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. 14, No. 2 (Spring 2005)

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Resistance Management from Around the Globe

Arthropod Resistance

Response of *Helicoverpa armigera* (Hübner) to different insecticides: a brief account

The ability of *Helicoverpa armigera* to adapt on the diverse cropping systems renders this as a very serious pest (Zalucki *et al*., 1986). The estimated yield losses vary with the crop and the effect on yield depends on pest density as well as timing of the infestation. Insecticides are being used primarily to control this pest. However, during the last few years, there have been reports regarding the ineffectiveness of different insecticides against this pest. Several workers have reported the development of resistance in this pest to several insecticides in various parts of the world, including India. A brief review of literature on the responses of larval & egg stages of *H. armigera* to different insecticides particularly with respect to development of resistance and/or increased tolerance to them from various parts of the world is given below.

DIFFERENTIAL SUSCEPTIBILITY OF LARVAE TO INSECTICIDES Larvae of *H. armigera* are the most damaging stage of this pest attacking almost all the field crops. Continuous and excessive use of insecticides has resulted into increased tolerance and/or development of resistance in this pest, against various groups of insecticides throughout the world.

The Annual Report of Queensland Department of Primary Industries for 1982-83 for the first time highlighted the development of resistance to synthetic pyrethroid insecticides in *H. armigera* in Emrald Irrigation Area, Australia from cotton and soybean fields (Queensland Department of Primary Industries, 1983). Studies under laboratory conditions on a strain of *H. armigera* from Australia showed that 3rd-instar larvae at discriminating concentrations exhibited 15-, 20-30- and 20- fold resistance to cypermethrin, deltamethrin and fenvalerate, respectively. Resistance to fenvalerate was found to be as high as 50-fold at double concentrations (Gunning *et al*., 1984).

Collins (1986) reported the failure of pyrethroid insecticides in controlling *H. armigera* on cotton in Thailand. Another study from Thailand revealed that *H. armigera* had developed 100-fold resistance to pyrethroids when compared with susceptible laboratory strain (McCaffery *et al.*, 1986). A third study from Thailand showed that strain of *H. armigera* had developed 102-fold resistance to cis-cypermethrin, 82-fold resistance to trans-cypermethrin and 125-fold

resistance to fenvalerate as against susceptible laboratory strain (Ahmad and McCaffery, 1988).

McCaffery *et al*., (1988) also observed resistance to cypermethrin and fenvalerate using topical application method in strains of *H. armigera* from Indonesia. Field collected larvae of *H. armigera* from cotton-growing areas of south Sulawesi, Indonesia, in 1987-88 showed 86-, 65- and 20- fold resistance to cis-cypermethrin, cis/trans-cypermethrin and fenvalerate, respectively when compared with susceptible laboratory strain (McCaffery and Walker, 1991).

Gu and Han (1989) reported high levels of fenvalerate resistance in *H. armigera* from cotton in Jiangsu Province, China, in 1985-88. Another study from China indicated that a strain of *H. armigera* was found to show pyrethroid resistance up to 33.3-fold in 1989 and resistance increased up to 88.9-fold in 1990 (Shen *et al*., 1992). A third study from Hebei Province, China in 1993 indicated that field-collected *H. armigera* larvae exhibited resistance to fenvalerate (Zhao *et al.*, 1994).

Wei *et al*. (1993) reported that resistance in *H. armigera* against fenvalerate, cypermethrin, decamethrin and cyhalothrin vary between 9.7 to 63.1 times in Guan county, Dinzhou city, Gucheng city and Handan city of Henei Province, China in 1991-92. Another report from China indicated that *H. armigera* from cotton fields in 1989 had developed resistance to deltamethrin and fenvalerate (Bin, 1994). Fenvalerate resistant *H. armigera* larvae when treated at 3rd instar stage for 21 generations showed 141.6-fold increased resistance to fenvalerate (Xianlin *et al*., 1996).

Jaarsveld (1994) recorded pyrethroid resistance for the first time in *H. armigera* in South Africa in 1993. Results from laboratory bioassays carried out between 1985 and 1996 using 3rd and 4th instar larvae of *H. armigera* in Cote d'Ivore showed that the pest had developed more than 12-fold and 22-fold resistance to cypermethrin and deltamethrin, respectively, when compared to susceptible strain of 1985 (Vassal *et al.*, 1997).

The first report of pyrethroid resistance in *H. armigera* in India was recorded from Andhra Pradesh in 1987 (Dhingra *et al*., 1988). McCaffery *et al*. (1989) reported high levels of resistance to the pyrethroids cypermethrin and fenvalerate in field-collected larvae of *H. armigera* from cotton, pigeon pea and chickpea areas of Andhra Pradesh, India, in 1986-88. Another study from Guntur region of Andhra Pradesh, India indicated that the pest had developed 79-fold resistance to fenvalerate (Venkataiah *et al.*, 1990). A third study from Andhra Pradesh on field-collected larvae of *H. armigera* indicated that the pest had developed tolerance to cypermethrin, fenvalerate and deltamethrin (Sudhakar, 1991).

Mehrotra (1990) reported that because of injudicious use of pyrethroids, *H. armigera* showed high degree of resistance to cypermethrin, fenvalerate and deltamethrin in India. Populations of *H. armigera* from Haryana were found to show increase in resistance to cypermethrin (Phokela *et al*., 1990). Further findings, using the discriminating dose method, indicated that larvae of *H. armigera* from cotton, sorghum, pigeon pea and chickpea growing areas of Andhra Pradesh exhibited changes in cypermethrin resistance during 1986-91 (Armes *et al*., 1992). Findings of Reddy *et al*. (1991) revealed that the strains of *H. armigera* from Guntur and kurnool districts of Andhra Pradesh, India were found to have developed 18.7-, 7.8- and 9.2- fold and 10.5-, 4.8- and 6.4- fold resistance to cypermethrin, decamethrin and fenvalerate, respectively in comparison with the strain from Srikakulam district.

Populations of *H. armigera* from cotton growing areas of Punjab, India were found 11.6- and 3.2- fold resistant to cypermethrin during 1990-91 and 1991-92, respectively in comparison with a population from Delhi (Mehrotra and Phokela, 1992). Lande and Sarode (1995) reported that populations of *H. armigera* from Maharashtra, India in 1991-92 were found to develop 1.76-, 5.46- and 9.97-fold resistance to cypermethrin, deltamethrin and fenvalerate, respectively in comparison with a susceptible laboratory population. However, no evidence of resistance was found in a study from Hisar, Haryana (Baruah, 1987).

Larval population of *H. armigera* from Hisar, Haryana India was found to develop 12.79- and 2.73-fold resistance to fenvalerate and cypermethrin, respectively when compared with kaul, Kaithal population under laboratory condition (Lal, 1998). Several other workers have also reported the increased tolerance and/or development of pyrethroid resistance in *H. armigera* from other parts of the world *viz.* formerly USSR (Sukhoruchenko, 1996), Bangladesh (Ali, 1994), Nepal (Armes, 1995), New Zealand (Cameron *et al*., 1995), Japan (Guilin and Yunxi, 1996), and Pakistan (Ahmad *et al*., 1997; Ahmad *et al*., 1998).

There are also reports of increased tolerance/development of resistance to organochlorine group of insecticides in *H. armigera* larvae.

A strain of *H. armigera* from South Africa exhibited 2-fold tolerance to endosulfan as compared with susceptible laboratory strain (Basson *et al.*, 1979). *H. armigera* from Thailand has also exhibited 125-fold resistance to DDT and 2-fold to endosulfan when compared with susceptible laboratory strain (McCaffery *et al.*, 1986; Ahmad and McCaffery, 1988). Gunning *et al*. (1990) reported that field populations of *H. armigera* were found to show up to 500-fold resistance to DDT during 1974-78. Another report of Gunning and Easton (1994) from Australia revealed that field-collected 3rd-instar *H. armigera* larvae from New South Wales and Queensland when

treated topically showed increase in endosulfan resistance from 50-fold in 1974 to 163-fold in 1984. The report from south Sulawesi Indonesia revealed that pest had developed 203-and 5-6-fold resistance to DDT and endosulfan, respectively in 1987-88 when compared with susceptible laboratory strain (McCaffery and Walker, 1991).

The population of *H. armigera* from Ghuteli region, Gujarat, India had developed 2.37-fold resistance to endosulfan when compared to Anand population (Mehta *et al*., 1992). Lal (1998) reported that larval population of *H. armigera* from Hisar region, Haryana, exhibited 0.91-fold resistance to endosulfan as compared to Kaul, Kaithal population. Development of resistance/increased tolerance in *H. armigera* against organochlorine insecticides has also been reported from other parts of the world *viz*.; USSR (Sukhoruchenko, 1982; Sukhoruchenko, 1996), Nepal (Armes, 1995), Pakistan (Ahmad *et al*., 1995; Ahmad *et al*., 1998).

Reports regarding tolerance or resistance in *H. armigera* to carbamate and organophosphate insecticides have also been documented by various workers.

The strains of *H. armigera* from Thailand exhibited 27-fold resistance to carbaryl (McCaffery *et al*., 1986), 28-fold to DDT and 2-fold to diazinon and monocrotophos (Ahmed and McCaffery, 1988) when compared with susceptible laboratory strain.

The population of *H. armigera* from Anand region, Gujarat, India had developed 4.58- and 3.52-fold resistance to carbaryl and monocrotophos, respectively when compared to Ghuteli region population while Ghuteli population exhibited 2.37-and 1.67-fold resistance to quinalphos and triazophos, respectively when compared to Anand region population (Mehta *et al*., 1992).

Resistance to quinalphos in 3rd instar larvae of *H. armigera* from Hisar, Haryana, India was found to be 2.70-fold when compared with Kaul, Kaithal population (Lal, 1998). *H. armigera* populations has also been reported to develop, resistance from other parts of the world *viz*.; formerly USSR (Sukhoruchenko, 1982; Sukhoruchenko, 1996), Australia (Kay *et al.*, 1985), Nepal (Armes, 1995), Pakistan (Ahmed *et al*., 1995; Ahmed *et al*., 1998), Japan (Guilin and Yunxi, 1996).

DIFFERENTIAL SUSCEPTIBILITY OF EGGS TO INSECTICIDES Various workers have reported that egg stage is the most vulnerable or susceptible stage to the insecticides having ovicidal action (Smith and Salkeld, 1966; Singh *et al*., 1982). Stoeva (1979) observed that methomyl gave 100% mortality of *H. armigera* eggs at 0.15% under laboratory condition. Ying (1982) found triazophos and quinalphos to be highly effective against the eggs of *H. armigera*. Hargreaves and Cooper (1984), using spray method against eggs of *H. armigera* on tomato in Queensland in 1980 found that methomyl gave highest ovicidal action at 0.0125-0.05% concentration followed by fenvalerate and sulprofos at 0.005 and 0.072%, respectively in comparison with deltamethrin, endosulfan, methamidophos, monocrotophos, cypermethrin and prothiofos.

Laboratory studies carried out in Central Asia, formerly USSR in 1979-81 by spraying infested cotton leaves having eggs of *H. armigera* showed that 3 days after treatment deltamethrin and fenvalerate caused 100 and 93.3% egg-mortality, respectively (Khodzhaev and Eshmatov, 1983).

Ovicidal toxicity of insecticides under laboratory conditions against *H. armigera* eggs in descending order were permethrin (89.9%), quinalphos (88.4%), monocrotophos (72.3%), phenthoate (55.6%), phosalone (29.0%) and chlorpyrifos (19.6%) (Vekaria and Vyas, 1985). Watson *et al*.(1988), reported that egg mortality decreased with the increase of egg age and chlorpyrifos was found to have highest ovicidal effect.

Laboratory tests against eggs of *H. armigera* revealed that methomyl, triazophos, monocrotophos and quinalphos at concentrations of 0.05 and 0.025% and fenvalerate at 0.008 and 0.004% proved to be very effective (Ahmad *et al*., 1990).

