 (2.58-9.04)	1.31	20.37[c]	3.15
ON2	559	Everett	3.48 (2.87-4.23)	1.4	9.11	2.49
ON3	545	Central Elgin	3.60 (2.01-7.48)	1.13	14.10[c]	2.57
ON4	999	Burford	12.67 (10.26-15.66)	0.96	8.96	9.05
ON5	417	Dexter	17.87 (14.75-21.95)	1.8	3.26	12.76
MB1	428	Winkler	2.12 (1.67-2.61)	1.66	6.57	1.51
MB2	542	Portage la Prairie	3.45 (1.77-5.93)	1.69	17.19[c]	2.46

[a] Tolerance ratio equals LC50 of field population/LC50 lab susceptible population.
[b] 'a' and 'b' constitute collections from separate fields on the same farm.
[c] Significant at α=0.05.

ACKNOWLEDGEMENTS We thank J. Whistlecraft (Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, London, Ontario) for the insecticide-susceptible CPB stain, as well as the numerous growers and government/extension personnel for CPB collections. We also thank Bayer CropScience Canada and BASF Canada Inc. for their contributions to this research.

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Table 2. Susceptibility of Colorado potato beetle (CPB) first instar larvae from populations originating from several Canadian provinces (PE=Prince Edward Island; QC=Quebec; ON=Ontario) to metaflumizone (BAS 320I).

Population	*n*	Region (County)	LC50 (95% C.L.)	Slope	c^2	Tolerance Ratio[a]
Lab Susceptible	547	--	6.10 (5.06-7.15)	2.34	11.55	1
PE3	436	Prince/Brae	4.50 (3.09-5.92)	1.46	3.88	0.74
ON4	401	Burford	8.80 (7.73-10.08)	3.22	2.33	1.44
ON5	524	Dexter	6.36 (4.91-7.94)	1.63	11.97	1.04

[a] Tolerance ratio equals LC50 of field population/LC50 lab susceptible population.

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Resistance Management News

Politics and Pesticides Resistant Managements in Iraq

Pesticide use has increased tremendously out of precise pest's identification and economic threshold determination in Iraq in the last three years. The Iraqi farmers buy only the cheap, broad spectrum insecticides due to their exhausted economic situation. They use high dosages and repeat applications many times in order to get a certain level of pest control and besides that, they depend on either their own strive or advice from the uneducated people who work in agricultural shops.

Cypermethrin and Alpha-Cypermethrin have become the dominant insecticides used by the farmers in Iraq. The dealer or wholesaler imports over 500-1000 tons of these insecticides yearly from different cheap origins (India, China, Egypt, Jordan, Lebanon, Turkey ...etc) and they are very bad quality. These insecticides have been used to control plant and animal pests. The misuse of these two pyrethroid insecticides and others damage the agro-ecosystem completely by killing the biological control agents, (predators and parasites) and establishing resistant strains of crop pests and also cross resistant to another insecticides chemical group. The country of origin of these insecticides, lack of quality control measures, high level of impurities, solvents used (xylen mainly), besides the frequent application of these insecticides on daily consumed crops such as tomato, cucumber, pepper, potato and leafy vegetables encourage increasing cancer cases in the area where these insecticides have been used and this was mentioned by the MOH without referring to these insecticides. The misleading label information which are designed by the wholesalers and retailers, the pesticide mixtures prepared by retailers, in addition to cheating these pesticides either by transfering the contents of the cheap products to another container belonging to well-known companies or importing insecticides that are less concentrated than what is mentioned on the label are the main characteristics of the Iraqi pesticides market.

The farmers, consumers, and the operators are the victims of this kind of chaos and mess which we face daily in Iraq.

The case I mention above was only in the years after the falling of the ex-regime (after April 2003) and when our borders are opened to both legal and illegal materials introduction to Iraq (Hi bye borders).The National Committee For Pesticides Registration and Approval (NCFPRA) had very strict laws which controled import of any pesticides entering the country and also put a very good quarantine before April 2003.

I am writing such a theme to show the damaging influence of politics on the agro-ecosystem and natural balance of pests in Iraq and in the meantime raise this problem to the scientists who are interested in such resistant dilemma which we faced three years ago in Iraq, asking for their suggestions and solutions. I am ready to answer any related questions that can help the farmers and consumers overcome this tragic story.

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Abstracts

Horseweed (*Conyza Canadensis*) Has Evolved in Glyphosate Resistance in China

ABSTACT Horseweed (*Conyza canadensis*), an invasive alien weed, is one of main weeds in orchards in China. Glyphosate has been used for the controlling annual and perennial weeds in orchards for more than 25 years in China. We firstly report that horseweed has evolved in glyphosate resistance in China. The seeds of 45 populations were collected from different orchards with different glyphosate application history. The pot seedlings with 5-7 leaves were treated with glyphosate dosage at 35, 70, 140 280, 560, 1120, 2240, 4480 and 8960 (ai)g ha^{-1}. The dosage dependence response curve of each population was set up by Log-logistic dose response regression equation. The ED_{50} value of each population was calculated and compared with 10 typical susceptible and resistant populations from America. The results showed that the different population had different glyphosate-resistant level which increased with prolonging of glyphosate application years. Two populations with the highest resistance level at 8.28 and 7.95 fold were found in Ningpo, Zhejiang Province where glyphosate was ever applied twice a year for more than fifteen years.

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Symposia

RESISTANCE 2007: An International Conference

16-18 April, 2007
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This major international conference, the fifth in an ongoing series, will review the latest research on the origins, nature, development and prevention of resistance to insecticides, fungicides and herbicides. It will provide a forum for researchers, consultants, regulators and industrialists to present and discuss approaches to overcoming this increasingly important constraint to effective crop protection.

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The 1st international electronic scientific biennale Colorado potato beetle-2007

Laboratory of biochemistry of insect adaptability
Institute of biochemistry and genetics of Ufa Scientific center, RAS

The Institute of biochemistry and genetics will hold in 2007 the 1st international electronic scientific biennale Colorado potato beetle-2007 (Ufa, 2007, fall) publishing the full texts of materials in the electronic and reprinting version.

The conference will review the following themes:
1. Resistance of Colorado potato beetle to insecticides
2. Mechanisms of insecticide resistance
3. Resistance of potato varietes (including transgenic)
4. Colorado potato beetle ecology and biology
5. Natural enemies and diseases of Colorado potato beetle
6. Population structure of Colorado potato beetle
7. Phylogeny of Subfamily Chrysomelinae

If you consider to include other themes, we`ll be glad to discuss your proposals.

The conference language - Russian and English.

Interested parties should contact
Maksim B. Udalov: udalov-m@yandex.ru

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The next two **submission deadlines** are:

Monday, March 19, 2007
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