Patel and Patel (1989) reported better ovicidal activity of fenvalerate (0.02%) and quinalphos (0.05%) than endosulfan, monocrotophos, cypermethrin and decamethrin against eggs of *H. armigera* under laboratory conditions. Methomyl was found to be an effective ovicide against eggs of *H. armigera* (Gunning *et al*., 1992).

Mala *et al*. (1993) reported that methomyl (0.048%), triazophos (0.08%) and thiodicarb (0.75%) were very effective ovicides (nearly 100% mortality) against different aged eggs of *H. armigera viz*. 1-day-old, 2-day-old and 3-day-old eggs using spray and dipping method.

Methomyl was found to be more effective ovicide against eggs of *H. armigera* compared with endosulfan and percent hatchability decreased with increased concentrations (Ramesh and Khan, 1996). Laboratory studies against eggs of *H. armigera* revealed that eggs of 3-day old age were found most sensitive to ovicides when compared to eggs of early age. Profenofos at 400 ppm gave 95, 70 and 100 per cent egg mortality for 0-24hrs; 24-48hrs and 48-72hrs ages, respectively (Pachori and Sharma, 1996).

Laboratory tests conducted by Sharma *et al*. (1996) using spray method on field-collected freshly laid eggs reported methomyl 40 SP and profenofos 50 EC as the best ovicides.

CONCLUSION It is evident from the literature reviewed

that *H. armigera* has developed resistance/tolerance to various group of insecticides throughout the world. In this context, an Integrated Resistance Management (IRM) strategy seems to be the most promising. The present day Integrated Pest Management (IPM) still relies heavily on chemicals intended for controlling the damaging stage i.e. the larva. However, under field conditions the observed control is a combination of the result of toxic action of chemicals on all the stages. The contribution of ovicidal action to the total effect has been well studied (Kathuria *et al*., 2000). In addition, regular updating of the strategy and tactics of pest management with the introduction of new concepts and approaches together with the introduction of new insecticides (or ovicides) is required in order to have better management of pests. To achieve this objective a clear understanding of the factors affecting susceptibility of *H. armigera* in response to chemicals, mechanisms of action of different insecticides (or ovicides), their scope and limitations etc. is quite necessary so that these plant protection tools may be employed more efficiently.

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V. Kathuria*, R. K Saini, and Pala Ram
Department of Entomology
CCS Haryana Agricultural University
Hisar-125 004, India

*For correspondence:
TERI School of Advanced Studies
Habitat Place, Lodhi Road, New Delhi - 110 003

Efficacy of some pesticides against spider mite, *Tetranychus urticae* Koch and its predatory mite, *Amblyseius longispinosus* (Evans)

ABSTRACT Phytophagous and predatory mites are available to the plant fauna at the same period. Vegetable crops are the worst suffer due to these phytophagous mites. Among the phytophagous mite, the spider mite, *Tetranychus urticae* Koch are potential pests and attack on a wide range of vegetable crops. *Amblyseius longispinosus* (Evans) are the potential predatory mite of *T. urticae* Koch. The indiscriminate use of pesticides results to outbreak of *T. urticae* due to killing of predatory mites. An field experiment was carried out to find out the efficacy of some commercially available pesticides viz. azadirachtin, dicofol, ethion, endosulfan, fenvalerate, monocrotophos and wettable sulphur at their recommended doses against spider and predatory mite in the laboratory condition on okra crop, variety Arka Abhay with three replication. The results indicate maximum mortality in dicofol 81.35% followed by monocrotophos, endosulfan and ethion 79.72%, 79.37% and 78.00% respectively where as less mortality was observed in fenvalerate 50.60% followed by azadirachtin and wettable sulphur 50.84% and 69.70% respectively against the spider mite. Where as ethion and dicofol responded maximum mortality and azadirachtin and sulphur responded less mortality against the predatory mite, *A. longispinosus* (Evans). On the basis of this trail azadirachtin and sulphur are safer to the predatory fauna and eco-friendly also.

KEYWORDS spider mite, predatory mite, vegetable, pesticides.

INTRODUCTION The spider mite, *Tetranychus urticae* Koch are potential pests and attack on a wide range of vegetable crops including brinjal, beans, tomato, okra, soybean & cucurbits. Vegetables are important rich source of minerals and micronutrients. But pest and disease are important production constraints of vegetables in tropical & sub-tropical regions. During recent years the mite damage to vegetable crops has been recognized as one of the limiting factors in attaining increased productivity of vegetables throughout the country (Singh, 1994).

The losses due to mites were reported from Bihar 36.8 to 83.2 per cent in different vegetable crops (Lall & Dutta, 1959). Here I discuss the mite species that cause damages to most vegetable crops are *T. urticae* Koch. *T. macfarlanei* Baker & Pritchard, *T. ludeni* Zacher and *T. neocaledonicus* André.

Studies on mite problem in India have gained importance and growers begun to realize their pest status. *T. urticae* Koch. was identified as polyphagous mite This mite active throughout the year (Rahman & Sapra, 1990). *Tetranychus urticae* Koch having wide range of host affinity of twenty-seven vegetable crops in India, as well as little potential predatory was also in the record from the vegetable crops. *A. longispinosus*. It was the potential predatory mite on the *T. urticae* Koch. in natural condition (Singh, 1994).

MATERIALS AND METHODS The field experiment was conducted in randomized block design at Vegetable research farm, Institute of Agricultural Science, BHU, Varanasi, India. The plot size was 2 x3 mt and row to row spacing was maintained at 50 cm apart the field in all three replications and cropped area 176 sq. meters and 144 sq. meters respectively. The seven pesticides were used at their prescribe doses. The control plot was treated with water. Five okra plants from each plot were randomly selected tagged. Total ten okra leaves were taken from upper, middle and lower portion of tagged plants. The live mite population was counted with help of stereoscope binocular microscope, after one, three, seven and fourteen days of treatment. The corrected per cent mortality was analyzed through Abbott's formula (1925).

The methods applied to know the relative toxicity of some selected commonly used pesticide viz., azadirachtin, dicofol, ethion, endosulfan, fenvalerate, monocrotophos and wettable sulphur against *T. urticae* Koch and *A. longispinosus* (Evans).

Test mite pest

T. urticae Koch natural population was maintaining on okra crop under the field condition for the pesticidal experiments *A. longispinosus* (Evans) was initiated by collecting adults from the field. The predatory mites were brought to the laboratory and released onto spider mite in poly-house. The mites from such culture were used in the laboratory study.

Pesticides

Commercial grade pesticides were prepared at their recommended concentration for field application as well as for laboratory trail.

Predatory mite

The leaves were dipped up-to five seconds in the pesticides solutions to ensure complete welting and allowed them to dry. The leaves were kept on moist cotton wool for an adequate period. The adult predatory mites about 2-5 days old were placed on

clean and dry treated leave. The treated leaves were observed and mortality of predatory mite *A. longispinosus* (Evans) was recorded after 7, 14, 21 and up-to 28 hours. The leaf treatments with pesticides were initiated through Leaf Dipped Method (FAO Method No. 10a) (FAO, 1980).

Result

The present study was aimed in this direction to know the relative toxicity of pesticides against *T. urticae* Koch and *A. longispinosus* (Evans) in the field and laboratory condition.

Effect of pesticides against spider mite *Tetranychus urticae* Koch

Azadirachtin (0.03%), dicofol (18.5EC), ethion (50EC), endosulfan (35EC), fenvalerate (20EC), monocrotophos (35EC) and wettable sulphur 80 WP were spayed at their recommended concentration and responded significant variation in present mortality at 1,3, 7 and 14 days after treatment.

Table. No. 1. Efficacy of various pesticides against spider mite, *Tetranychus urticae* Koch at field condition.

Pesticides	Concentration	Mean percent mortality (in days)			
	(%)	1	3	7	14
Azadirachtin 0.03%	0.23	36.46	75.33	76.8	14.76
		(37.14)**	-60.29	-61.26	-22.57
Dicofol (18.5EC)	0.04	81.9	99.4	100	44.13
		-64.82	-86.22	-88.19	-41.62
Ethion (50EC)	0.05	83.63	97.93	98.2	32.26
		-66.13	-82.88	-83.28	-34.61
Endosulfan (35EC)	0.05	79.2	88.13	87.86	62.3
		-62.86	-72.76	-72.57	-52.14
Fenvalerate (20EC)	0.005	45.33	63.03	64.13	29.9
		-42.32	-52.61	-53.27	-33.12
Monocrotophos (36EC)	0.05	85.43	90.63	91.2	41.53
		-67.57	-72.21	-77.83	-40.12
Wettable sulphur (80WP)	0.25	60.53	87.6	88.3	42.4
		-51.08	-69.48	-70.07	-40.62
Control (Water solution)	----	9.06	8.73	7.1	0.83
		-17.37	-17.04	-15.39	-3.03
C.D	at	1%	5%	Significance	
Days		3.056	2.325	s	
Pesticides		4.322	3.288	s	
Days x Pesticides		8.644	6.577	s	

* Mean of three replication.
** Figure in parentheses is arcsine percentage transformation
***For comparing Pesticides mean

After one day less mortality was observed in azadirachtin 36.46% and significant difference were observed monocrotophos (85.43%), ethion (83.63%), dicofol (81.90%), endosulfan (79.20%), wettable sulphur (60.53%) fenvalerate (45.33%). Maximum mortality was recorded with monocrotophos (85.43%) and ethion (83.63%).

Three days after maximum per cent mortality was recorded in dicofol (99.40 %) against spider mite. Ethion and monocrotophos has no significant difference 88.13 and 87.60 per cent respectively. Azadirachtin showed less mortality 75.33 per cent but the performance was encouraging.

After the seven days the performance of percent mortality was on the similar pattern as three days after. But after fourteen days the per cent mortality was reduced. Endosulfan, dicofol, wettable sulphur and monocrotophos still they showed their response. They have proved their residual response. The residual response of azadirachtin was reduced with in seven days.

Effect of pesticides against predatory mite *Amblyseius longispinosus* (Evans)

Predatory mite, *Amblyseius longispinosus* (Evans) adults were exposed all pesticides on same concentration which was applied on the spider mites at 7, 14, 21, and 28 hours.

Table No. 2. Efficacy of various pesticides against predatory mite, *Amblyseius longispinosus* (Evans) in the laboratory condition.

Pesticides	Concentration	Mean percent mortality (in hours)			
	(%)	7	14	21	28
Azadirachtin 0.03%	0.23	18.35	14.10	8.68	---
		(25.33) **	(21.94)	(17.11)	
Dicofol (18.5EC)	0.04	99.51	84.40	71.48	30.47
		(86.45)	(66.76)	(57.71)	(33.50)
Ethion (50EC)	0.05	92.45	79.73	80.31	33.90
		(74.07)	(76.22)	(63.64)	(35.58)
Endosulfan (35EC)	0.05	86.48	9.18	------	------
		(68.56)	(17.62)		
Fenvalerate (20EC)	0.005	32.77	32.06	16.10	0.35
		(34.87)	(28.74)	(28.74)	(6.30)
Monocrotophos (36EC)	0.05	68.50	73.92	73.21	10.64
		(55.85)	(59.30)	(58.83)	(15.69)
Wettable sulphur (80WP)	0.25	14.25	8.93	4.83	-----
		(22.06)	(17.33)	(12.61)	
Control (Water solution)	-----	-----	-----	-----	-----
C.D***	at	1%	5%	Significance	
Hours		2.993	2.214	s	
Pesticides		3.959	2.928	s	
Days x Pesticides		7.917	5.859	s	

*Mean of three replication in each replication 15 adults was released.
**Figure in parentheses is arcsine percentage transformation.
***For comparing pesticides treatment mean.

After seven hours very less mortality were observed of predatory mites in wettable sulphur 14.25 % and azadirachtin 18.35 %. Maximum mortality were showed in dicofol 99.51 % and followed by ethion, endosulfan, monocrotophos and fenvalerate i.e. 92.45, 86.48, 68.50 and 32.77 per cent respectively. The per cent mortality after 14 hours was observed as a same pattern as 7 hours after. But only the endosulfan was shown the lowest affect on predatory mites like wettable sulphur. There was no significant difference in mortality between wettable sulphur and endosulfan. After 21 hours the endosulfan showed no mortality. Ethion, monocrotophos and dicofol performed well and showed their affect with significant difference 80.31, 73.21 and 71.48 per cent, respectively. Ethion, dicofol and monocrotophos showed maximum mortality after 28 hours 33.90, 30.47 and 10.64 per cent, respectively.

These pesticides proved their detrimental performance to the predatory mites.

DISCUSSION Chemical control of vegetable crops by using synthetic pesticides has been widely practices in almost all the third world countries. Summer vegetable crops suffer various serious by spider mite in India, Another factor enhancing mite population is the dust deposition on foliage which are in close proximity to earthen road and dust from brick Kiln provide medium for attachment of mite were webs on smooth surface (Singh, 1994).

Spider mite

It is evident from the result that the increase in mite mortality is statistically significant even at one per cent probability level in comparison to control. The pesticides are arranged on the basis of relative toxicity in following descending order of performance: wettable sulphur, fenvalerate, azadirachtin, ethion, endosulfan, monocrotophos and dicofol.

In the present study, dicofol, monocrotophos, endosulfan and ethion have been found to be almost equally effective against spider mite, *Tetranychus urticae* Koch which has been supported by many workers (Bindra & Goyal, 1996; ChannaBasavanna, 1981; Jagdish and ChannaBasavanna, 1983; Sandhu *et al*., 1983; Pandey and Reddy, 1983; Singh *et al*., 1989; Singh and Singh, 1992).

Hence, plant product as above can be taken advantage for the evaluation for toxic level against spider mite. Azadirachtin (0.03%, 5 ml/liter) have been found effective up-to (76.80%) against *Tetranychus urticae* Koch at after seven days (Table 2). These finding were supported by work of Rai *et al*. (1993) and Patel et *al*. (1993).

Predatory mite

The toxicity of the some pesticides against predatory mites, *Amblyseius longispinosus* (Evans) clearly showed the maximum mortality in dicofol (99.51 %) in the laboratory condition. Ethion was also given the parallel response in mortality (92.45 %) and all pesticides showed significant variation among themselves in the respect of toxicity to predatory mites. In the present study ethion, dicofol, monocrotophos and endosulfan have been found to be equally effective against predatory mites, *Amblyseius longispinosus* (Evans) which has supported by Momen *et al*. 1997 and Stark *et al*. 1997. Azadirachtin have been found less effective on predatory mites, *Amblyseius longispinosus* (Evans) (Momen *et al*. 1997). Thus neem pesticides may prove to be one of the most promising botanical pesticides in reference to safer to the beneficial and predatory fauna (Mansour *et al*. 1987). Wettable sulphur and azadirachtin showed eco-friendly response to the predatory mite.

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S. P. Singh and R. N. Singh
Department of Entomology & Agricultural Zoology
Institute of Agricultural Sciences
Banaras Hindu University
Varanasi - 221 005

Development of Resistance in Helicoverpa armigera (Hubner) to Endosulfan in Jammu and Comparative Biology of its Resistant, Parental and Susceptible strains

ABSTRACT Selection of 3rd instar larvae of *Helicoverpa armigera* to various sub-lethal concentrations of endosulfan up to five generations resulted in maximum mortality at highest concentration in every successive generation. The resistance ratio for the fifth generation increased 5.22-fold as compared to first generation. Various biological and developmental attributes revealed that oviposition period, fecundity and female adult longevity differed significantly among endosulfan-selected, parental and susceptible strains of the pest. However, all other parameters remained statistically non-significant.

KEYWORDS Resistance, Endosulfan, Helicoverpa armigera, Biology

Helicoverpa armigera (Hubner) is one of the most destructive polyphagous pest that has attained the national importance as a key pest of various crops like cotton, chickpea, pigeon pea, maize, sorghum, tomatoes etc. grown in India. Control of *H.armigera* often lies mainly on the use of insecticides due to which the species has exhibited a propensity to develop tolerance against them in several countries. The first report of resistance in India (Reed and Pawar, 1982) indicated differential susceptibility to different insecticides among the larvae collected from various parts of the country. Presently, the development of resistance in *H.armigera* stands well documented (Bhatia, 1986; McCaffery *et al*., 1988; Mehrotra, 1989) to carbamates and organophosphates (Kranthi et *al*., 2001) and pyrethroids (Arora *et al*., 2003). Even the phenomenon of insecticide resistance mechanisms has been clearly understood (Mehrotra and Phokela, 1986; Brown and Brogdon, 1987; Roush and McKenzie, 1987). The phenomenon of resistance to almost all the conventional insecticides used for its control has extended to almost all the north Indian states viz. Delhi (Phokela *et al*., 1990), Punjab (Mehrotra and Phokela, 1992), Haryana (Pedegley *et al*., 1987) and very less insecticide use innocent region like Varanasi in Uttar Pradesh (Singh et *al*., 1994; Vaishampayan and Singh, 1995).

Though the distribution of this pest is ubiquitous in all the states of India, yet no work has been undertaken to assess the level of resistance in this pest to the most commonly used insecticides in Jammu and Kashmir, inspite of the fact that at many occasions in the past, farmers have approached the Plant Protection specialists in Department of Agriculture, Government of Jammu and Kashmir and the entomologists working in the Agricultural University with information of insecticidal control failure against this pest even after spraying repeatedly at much higher dosage of endosulfan than recommended for its control in various crops like chickpea, tomato, cole crops, pea etc. A need was, therefore, realized to quantify the potential of development of resistance to endosulfan and the comparative fitness costs in this pest.

MATERIALS AND METHODS

The insect and its rearing: The culture of *H.armigera* was initiated by collecting about 200 larvae from the farmers' fields in and around Jammu during the last week of November 2002 in scintillation vials and brought to the laboratory & maintained at 26 ± 2°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 named as parental stock and the other was used for exposure to endosulfan. Hundred pupae of susceptible strain of *Helicoverpa 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 endosulfan was used to prepare one per cent stock solution in acetone from which further dilutions were prepared subsequently.

Bioassay and Laboratory Selection: Sub-lethal concentrations of insecticides prepared on the basis of recommended concentrations of various insecticides (Simwat, 1994) were applied to the thoracic dorsum of each 3rd instar larva of susceptible as well as field

collected strain of *Helicoverpa armigera* separately with the help of a Merck micropipette @ 1.0µl per larva. Six to seven concentrations of endosulfan 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 petriplates (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 the F1 generation and the exposures and the selections were conducted subsequently up to 5 generations. The parental and susceptible strains were also maintained through without exposure 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 8.0 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 endosulfan-selected, parental, and susceptible strains were desexed so as to get atleast 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 per cent sucrose solution to serve as food to initiate the studies on the various biological and developmental attributes of endosulfan-selected, parental and susceptible strains.

Statistical analysis of the data The mortality data was analysed to work out LC_{50} value at 95 per cent confidence interval by SPSS 8.0. The data on the various parameters of the biology of the insecticide-selected, parental and susceptible strains was 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 per cent concentration (Table 1) was 16.67 per cent among all the concentrations used. Similarly, the lowest survival of 13.33, 14.00, 12.00 and 10.00 per cent were obtained in the 2nd to 5th generation, respectively. Lande and Sarode (1993) reported a survival of 30.0 per cent at 0.50 per cent concentration of endosulfan.

After subjecting the data to probit analysis, the results obtained in the Pearson Goodness of Fit chi-square 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 6.04 ± 0.20) 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 0.15795 which subsequently increased to 0.82463 in the 5th generation and the insect developed 5.22-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 endosulfan used on various vegetable crops in Jammu. Though several workers (Lal, 1998; Patel and Koshiya, 1999; Kapoor *et al.*, 2000) have come out with the report of development of resistance to endosulfan 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 endosulfan 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.

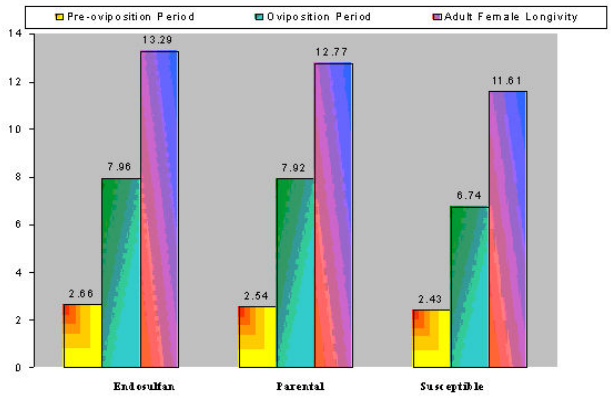

Figure 1. Differential biological attributes in various strains of Helicoverpa armigera

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

Generation	Conc. of Endosulfan used	Mortality (%)	Residual population Rejected(X) Selected(√)
F_1	0.10	50.00	X
	0.15	56.66	X
	0.20	63.33	X
	0.25	70.00	X
	0.30	76.66	X
	0.35	83.33	√
F_2	0.25	46.66	X
	0.35	53.33	X
	0.45	60.00	X
	0.55	66.66	X
	0.65	73.33	X
	0.75	80.00	X
	0.85	86.66	√
F_3	0.80	70.00	X
	0.85	74.00	X
	0.90	78.00	X
	0.95	80.00	X
	1.00	82.00	X
	1.05	86.00	√
F_4	1.00	68.00	X
	1.05	72.00	X
	1.10	76.00	X
	1.15	80.00	X
	1.20	84.00	X
	1.25	88.00	√
F_5	1.15	78.00	X
	1.25	80.00	X
	1.35	82.00	X
	1.45	84.00	X
	1.55	84.00	X
	1.65	90.00	√

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

Generation	Heterogeneity $\chi^2_{(n-2)}$	Regression equation	Slope±S.E.	Fiducial limits*	LC50 (%)	Resistance ratio
I	$\chi^2_{(5)}$=8.246	Y=-0.9543+6.04193x	6.04±0.20	0.08315 – 0.21036	0.15795	1
II	$\chi^2_{(6)}$=4.280	Y=-.97440+2.56410x	2.56±0.19	0.29639 – 0.44782	0.38002	2.4
III	$\chi^2_{(5)}$=0.493	Y=-1.68209+2.68030x	2.68±0.29	0.53039 – 0.69678	0.62757	3.97
IV	$\chi^2_{(5)}$=0.519	Y=-1.60482+2.12960x	2.12±0.28	0.63418 – 0.83929	0.75358	4.77
V	$\chi^2_{(5)}$=3.478	Y=-1.38626+1.68110x	1.68±0.44	0.66831 – 0.9399	0.82463	5.22

*at 95% confidence interval

Comparative Biology

Out of eleven biological parameters, only three parameters, viz., oviposition period, fecundity and adult-female longevity had been adversely affected in the endosulfan-selected *H. armigera*. On the other hand, no significant differences in other parameters were observed in all the three strains of test insect.

Oviposition period: The mean oviposition period of endosulfan-selected and parental strains was 7.96± 0.38and 7.92± 0.36 days, respectively, whereas the corresponding figure for susceptible strain was 6.74 ± 0.35 (Table 3).Both the endosulfan-selected and parental strains were significantly different from susceptible strain of *Helicoverpa armigera* with respect to this parameter. These differences are probably due to the reproductive disadvantages in the resistant female moths (Dong *et al*., 1996) and due to the variations observed by Xia *et al*. (2001).

Table 3. Biological attributes of insecticide-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 (%)	Adult Longevity (days) Male	Female
Endosulfan	2.66±0.52^{ab} (2.0-3.0)	7.96±0.38^{b} (7.8-8.2)	2.60±0.72 (1.7-3.3)	356.3±2.62^{b} (350-365)	3.95±0.74 (3.0-4.8)	93.4±1.42 (90.0-96.0)	10.19±0.66 (9.10-12.0)	13.29±0.77^{c} (12.2-14.0)
Parental	2.54±0.50^{ab} (2.0-2.8)	7.92±0.36^{b} (7.7-8.5)	2.31±0.68 (1.6-3.0)	379.4±2.95^{b} (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±0.78^{b} (11.6-13.9)
Susceptible	2.43±0.43^{a} (2.0-2.7)	6.74±0.35^{a} (6.6-7.0)	2.44±0.60 (1.6-2.8)	703.8±4.0^{a} (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±0.55^{a} (10.9-12.1)
			NS		NS	NS	NS	

- Mean ± S.E (m) of 10 pairs/individuals
- Figures in parenthesis represent range
- Means followed by the same letter in a column are not significantly different (p=0.05), Tukey's Multiple Comparison Test (HSD*)

Fecundity: The average fecundity of endosulfan-selected and parental strains were 356.3 (360-365) 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 endosulfan 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 endosulfan-selected strain was at par with that of the parental strain (Fig. 2) significant differences in the above figures revealed that the selection with endosulfan for five generations had ostentatious effect on the fecundity of this insect. A significant reduction in the fecundity of resistant females of *H.armigera* has been reported by Campanhola (1988) who produced 1200 eggs each as compared to 2500 eggs per susceptible female. It has also been suggested that the reduced fecundity was the consequence of metabolic resistance to insecticides. Further, it was advocated that the females of this pest have relatively higher tendency for the development of resistance to insecticides (Forrester *et al*., 1993; Glenn *et al*., 1994). Recently, these observations have been further strengthened by striking differences observed on the effective fecundity of resistant females of this pest, which was found decreased significantly (Xia *et al*., 2001). The present findings, thus, fall in line with the results of these workers.

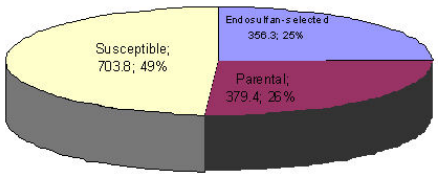

Fig. 2 Differential fecundity among various strains of *Helicoverpa armigera*

Adult longevity The average female longevity of the endosulfan-selelected, parental and susceptible strains was 13.29± 0.77, 12.77± 0.78 and 11.61± 0.55 days, respectively (Table 3). The female longevity exhibited significant difference among endosulfan-selected, parental and susceptible strains of *H.armigera*. Since no information is available in literature on this aspect,

it is opined that such differences might have occurred due to slight variations in the rearing conditions in the laboratory which otherwise require further confirmation.

ACKNOWLEDGEMENT Authors are thankful to Dr.R.M.Bhagat, Professor & Head, Division of Entomology, SKUAST-Jammu for providing facilities to conduct the present work, critically going through the manuscript and suggesting changes for its improvement.

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Munazah Yaqoob and R.K.Arora
Division of Entomology, Faculty of Agriculture
Sher-e-Kashmir University of Agricultural Sciences & Technology-Jammu
Udheywalla, Jammu-180 002. INDIA
E mail: rkarora_003@yahoo.co.in

The first report of resistance of Colorado potato beetle to phosalon and endosulfan from Iran

INTRODUCTION Colorado potato beetle, *Leptinotarsa decemlineata*, was a quarantine pest for Iran. This pest was introduced to Iran during 1984 probably by imported potatoes. Nowadays Colorado potato beetle (CPB) has become the most important defoliator of potatoes in Iran. CPB in Iran are controlled primarily with conventional insecticides since there are not any natural enemies for CPB control. Review of history of

CPB resistance in other countries shows that this pest has a powerful mechanism for pesticide resistance. Many populations of CPB around the world have developed resistance to nearly all registered insecticides (Hare 1990; Heim et al., 1990; Roush and Tingey 1991). It's about 15 years that recommended insecticides for CPB control have not changed in Iran. Recommended insecticides in Iran for CPB control are endosulfan and phosalon. Because of the long-time use of these insecticides in the fields, it is expected that CPB shows resistance to them. The current status of CPB resistance to insecticides is unclear in Iran, but growers in some regions report failure in CPB control with endosulfan and phosalon. Many of them no longer use these insecticides. To test the efficiency of recommended insecticides, some laboratory bioassays were carried out.

MATERIALS AND METHODS

Insects- The first generation adults of CPB were collected by hand from commercial potato fields in Hamedan province. Tests began <24 h after collection of beetles.

Chemical- Tests were done with technical grade of endusulfan (>96%) and phosalon (>95%). The solvent was pure acetone.

Preliminary tests- Dose selection is a very important element in each insecticide bioassay test. We did more than 5 preliminary tests to find proper doses. The process of dose selection was based on Robertson et al (1984) and Robertson and Preisler (1991).

Final test- Based on results of preliminary tests and availability of insects, seven (phosalon) and eight (endosulfan) concentrations resulting in mortalities of less than 10 to more than 90 percent and a zero concentration in each replicate were used as check. Ten insects were allocated to each concentration. Each dose-response had three replicates, so we used 240 (phosalon) and 270 (endosulfan) insects as test subjects. Each replicate was done with a new stock solution to remove any pseudoreplication (see Robertson and Preisler, 1991). A 1-µl droplet of solution was applied underside of the insects between coxa. For this process a precise microaplicator was used. All treated insects were placed in little transparent plastic container with fresh potato leaves and kept in 25 ± 1°C, photoperiod of 16:8 (L:D) and 70 ± 10 % relative humidity. Dead beetles were recorded after 24 h. To distinguish between dead and alive beetles, we used method of Zhao et al (2000).

Data analysis- Dose-mortality regression, LD's and their confidence Interval were estimated by probit analysis using POLO-PC software (LeOra Software, 1987).

RESULTS AND DISCUSSION The table shows the LD_{50} for tested insecticides. The recommended field rate for these insecticides in Iran is 0.525-0.7 kg (AI/ha). The produced concentration of field recommended rate is 1400 ppm (0.7 kg /500 litters of water). This means that a 1400 ppm concentration has a potential to kill all of CPB in the fields. Some new reports in Iran show that these insecticides, especially phosalon, can control CPB populations in recommended rate (Aghdam and Ghanbalani, 2002; Aghdam, 2004; Ranji *et al*., 2004). The estimated LD_{50} for these insecticides was very high in comparison to recommended field rate.

Table. Toxicity of phosalon and endusulfan to adults of *Leptinotarsa decemlineata* collected from Hamedan Province, Iran

	LD_{50}*	CL 95%	Slope (SE)
Phosalon	28.46	23.15-34.25	2.18
			-0.41
Endusulfan	18.85	14.37-23.15	1.84
			-0.64
* µg/beetle			

The convert of LD_{50}s to ppm produces 28465 and 18851 for phosalon and endusulfan respectively. A simple comparison between these new numbers and 1400 ppm (recommended field rate) shows that control of CPB is completely impossible with these insecticides. The proportion rates between LD_{50} and recommended rate are 20.3 (28465/1400) and 13.4 (18851/1400) for phosalon and endusulfan respectively.

Although there are not any generalized relationship between laboratory LDs and field application rates, the researchers know that minimum dosage that will cause 90% mortality in the field (MED90) is more than laboratory estimated LD_{90}. Williams 1973 (in Haverty and Robertson [1982]) believed that LD_{90} must be multiplied by 3 to obtain a field dosage. Haverty and Robertson (1982) show that this is not a general rule. They show that to find a field rate of permethrin for the western spruce budworm, estimated LD_{90} must be multiplied by 11.91. If we want to report a filed rate for these insecticides based on my bioassays, we must multiply LD_{90}s by a number; we suppose that rate of MED90/LD_{90} is equal to one. So, the field rates for phosalon and endusulfan become 110231 and 91548 ppm respectively. In some regions of Iran we controlled CPB with 1400 ppm of these insecticides but our results show that field rate of them in Hamedan is very high. In other words, CPB can not be controlled with recommended field rates of these

insecticides and this is a good criterion of CPB resistance to phosalon and endusulfan in the potato fields of Hamedan.

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H. Allahyari, P. A. Fard and K. Talebi
Department of plant protection
College of Agriculture
University of Tehran Karaj Iran

Development of a Bioassay to Detect Changes in the Susceptibility of Cotton Aphid, *Aphis gossypii* Glover, to Commercial Neonicotenoid Insecticides

ABSTRACT A bioassay was developed to detect changes in cotton aphid, *Aphis gossypii* Glover, susceptibility to commercially available neonicotenoid insecticides. Formulated Intruder (acetamiprid, Dupont), Trimax (imidacloprid, Bayer Crop Science), and Centric (thiamethoxam, Syngenta Crop Protection) were applied to cotton plants grown in a greenhouse. A series of rates of each insecticide were applied to plants in a spray chamber. After 24 h, the uppermost terminal leaf was removed from each plant and placed in a Petri dish lined with a 2% agar solution. Five cotton aphid nymphs were placed on each leaf and mortality was rated at 24 h. LC_{50} values averaged 0.0008, 0.0013, and 0.0020 kg ai/ha for acetamiprid, imidacloprid, and thiamethoxam, respectively. This experiment demonstrates the utility of utilizing live plant tissues to measure cotton aphid susceptibility to the neonicotenoid insecticides.

INTRODUCTION The cotton aphid (=melon aphid), *Aphis gossypii* Glover, is a pest of many crops worldwide (Blackmon and Eastop 1984). In the southern United States, insecticides are applied to a significant percentage of the cotton acreage every year for their control. Historically, cotton aphids have rapidly developed resistance to new insecticides soon after their release for commercial use. In a paper modeling the development of insecticide resistance in *Heliothis virescens* (F.), Mallet and Luttrell (1991) categorize pests into three groups depending on their reproductive potential and likelihood to develop resistance. They consider cotton aphids in the category with the potential to develop high levels of resistance in a relatively short period of time. This is based on the high reproductive potential of cotton aphids and their capacity for resurgence after an insecticide application.

Cotton aphid populations are generally maintained at low levels through the actions of natural enemies (Weathersbee and Hardee 1994). However, numerous applications of broad spectrum insecticides are often made during early- to mid-June in cotton to control tarnished plant bugs, *Lygus lineolaris* (Palisot de Beauvois) in the mid-southern U.S (Scott and Snodgrass 2000). Historically, pyrethroids, carbamates, and organophosphates were the insecticides of choice for those applications. Consequently, outbreaks of cotton aphid in mid- to late-June were usually the result of those applications because of the elimination of natural enemies (Slosser et al. 2001).

Recently, a new class of insecticides, the

neonicotenoids, has been introduced that is relatively soft on natural enemies, and provides good control of both tarnished plant bugs and cotton aphids (Tomizawa and Casida 2003). Imidacloprid (Trimax, Bayer Crop Science) was the first neonicotenoid labeled for use in cotton in the U.S. Since the introduction of imidacloprid, other neonicotenoids have been introduced. They include thiamethoxam (Centric, Syngenta Crop Protection), and acetamiprid (Intruder, DuPont) (Tomizawa and Casida 2003). Currently, these insecticides are applied over large acreages during June because of their activity against both cotton aphids and tarnished plant bugs. This combined with the historical ability of cotton aphids to rapidly develop resistance to new insecticides creates the need for a proactive program to monitor cotton aphid susceptibility to these compounds (Kerns and Gaylor 1993).

For a monitoring program to be effective it is important to develop a bioassay that is cost effective and can produce rapid results. Programs to monitor insect susceptibility to the pyrethroids, carbamates, and organophosphates rely on bioassays that take advantage of the contact activity of these compounds. However, the neonicotenoid's have little contact activity and must be ingested to provide mortality. Therefore, a bioassay technique was developed using live plant tissue to determine cotton aphid susceptibility to imidacloprid, thiamethoxam, and acetamiprid. Results of these bioassays will be discussed.

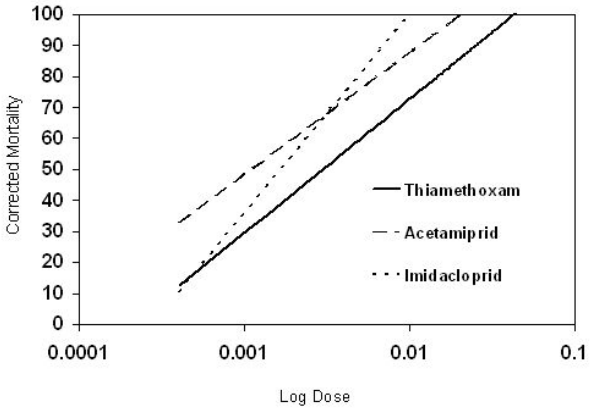
MATERIALS AND METHODS A bioassay procedure was developed to rapidly screen the susceptibility of cotton aphids to three commercially available neonicotenoid insecticides. The insecticides included acetamiprid (Intruder, DuPont), imidacloprid (Trimax, Bayer Crop Science), and thiamethoxam (Centric, Syngenta Crop Protection). Commercial formulations of each insecticide were used for bioassays. Serial dilutions of each insecticide were made to obtain six or seven concentrations along with a non-treated control. Insecticides were diluted in water to obtain 1000 ml of solution at the various concentrations and applied to actively growing plants in a spray chamber calibrated to deliver 46.8 L per hectare. Plants used for bioassays were grown in the greenhouse in Jiffy® (Jiffy Products, Shippagan, Canada) peat pellets until they had two true leaves. After treatment, the plants were set aside for 24 hours to allow complete penetration of the insecticide into plant tissue. After 24 hours, the upper most terminal leaf was removed from each plant and placed in a 9.0 cm Petri dish with a 2% agar solution. Ten Petri dishes were used for each treatment with three replications.

Cotton aphids used for bioassays were collected from a large block of non-treated cotton near Stoneville, MS. Aphids were collected by removing the terminals of heavily infested plants. Infested terminals were transported to the laboratory in plastic bags. In the laboratory, five late instar cotton aphid nymphs were transferred to each leaf with a small paint brush. The dishes were held in an environmentally controlled room at 26±2°C, 75±5 percent relative humidity, and a 14 hour light to 10 hour dark photoperiod. Mortality of cotton aphids was rated after 24 hours of exposure to the treated leaves. Data were log transformed and analyzed with Probit analysis (PROC PROBIT, SAS Institute, Version 8.2, Cary, NC). LC_{50} and LC_{90} values along with 95% fiducial limits were obtained for each insecticide.

RESULTS

Acetamiprid: Seven concentrations of acetamiprid were used to obtain a concentration mortality curve for cotton aphids. The concentrations ranged from 0.0004 to 0.028 kg ai/ha. The resulting concentration mortality curve had a slope±SE of 0.61±0.091 (X^2=3.15, d.f.=5, P=0.68) (Fig. 1). The LC_{50} (95% fiducial limits) and LC_{90} (95% fiducial limits) values were 0.0008 (0.00050 - 0.00119) and 0.0067 (0.00437 - 0.01321) kg ai/ha, respectively.

Figure 1. Dose mortality curves for acetamiprid, imidacloprid, and thiamethoxam against cotton aphids in a spray table bioassay.



Imidacloprid: Six concentrations of imidacloprid were used to obtain a concentration mortality curve for cotton aphids. The concentrations ranged from 0.0004 to 0.014 kg ai/ha. The resulting concentration mortality curve had a slope±SE of 1.04±0.133 (X^2=1.17, d.f.=4, P=0.88) (Fig. 1). The LC_{50} (95% fiducial limits) and LC_{90} (95% fiducial limits) values were 0.0013 (0.00100 - 0.00160) and 0.0044 (0.00325 - 0.00685) kg ai/ha, respectively.

Thiamethoxam: Six concentrations of thiamethoxam were used to obtain a concentration mortality curve for cotton aphids. The concentrations ranged from 0.002 to 0.056 kg ai/ha. The resulting concentration mortality

curve had a slope±SE of 0.63±0.114 (X^2=2.43, d.f.=4, P=0.66) (Fig. 1). The LC_{50} (95% fiducial limits) and LC_{90} (95% fiducial limits) values were 0.0020 (0.00100 - 0.00295) and 0.0149 (0.00979 - 0.03077) kg ai/ha, respectively.

DISCUSSION Results from these bioassays demonstrate the high level of efficacy each of these compounds have against cotton aphids at relatively low concentrations. Given the high level of efficacy at low rates and high reproductive capacity of cotton aphids, these compounds are likely to provide a high selection pressure for cotton aphids to develop resistance in the near future (Polumbo et al. 2001). Therefore, a comprehensive system to monitor for cotton aphid susceptibility to these compounds is needed. Results of the current study establish a relatively simple and rapid bioassay using live plants grown in the greenhouse as the carrier for delivery of the insecticides to the insects. This technique is not the ideal bioassay method for detecting changes in a population over time. However, similar techniques been developed to monitor changes in whitefly, *Bemisia* spp., susceptibility to neonicotenoids in Arizona (Williams et al. 1996). Historically, bioassays have relied on contact activity of the insecticide through the use of topical bioassays or surface treatments (i.e. glass vials/slides). However, the neonicotenoid insecticides have little contact activity and are most effective when ingested (Maienfisch et al. 2001). Currently, methods for delivering the active ingredients to the target site are limited. These bioassays demonstrate the effectiveness of using excised plant tissues from treated plants. With this bioassay, it will be important to use plant tissue of the same age and relative physiological condition to maintain homogeneity over time. Plant tissue age and physiological condition may influence uptake of the insecticide; and thereby, influence results of future bioassays.

In conclusion, this bioassay will provide an effective means for detecting and quantifying changes in cotton aphid susceptibility to the neonicotenoids. In the future, additional bioassays will be developed and cotton aphids will be obtained from various regions to test the repeatability of these techniques.

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J. Gore
United States Department of Agriculture, Agricultural Research Service Southern Insect Management Research Unit
Stoneville, MS

Detoxification of *Cyclocephala comata* Bates (Coleoptera: Scarabaeidae) to Pyrethroide and Phosphorated Insecticides

INTRODUCTION The State of Jalisco, Mexico is one of the most representative of this problem with an infested surface of approximately 200,000 hectares; affecting mainly the rain and stormwater harvesting areas. Inside the gender of whitegrubs the larvae of *Cyclocephala comata* Bates represent one of the main problems of phytosanitary character. To this day the main tools that the farmers have to combat this plague are the cultural and chemical control being the last one the most utilized, since in a beginning the organochlorine insecticides were used which were removed years ago of the market and to our days the active ingredients of common use are represented mainly by Organophosphates, Carbamates and recently the Pyrethroids have entered the market (PLM 1999 and PLM 2002). These products have been in the market for more than 15 years and have been used repetitively year after year showing high resistance levels since 1993 (Posos *et al*, 1995). For what to our days the dose has risen from 15 Kg. of insecticide up to 60 Kg. per

hectare without obtaining an effective control of the plague. Due to the fact that the resistance mechanisms developed by *C. comata* Bates to detoxification of the insecticides used in their control are unknown the main objectives of this work is to determine by the use of synergists the detoxification routes that *C. comata* has developed for the insecticides of the different toxicological groups used for its control.

MATERIALS AND METHODS The biological material of the population of *C. comata* was obtained from San Martin Hidalgo Jalisco, Mexico of commercial parcels of cultivations sowed in the region during the cycles Spring/Summer 2001 and 2002.

In the months of July and August, the collections of larvae of *C. comata* Bates carried out, were placed in black polyethylene bags, in which previously was added an earth proportion in mixture with organic matter and/or corn sprouts, so that the larvae had food and that at the same time similar conditions to their environment to be transferred to the laboratory. The biological material collected in field and settled down in the laboratory was placed in plastic boxes with earth and organic matter, and provided with food, selecting the larvae of the third instar according to the size so that they were the most homogeneous possible. They were weighed and separated in groups of 20 larvae, placed in boxes of disposable polypropylene with a mixture of earth and organic matter. We used the Technique of Topical Application proposed by the FAO. The insects were placed in polypropylene boxes provided with fresh food (Lagunes and Vázquez, 1994).

The evaluated treatments were the following ones: Chlorpyrifos and Diazinon (Organophosphates), Bifenthrin and Tefluthrin (Pyrethroids) in turn each one of the evaluated treatments were blended with the synergists Piperonyl Butoxide (PBO), Diethyl Maleate (DEM) and Merphos (DEF) in a proportion of 1:10.

For each insecticide treatment and of the mixture the active principles were used in technical grade using 8 dose ranges preparing 10 ml of each dose for each treatment. The active principles were diluted in acetone at 95% of purity grade reagent.

To be able to identify the detoxification routes involved bioassays they were run in two senses; The first ones to determine the lethal Dose 50 (LD_{50}) of the applied insecticides diluted only with acetone; in this case for each one of the involved insecticide treatments 6 dose ranges were run and 20 larvae were used for each dose range. To determine the second LD_{50} of the mixture of the insecticides with the three different synergists departing from the established LD_{50} for the insecticides; bioassay were run with the insecticide mixture and each one of the synergists, (DEF, DEM and PBO) in a proportion of 1:10 (1.0 gr a.i. of the insecticide for 10 gr a.i. of the synergist) having 6 - 10 smaller dose ranges starting from the determined LD_{50} for each one of the insecticides in question.

For each one of the carried out bioassays, 6 discriminatory doses and a witness without applying were run. To determine each one of the doses in question 20 larvae were used by dose. When mortality was presented in the witness without being applied we proceeded to calculate the % of mortality by means of Abbott's formula:

Once the bioassays were carried out, and the data of mortality calculated, they were analyzed by means of the Probit analysis method of maximum verisimilitude proposed by Finey 1971, These analyses were carried out using the SPSS Version 10.0 computer software.

RESULTS AND DISCUSSION The symptoms presented by the larvae of *C. comata* by means of which the approach of death was settled down 24 hours after having applied the insecticides are the following:

Lost of the mobility of the larva 3 hours after having applied the toxic. After six hours of having applied the insecticide the larva lost their form of "C" and took a yellow hyaline coloration. At 12 hours the larva lost swelling and movement, taking an intense yellow color. At 24 hours the movement of the larva was almost null, it turned flaccid and of clear brown color.

With what concerns to the Organophosphate insecticides as observed in Table 1 that for the case of Chlorpyrifos, it is observed that the main detoxification route is through FOM with a 17X, followed by esterase's with 9X, it is necessary to point out that in this case for the Glutation-S-Transferase are not strongly involved in the resistance mechanism for the Chlorpyrifos since in this case the synergism proportion was of only 4X.

Table 1. Lethal Dose average (LD_{50}), Synergism proportion and determination coefficients (R^2) and Tests of Squere Chi (X^2) of the Organophosphate insecticides used for the control of *C. comata* Bates.

Treatment	LD_{50} Mg/g	Synergism Proportion	R^2	X^2	Detoxification route
Chlorpyrifos	0.99029		0.88	0.0077	
Chlorpyrifos + PBO	0.05707	17.39X	0.88	0.1184	OXIDASES
Chlorpyrifos + DEF	0.80532	9.21X	0.96	0.1207	ESTERASES
Chlorpyrifos + DEM	0.46982	4.77X	0.77	0.0643	G-S TRANSFERASES
Diazinon	1.95919		0.88	0.0388	
Diazinon + PBO	0.97939	2.00X	0.99	0.189	OXIDASES
Diazinon + DEF	1.4579	1.34X	0.9	0.0368	ESTERASES
Diazinon + DEM	0.26783	7.31X	0.96	0.132	G-S TRANSFERASES

It is necessary to point out that this behavior of the insect to unfold Chlorpyrifos coincides with the works carried out by Georghiou et al., 1980 Bisett et al., 1991 and 2000).

For the case of the Diazinon, it is observed that the main detoxification route is through the Glutation-S-transferase with a proportion of 7X, what coincides with the information that reports Lagunes and Villanueva (1994), and on the other hand Rodríguez et al., (1999) found that in the glutation-S transferase a

high frequency exists for this resistance mechanism as happens in the case of the diazinon.

In the case of Table 2 that corresponds to the detoxification routes used by *Cyclocephala comata* to metabolize the pyrethroide insecticides, it is observed that the main route used by the insect is through FOM with a synergistic proportion of 10X for Bifenthrin and 23X for Tefluthrin, followed by the metabolic route of esterase's with a proportion of 10X in bifenthrin and 14X in Tefluthrin. It is necessary to point out that for this case bifenthrin has been only one year in the market and to this day it already presents characteristic samples of crossed resistance with organochlorine. What coincides with Bisset and Rodríguez 2000 where they demonstrated that the main routes of pyrethroid detoxification are through FOM and Esterasas.

Table 2. Lethal dose average (LD_{50}), Synergism proportion and determination coefficients (R^2) and Tests of Square Chi (X^2) of the pyrethroid insecticides used for the control of *C. comata* Bates.

Treatment	LD_{50} Mg/g	Synergism Proportion	R^2	X^2	Detoxification route
Bifenthrin	0.58925		0.84	0.0126	
Bifenthrin + PBO	0.05592	10.53X	0.7	0.2027	**OXIDASES**
Bifenthrin + DEF	0.05708	10.32X	0.87	0.085	**ESTERASES**
Bifenthrin + DEM	0.18746	3.14X	0.7	0.0453	G-S TRANSFERASES
Tefluthrin	0.80359		0.78	0.034	
Tefluthrin + PBO	0.03478	23.10X	0.75	0.071	**OXIDASES**
Tefluthrin + DEF	0.17826	14.97X	0.82	0.2093	**ESTERASES**
Tefluthrin + DEM	0.05368	4.50X	0.73	0.1099	G-S TRANSFERASES

CONCLUSIONS

1. Chlorpyrifos this being seriously affected mainly by Oxidases and in smaller proportion by esterases.
2. Diazinon this being affected strongly by the Glutation-S-transferase.
3. Bifenthrin this being affected mainly by Oxidases and esterases in the same proportion and in a smaller scale the Glutation-S-Transferase.
4. Tefluthrin this being affected drastically by the oxidizer system and in smaller proportion by the esterases and in minimum scale the Glutation-S-Transferase.
5. The larvae of *C. comata* have developed metabolic and physiologic resistance through Microsomal Oxidases of Mixed Function and in a smaller proprcion to the esterases, being these the main mechanisms of detoxification of the organophosphate and pyrethroid insecticides.

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P. P. Ponce, J. S. Santana , J. L. Martínez Ramírez, C. M. Duran Martínez, G. E. Cabral, and F. P. Luna
Universidad de Guadalajara, CUCBA
Departamento de Produccion Agricola

E. G. Rodríguez
Universidad Autonoma Agraria Antonio Narro

Premilinary data on resistance appearance of Pollen beetle PB (*Meligethes aeneus* F.) to selected pyrethroids, organophosphorous and chloronicotynyls insecticide, in 2004 year in Poland.

INTRODUCTION Poland is a major producer of rape (*Brassica napus*). This plant is cultivated on about 600 000 ha in this country but the average crop is low - 1000 000 t/year. Pollen beetle (PB) (*Meligethes aeneus* F.) is the most serious rape pest in Poland (on average 15% of yield losses per year) and considered to be the pest with the high likelihood of developping insecticide resistance. All classes of insecticide have been widely used to control PB in Poland. The long period of intensive selection pressure has lead to PB resistance to some chemical classes of insecticide.

For many years pyrethroids (deltamethrin, cypermethrin, alpha-cypermethrin, beta-cyfluthrin, bifenthrin, zeta-cypermethrin, lambda-cyhalothrin, esfenvalerate, fenvalerate, ethofenprox), organophosphorous (chlorpirifos, phosalone) held the primary place in the PB control in Poland. Over a period of 26 years these classes of insecticides have been most commonly used for controlling PB in Poland (97% of chemical sprays in rape cultivations). Nowadays the use of pyrethroids in Poland is systematically decreasing. The cases of observed PB resistance to pyrethroides classe under practical conditions increased.

Bioassays of alpha-cypermethrin, acetamiprid (chlotonicotynyls) and chloropiryphos + cypermethrin for resistance monitoring in PB were performed in western region of Poland (Institute of Plant Protection in Poznan) in 2004.

METHODS

Laboratory tests. In laboratory tests the standard method recommended by Insecticide Resistance Action Committee (IRAC method nr.7) was used.

The three insect populations originated from western Poland: Winna Gora, Skoki, Nowy Tomysl were selected. A representative sample of PB beetels in selected field populations and sufficient non-infested, untreated leaves were collected for testing. Chemicals:

- pyrethroide alpha-cypermethrin (Fastac 100 EC) 25, 33, 50 ppm concentrations were tested (recommended concentration in Poland: 25-50 ppm)
- chloronicotynoide acetamprid (Mospilan 20 SP) 50, 66, 100 ppm concentration were tested (recommended concentration in Poland: 50-100 ppm)
- phosphoroorganic + pyrethroide chlorpirifos + cypermethrin 500, 1000 ppm concentration of chorpirifos were tested (recommended concentration in Poland: 500-1500 ppm)

Accurate dilutions of the tested active substances from commercially available products were used in determined doses.

Rape inflorescence and leaves were dipped in water for untreated control and other leaves in tested insecticide concentration liquid for about five seconds and placed on paper towel to let it dry up. Untreated and treated dry leaves were placed into a 1 litre jar with 10 cm diameter filter paper and 100 PB beetles were placed in each jar. 3 replicates were conducted for each concentration and control.

A final assessment - lethal effects of active substance of insecticides were determined after 120 hours of application and expressed as percent mortality at each dose, correcting for untreated (control) mortalities using Abbott's formula (Abbot 1925). Untreated mortality was quoted.

At each assessment, beetles were classed as either: (a) unaffected, giving a normal response (such as taking a co-ordinated step) when gently stimulated by touch, or (b) dead or affected, giving an abnormal response to stimulation. Corrected Mortality = 100 x (P-C/100-C) where P = % mortality in treatment, C = % mortality in controls. Testes were performed in the laboratory conditions: 22-24° C and photoperiod of 16:8 (L:D).

2004 year results and discussion

Table 1.Susceptibility of PB beetles to pyrethroide - alpha-cypermethrin in 2004 (laboratory tests)

populations	active substance		
	25 ppm	33 ppm	50 ppm
	-% mortality	-% mortality	-% mortality
Winna Gora	25	38	45
Skoki	15	25	30
Nowy Tomyśl	10	20	40

Table 2. Susceptibility of PB beetles to chloronicotinyle insecticide (acetamiprid, Mospilan 20 SP) in 2004 (laboratory tests)

populations	active substance		
	50 ppm	66 ppm	100 ppm
	-% mortality	-% mortality	-% mortality
Winna Gora	78	90	95
Skoki	70	88	93
Nowy Tomyśl	75	85	90

Table 3. Susceptibility of PB beetles to organophosphorous + pyrethroide, (chloropiryfos + cypermethrin) Nurelle D 550 EC in 2004 (laboratory tests)

populations	active substance	
	500 ppm	1000 ppm
	-% mortality	-% mortality
Winna Gora	100	100
Skoki	100	100
Nowy Tomyśl	99	100

Populations from all three provinces demonstrated high level of resistance to pyrethroide insecticide, and some level of resistance to chloronicotinyle insecticide. In laboratory studies (2004) the pyrethroid insecticide and chloronicotinyle were less effective in controlling PB beetle. Survival at 25, 33,and 50 ppm concentration in case of pyrethroide and 50, 66 and 100 ppm in case of chloronicotinyle indicated occurence of resistance in tested populations.

The results indicated that populations tolerant to pyrethroids and chloronicotinyles were no cross-resistant to organophosphorous. The widespread use of pyrethroids in Poland can lead to control failure. Understanding the conditions which favour the development, the causes, and the mechanism of resistance are the crucial challenge for the future of pyrethroids and chloronicotinyle insecticides use to PB control in Poland.

The constant monitoring of PB susceptibility level to insecticides used in Poland and future studies on mechanisms of PB resistance to them will allow to enterprise a better strategy for delaying PB resistance. At present the general principles of strategy involve the limited use of pyrethroide group and rational application of all recommended insecticides and their rotation including different modes of their toxic action.

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P. Wegorek
Institute of Plant Protection in Poznan - Poland

Utilization of Trap Cropping, Neem and Nuclear Polyhedrosis Virus - a pest diversionary approach for the Management of Insecticide Resistance of *Helicoverpa armigera* (Hubner) in Cotton

ABSTRACT Push-pull strategy was used for the management of insecticide resistant *Helicoverpa armigera* (Hubner) with conjunctive use of trap crops, neem and Nuclear Polyhedrosis Virus in cotton. Application of NSKE on cotton leaving trap crops and restricted application of NPV on okra under trap cropping system in cotton was highly effective in reducing the incidence of *H. armigera* and damage to fruiting bodies, boll, locule and inter locule basis over cotton sole crop. The preference of *H. armigera* was towards okra as a trap crop compared to cotton. Application of NSKE on cotton diversified the *H. armigera* towards untreated okra. The synthetic pyrethroids resistance in field survived *H. armigera* at the end of the season was reduced from 89.2 - 94.5 percent to 86.0 - 92.0 percent.

KEYWORDS *Helicoverpa armigera*, Insecticide Resistance Management, push-pull strategy, cotton

INTRODUCTION American bollworm Helicoverpa *armigera* (Hubner) (Lepidoptera: Noctuidae) has become a very serious threat to cotton production in many countries. It has developed resistance to several pesticides used in India (Regupathy *et al*., 2004). So there is an urgent need for eco-friendly management practices to tackle this pest. The basic principle in pest management through cropping system is that any change in the vegetation will influence the population of pests and natural enemies (Altieri and Letourneau, 1982). In recent years use of various neem formulations are proved to be worthy in reducing

resistance to different insecticides in this pest (Regupathy and Ayyasamy, 2004). The virus infection was found to increase the susceptibility of *H. armigera* to the insecticides (Geetha *et al*., 1999 and Leethial and Regupathy, 2004). But, use of Nuclear Polyhedrosis Virus (NPV) on larger scale and on cotton due to leaf alkalinity possess certain practical problems (Ignoffo *et al.*, 1972 ; Young and Yearian, 1977 and Mcleod *et al*., 1977). Significant beneficial effects can be obtained when cultural methods, botanicals and biocontrol agents were combined. Push and pull strategy involves "pushing" the insects away from the economic crop and pulling them unto a trap crop where their population is reduced by a biological control agent. Keeping in view, investigations were carried out under field conditions to evaluate the push- pull strategy with conjunctive use of trap crop okra, neem and NPV for proper management of *H. armigera* in cotton.

MATERIALS AND METHODS Field experiment was conducted on cotton at Agricultural Research Station, Srivilliputtur, Tamil Nadu Agricultural University, Tamil Nadu during Summer, 2003 (March-July). The experiments were laid out in a randomized block design with three variants *viz*., trap crop (okra), neem seed kernel extract (NSKE5%) spray restricted on cotton (SVPR 2) leaving trap crop and HaNPV spray restricted on trap crop okra leaving cotton. The experiment was replicated three times by maintaining a plot size of 5 m x 5 m. In each plot having 10 rows of cotton (45 x 30 cm), fifth row was substituted with trap crop okra (Arka Anamika), which was sown simultaneously on the other side of the ridge without any loss to cotton cropped area. The trap cropping system was compared with the cotton sole crop. All the plots received recommended agronomic practices of the region except the treatment operations. NSKE 5% was applied on cotton leaving trap crop to diversify the pests to trap crop before each application of HaNPV commencing from 53 days after sowing (DAS) at weekly interval upto maturity phase (102 DAS). The application of HaNPV on trap crop okra / cotton sole crop (@ 500 LE) was commenced from one week after the application of NSKE spray (60 DAS) at weekly interval upto the maturity of the trap crop.

The bollworm incidence was assessed on the basis of egg, larval population and percent damage on fruiting bodies (squares, flowers and bolls), open bolls, locules and inter locules. Eggs and larvae were counted on 10 randomly selected tagged plants per plot. The total number of fruiting bodies and those damaged by bollworms were counted at ten randomly selected plants per replication. The total number of bolls collected from ten randomly selected plants per plot at each picking was assessed for number of damaged bolls, number of locules damaged, inter locule boring and percentage was worked out. Kapas were picked out at ten days interval from each plot and the yield was expressed in terms of q/ha. The preference ratio (PR) of pests on cotton and trap crop okra was worked out by using the formula given by Saminathan and Regupathy (2003). The efficacy of HaNPV on trap crops was assessed by counting total larval population and virosed larvae after respective treatment from ten randomly selected plants and the percent mortality was calculated. The resistance frequency of F1 field population of *H. armigera* before first spray and F1 field survived population at the end of the crop to synthetic pyrethroids was monitored using discriminating dose (DD) assays and the per cent resistance was calculated by using the formula given by Regupathy and Dhamu (2001).

RESULTS AND DISCUSSION In cotton sole crop (untreated check), the mean egg and larval population was 20.6 and 18.1 per ten plants respectively and percent damage of 19.7, 32.1 and 29.1 on fruiting bodies, boll and locule basis respectively. Combined use of trap crops, NSKE application on cotton and NPV application on trap crops reduced the incidence of *H. armigera* on cotton compared to cotton sole crop. Cotton (NSKE treated) + okra (NPV treated) effected 48.1 and 66.3 per cent reduction of eggs and larvae and 35.5, 38.0 and 38.5 per cent reduction of fruiting bodies, boll and locule damage respectively compared to cotton sole crop (untreated check). This was reflected in yield. Cotton (NSKE treated) + okra (NPV treated) plots recorded the maximum yield of 14.6 q/ha compared to 7.7 q/ha in cotton sole crop (untreated check). The efficacy of other treatments in reducing the incidence and damage of *H. armigera* was found in descending order such as cotton sole crop (NSKE and NPV treated) > cotton sole crop (NSKE treated) [3] cotton (NSKE treated) + okra (NPV untreated) > cotton (NSKE untreated) + okra (NPV treated) > cotton (NPV treated) [3] cotton (NSKE untreated) + okra (NPV untreated) [3] cotton sole crop (untreated).

Table 1: Effect of push-pull strategy with conjunctive use of trap crops, neem and HaNPV on bollworm incidence in cotton (Srivilliputtur, Summer 2003)

Treatments	Mean no. of eggs per 10 plants#	% reduction over untreated check*	Mean no. of larvae per 10 plants#	% reduction over untreated check*	% damage on fruiting bodies*	% reduction over untreated check*	% boll damage (open boll basis)*	% reduction over untreated check*	% locule damage*	% reduction over untreated check*	% inter locule damage *	% reduction over untreated check*	Kapas Yield (q/ha)
Cotton (NSKE treated) + okra (NPV treated)	10.7 (3.3)[b]	48.1 (43.8)[c]	6.1 (2.5)[a]	66.3 (54.5)[a]	12.7 (20.8)[a]	35.5 (36.5)[a]	19.9 (26.4)[a]	38 (38.0)[a]	17.9 (24.9)[a]	38.5 (38.3)[a]	9.9 (18.3)[a]	33.6 (35.3)[b]	14.6[a]
Cotton (NSKE treated) + okra (NPV untreated)	8.8 (3.0)[a]	57.3 (49.1)[a]	7.6 (2.8)[b]	58 (49.6)[c]	15.3 (23.0)[cd]	22.3 (28.1)[d]	22.4 (28.2)[b]	30.2 (33.3)[b]	21 (27.2)[b]	27.8 (31.8)[b]	8.8 (17.2)[ab]	40.9 (39.7)[a]	13.4[b]
Cotton (NSKE untreated) + okra (NPV treated)	19.1 (4.4)[d]	7.3 (12.2)[a]	12.3 (3.5)[c]	32 (34.4)[d]	18.1 (22.1)[ef]	8.1 (16.5)[b]	27 (31.2)[c]	15.9 (23.4)[c]	26 (30.6)[c]	10.7 (19.0)[b]	14.5 (22.3)[c]	2.7 (9.1)[a]	8.5[b]
Cotton (NSKE untreated) + okra (NPV untreated)	16.3 (4.1)[c]	20.9 (27.1)[b]	17.5 (4.2)[d]	3.3 (10.4)[e]	19.6 (26.2)[f]	0.5 (3.7)[a]	31.2 (33.9)[d]	2.8 (9.6)[b]	29 (32.5)[d]	0.3 (3.5)[a]	13.3 (21.3)[c]	10.7 (19.1)[c]	7.8[b]
Cotton (NSKE and NPV treated)	11.1 (3.3)[b]	46.1 (42.7)[c]	6.7 (2.6)[ab]	63 (52.5)[b]	13.5 (21.4)[ab]	31.5 (34.1)[c]	20.3 (26.7)[a]	36.8 (37.3)[a]	18.2 (25.2)[a]	37.5 (37.7)[a]	8.6 (17.0)[a]	42.3 (40.5)[a]	14.2[a]
Cotton (NSKE treated)	9.6 (3.1)[ab]	53.4 (46.9)[b]	7.9 (2.8)[b]	56.4 (48.6)[c]	14.7 (22.5)[bc]	25.4 (30.2)[c]	22.6 (28.3)[b]	29.6 (32.9)[b]	21.7 (27.7)[b]	25.4 (30.2)[b]	10.3 (18.6)[b]	30.9 (33.7)[b]	13.8[a]
Cotton (NPV treated)	19.3 (4.4)[d]	6.3 (14.5)[a]	12.3 (3.5)[c]	32 (34.4)[d]	16.8 (24.1)[de]	14.7 (22.5)[b]	26.9 (31.2)[c]	16.2 (23.7)[c]	25.7 (30.4)[c]	11.7 (19.9)[b]	14.1 (22.0)[c]	5.4 (13.3)[a]	8.8[b]
Cotton sole crop (untreated check)	20.6 (4.5)[d]	-	18.1 (4.3)[d]	-	19.7 (26.3)[f]	-	32.1 (34.5)[d]	-	29.1 (32.6)[d]	-	14.9 (22.9)[c]	-	7.7[b]

Figures in parentheses are square root transformed values * Figures in parentheses are arcsine transformed values

Means in a column followed by same letter(s) are not significantly different (P = 0.05) by DMRT

Application of NSKE on cotton diversified the *H. armigera* towards untreated trap crops. The egg and larval preference of *H. armigera* on cotton: okra increased from 1: 1.45 to 1: 3.29 and 1: 1.39 to 1: 3.34 towards okra respectively (Table 2). The percent recovery of NPV infected larvae on okra and cotton

ranged from 34.2 to 43.5 and 18.9 to 20.3 per cent in the respective treated plots (Table 3). The extent of resistance before spraying was 92.4, 91.8, 92.0, 94.5 and 89.2 per cent to cypermethrin, fenvalerate, deltamethrin, lambda-cyhalothrin and beta-cyfluthrin respectively. The resistance of field survived population at the end of the season was 89.1, 88.0, 86.9, 92.0 and 86.0 per cent to cypermethrin, fenvalerate, deltamethrin, lambda-cyhalothrin and beta-cyfluthrin respectively (Table 4).

Table 2.Effect of trap crops and restricted application of NSKE on cotton on the preference of cotton bollworms (Srivilliputtur, Summer 2003)

Cropping system	NSKE 5% spray on cotton	Crops	*H. armigera*			
			Eggs		Larvae	
			P	PR	P	PR
Cotton + okra	Cotton untreated with NSKE	Cotton	16.32	-	17.53	-
		Okra	23.77	01:01.4	24.37	01:01.4
	Cotton treated with NSKE	Cotton	8.75	-	7.6	-
		Okra	28.82	01:03.3	25.42	01:03.3
Cotton sole crop	Cotton untreated with NSKE	Cotton	20.62	-	18.13	-
	Cotton treated with NSKE	Cotton	9.62	-	7.93	-
P – Mean population per ten plants			PR – Preference ratio			

Push-pull strategy was found effective through integration of trap crops and bio control agents against maize stem borer *Chilo partellus* Surrinae (Swin) and integration of alarm pheromone and biocontrol agents against cotton aphid, *A. gossypii* (Pickett *et al.*, 1986). In the present study, application of NSKE on cotton in conjunction with NPV on trap crops under trap cropping system in cotton was most effective in controlling *H. armigera*. In our study okra acted as a good trap crop for *H. armigera* and the efficiency of the trap crop was improved by applying NSKE on cotton. Similar diversion of *A. devastans*, *B. tabaci*, *A. gossypii* and semiloopers from cotton to okra was observed when the non-edible oil formulations were applied on the main crop (Saminathan and Regupathy, 2003). In the conjunctive use of trap crops, NSKE on cotton and NPV on trap crops, the resistance of the field collected population of *H. armigera* to the pyrethroids monitored through discriminating (DD) dose showed lesser percent survival compared to the survival of the field-collected population before spraying. This might be due to the NPV infection of *H. armigera* collected from the field sprayed with microbials. The carry over of diseases through adults plays important role in the vertical transmission of virus over generations (Nair and Jacob, 1985 and Subramanian, 2003). Botanicals may be used to increase the susceptibility of the target pest. The exposure to a stressor might influence the susceptibility of the host to an active pathogen. The biologically active compounds from the plant products penetrate the gut wall, which allows the easy penetration of the pathogen into the haemocoel (Steinhaus, 1963). The non-chemical methods used in the present study in cotton provide scope for relaxation in selection pressure of *H. armigera* to certain extent. Hence, the percent survival is reduced at the end of the season, though significant is less than that of survival of larvae collected before first spraying. Combined use of botanicals with microbial pesticides and chemical insecticides increases the efficacy and also reduces the cost per application and delay the development of resistance.

Table 3. Effect of HaNPV spray on trap crops and cotton (Srivilliputtur, Summer 2003)

Treatments	Trap crop / cotton	Per cent NPV infected larvae on trap crops / cotton
Cotton (NSKE treated) + okra (NPV treated)	Okra	34.2 (35.8)^b
Cotton (NSKE treated) + okra (NPV untreated)	Okra	1.3 (6.7)^a
Cotton (NSKE untreated) + okra (NPV treated)	Okra	43.5 (41.2)^b
Cotton (NSKE untreated) + okra (NPV untreated)	Okra	1.1 (5.1)^a
Cotton (NSKE and NPV treated)	Cotton	20.3 (26.7)^c
Cotton (NSKE treated)	Cotton	0.76 (5.0)^a
Cotton (NPV treated)	Cotton	18.9 (25.7)^c
Cotton sole crop (untreated check)	Cotton	0.8 (5.2)^a

Figures in parentheses are arcsine transformed values
Means in a column followed by same letter(s) are not significantly different (P=0.05) by DMRT

Table 4. Effect of push - pull strategy with conjunctive use of trap crops, neem and HaNPV against resistance frequency (RF) of *H. armigera* to synthetic pyrethroids (Srivilliputtur, Summer 2003)

Synthetic Pyrethroids	DD dose (µg/µl)	% resistance of F_1 field population before first spray	% resistance of F_1 field survived population after last spray
		% resistance ±SE	% resistance ±SE
Cypermethrin	0.1	92.4 ± 3.6	89.1± 4.6
Fenvalerate	0.2	91.8 ± 3.9	88.0 ± 4.6
Deltamethrin	0.0125	92.0 ± 3.8	86.9 ± 5.0
Lambda -cyhalothrin	0.025	94.5 ± 3.1	92.0 ± 3.8
Beta-cyfluthrin	0.2	89.2 ± 4.1	86.0 ± 5.3

SE- Pooled binomial standard error (±)

ACKNOWLEDGEMENT The financial support from the Common Fund for Commodities (CFC) Europe, International Cotton Advisory Committee (ICAC) U.S.A and Natural Resource Institute (NRI), U.K. is acknowledged.

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P. Duraimurugan and A. Regupathy
Department of Agricultural Entomology
Tamil Nadu Agricultural University
Coimbatore 641 003
India

Studies to Elucidate Antibiosis Resistance in Selected Tomato Accessions against Fruitworm, *Helicoverpa armigera* Hubner (Lepidoptera:Noctuidae)

INTRODUCTION Tomato, an important tropical vegetable crop suffers mainly from tomato fruitworm (TFW) *Helicoverpa armigera* (Hubner), a polyphagous pest. It is the devastating pest of tomato and is capable of causing up to 88% damage to fruits (Singh and Narang, 1990). The only practical method to control this pest is to have a persistent insecticide over the foliage and fruiting bodies. As tomato is picked at short intervals, maintenance of insecticidal film is both uneconomical and hazardous. The ultimate solution to manage these pests lies in evolving and using insect resistant varieties which are of immense value in the integrated pest management programme. We have attempted to elucidate the antibiosis resistance mechanism in selected tomato accessions which exhibited relatively low infestation of TFW.

MATERIALS AND METHODS

Insect and Plant Materials

Larvae of *H. armigera* were reared on a meridic diet, prepared from chickpea (*Cicer arietinum*) flour. All insects were reared in controlled conditions at room temperature (25°C-30°C) and humidity (60% to 75% RH) with 12 h light 12 h dark photoperiod. After hatching, larvae were placed with diet until the early third instar. They were then individually reared to pupation in clear plastic cups (4.5 cm high and 3 cm wide) with lids. On pupation, they were sexed and placed in insect cages (120 X 90 X 90 cm) for adult emergence, from which the larvae were used for experiments. Tomato accessions, categorized on their reaction to TFW (Srinivasan, 2003) as being resistant (LE 355 and LE 1223), moderately resistant (LE 2, LE 10 and LE 104), moderately susceptible (LE 3, CO 3, PKM 1, LE 18 and LE 887) and highly susceptible (LE 4), were grown in a greenhouse. After six weeks, seedlings were transplanted to plastic pots (18 cm dia and 17 cm in height) and were watered daily. Fruit that were harvested were used for larval bioassays.

Larval Bioaasays

The third instar larvae were taken from the nucleus culture, which was maintained on semi synthetic diet under controlled conditions. The larvae were released in plastic cups containing known weight of fresh fruits of different tomato accessions. Test of each accession were replicated five time involving twenty-five larvae in each replicate. Before releasing the larvae in the cups, the initial fresh weight of larvae was taken. The weight of larvae, uneaten fruits and faecal matter was taken when the larvae entered fourth instar stage. The insect's weight gain/loss was calculated by subtracting its initial weight from its weight at the end of the experiment. Food ingested was determined by subtracting the weight of uneaten food from weight of food provided. All faeces were separated from uneaten food and weighed. From the results, following indices were obtained as proposed by Waldbauer (1968).

- Relative growth rate (RGR) = Weight gained by the larva / (Weight of initial biomass X day)
- Relative consumption rate (RCR) = Weight ingested by the larva / (Weight of initial insect biomass X day)
- Efficiency of conversion of ingested food to body substance (ECI)

- ECI = [(Weight gained by the larva) / (Weight of food ingested)] X 100

- Efficiency of conversion of digested food to body substance (ECD)

- ECD = [(Weight gained by the larva) / {(Weight of food ingested) - (Weight of faeces)}] X 100

- Approximate digestibility (AD)

- AD = [{(Weight of food ingested) - (Weight of faeces)} / (Weight of food ingested)] X 100

RESULTS AND DISCUSSION The growth rate indicates that the rate at which the digested food is available to the insect and ultimately the rate of increase in weight per gram body weight per day. The results on growth rate revealed that the highest growth rate (1.198) was recorded with larvae fed on LE 887, which was significantly superior to all other accessions (Table 1). This was followed by larvae fed on LE 2, while larvae reared on LE 355, LE 3, LE 18 and LE 10 recorded significantly lower RGR values. This indicates that the accessions LE 3, LE 10, LE 18 and LE 355 are the most inferior food for the development of *H. armigera*. In general, tomato is not preferred by most of the lepidopteran insects because of its poor nutritional qualities as well as toxicity due to secondary metabolites (Singh and Parihar, 1988; Katole, 1992; Singh and Sehgal, 1993 and Ming and Jun, 2001).

Table 1. Consumption and utilization of different tomato accessions by larvae of *H. armigera*

S. No	Cultivar	RGR	RCR	ECI (%)	ECD (%)	AD
1	LE 4	0.206 ^{ab}	2.36 ^{ab}	19.42 ^{ab}	24.09 ^{a}	82.33 ^{bcd}
2	Co 3	0.512 ^{ab}	5.778 ^{abc}	9.19 ^{ab}	13.66 ^{a}	70.80 ^{bcd}
3	LE 2	0.802 ^{bc}	11.028 ^{d}	6.09 ^{ab}	11.32 ^{a}	59.78 ^{ab}
4	LE 1223	0.446 ^{ab}	7.06 ^{bc}	7.08 ^{ab}	29.49 ^{a}	37.36 ^{a}
5	PKM 1	0.610 ^{ab}	6.038 ^{abc}	11.22 ^{ab}	15.16 ^{a}	59.49 ^{ab}
6	LE 887	1.198 ^{c}	10.494 ^{cd}	6.27 ^{ab}	12.73 ^{a}	38.11 ^{a}
7	LE 104	0.298 ^{ab}	5.10 ^{ab}	6.82 ^{ab}	7.57 ^{a}	90.14 ^{d}
8	LE 355	0.100 ^{a}	1.22 ^{a}	23.25 ^{a}	37.39 ^{a}	61.89 ^{abc}
9	LE 3	0.132 ^{a}	3.11 ^{ab}	4.44 ^{ab}	4.90 ^{a}	89.61 ^{d}
10	LE 18	0.118 ^{a}	3.41 ^{ab}	2.04 ^{a}	3.73 ^{a}	87.01 ^{cd}
11	LE 10	0.150 ^{a}	1.296 ^{a}	11.84 ^{ab}	27.25 ^{a}	43.98 ^{a}

Means followed by same letter(s) are not significantly different by DMRT (p=0.05)

The relative consumption rate (RCR) indicates the rate of feeding in relation to the weight of insect in a definite time. The results of relative consumption rate (RCR) showed that the accession LE 2 recorded the highest RCR value (11.03), which was significantly higher to all other accessions. This was followed by LE 887; LE 10 and LE 355 showed the least RCR values and were significantly different from other accessions. The highest consumption in LE 2 might be due to the presence of tender and juicy fruits. Waldbaeur (1968) observed that the rate of feeding in insects is limited by the response to bulk water content and other physico-chemical properties of whole fresh food. Singh and Sehgal (1993) also observed a similar trend with different host plants against Spilosoma obliqua Walker.

The measurement of efficiency of conversion of ingested food (ECI) indicates the overall efficiency of the insect to utilize the food for growth. It fluctuates with approximate digestibility and efficiency of conversion of digested food. Highest ECI value of 23.25 was observed with larvae fed on LE 355, which was on par with LE 18 recording 22.04%. The data on efficiency of conversion of digested food (ECD) showed that the tomato accessions did not show statistical variations. However, LE 355 recorded the highest value of 37.39%. From these results, it is clear that the accession LE 355 is comparatively superior host plant to tomato fruitworm, though it has recorded the lowest RCR and RGR.

Highest digestibility was exhibited for LE 104 (90.14%), which was on par with LE 3 (89.61%). This was followed by LE 18 recording 87.01%. The least digestibility was observed in larvae fed with LE 1223 (37.36%), which was on par with LE 887 and LE 10 recording 38.11 and 43.98%, respectively. The variation in the digestibility of food materials is dependent on the physical and chemical nature of host plants. Waldbaeur (1964) had suggested the variation in digestibility comprise the factors such as nutrient deficiency, imbalance, and highest content of crude fibres and deficiency of water. Thus, the results on consumption rate, growth rate and digestibility indicated that they did not have any definite, conclusive patterns with the resistance reaction of the selected tomato accessions; hence, the resistance reaction of the selected tomato accessions may collectively be attributed to secondary metabolites which are correlated to antibiosis and glandular and non-glandular trichomes which are correlated to antixenosis, but with no single factor having control over the resistance reaction.

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R.Srinivasan and S.Uthamasamy
Department of Agricultural Entomology
Tamil Nadu Agricultural University, Coimbatore 641 003
TN., INDIA

Susceptibility evaluation of termites, *Odontotermes obesus* (Rambur) against the fungal entomopathogens.

ABSTRACT Subterranean termites, *Odontotermes sp*. is identified as an important pest in central and north India causing damage to the extent of 60 per cent in several economically important crops like Groundnut, Maize, Wheat, Sugarcane and Cotton. Increased environmental concern due to several unintended repercussions of persistent organochlorines and cyclodiens like Aldrin, Dieldrin, Chlordane and Heptachlor though, the most effective and long lasting insecticides for the control of termites, has prompted for need of screening of safer, ecofriendly alternative, like biocontrol agents. Present contribution reports the *in vitro* efficacy of two of the most potent fungal entomopathogens, *Metarhizium anisopliae* and *Beauveria bassiana* along with Chlorpyriphos, the standard check against assorted population of worker termites.

Chlorpyriphos, the standards check registered lowest median lethal concentration and time to inflict 50 per cent mortality in worker termite population. *Beauveria bassiana* and *Metarhizium anisopliae* strain M.a.-4 showed promising traits as a potent biocontrol agent, in all the growth phases. The potency of entomopathogens increased from growth phase I (21days old culture) to phase II (28 days old culture) and then declined slightly in phase III. (34 days old culture) though, statistically not comparable with standard check.

INTRODUCTION Subterranean termite is a polyphagous pest infesting several economically important crops, agroforestry, human structures and almost all the human belongings causing damage worth billions of rupees (Mishra, 1999). Chotani, 1997 reported various economically important genera of termites including *Odontotermes sp.* attacking annual as well as perennial crops especially in the semi arid and sub humid tropics inflicting significant yield losses.

Though, application of persistent insecticides at the site of active infestation is the most recommended management practice, very little is known about their toxicity, longevity, vapor pressure, water solubility or degradability in the environment. (Smith, 1979). Increased environmental concern about the adverse effects of organochemicals has made it imperative to reveal an effective, economical and safe alternative to the agro chemicals. (Logan *et al.*, 1990).

Promising traits of muscardine fungus, *Metarhizium anisopliae* (Metsch.) and *Beauveria bassiana* (Bals.) viz., viability of conidia in soil and the ability of fungal propagules to penetrate the cuticle of termites (Strack, 2002) along with slow acting nature like successful chemicals, ability of fungal spores to spread among social termites and self replicability has rendered it a status of most potent bio control agent against termites, *Odontotermes sp.* (Milner *et al.*, 1996, Grace *et al.*, 1992).

Thus, present study was framed with an attempt to evaluate the *in vitro* bioefficacy of entomopathogenic fungus against assorted population of worker termites, *Odontotermes obesus* (Rambur).

MATERIALS AND METHODS Collection of termites was done with the help of 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°C with relative humidity of 80 - 90 per cent. Due care was taken for acclimatization of worker termites before proceeding for the bioassay (Gurusubramanian *et al*., 1999).

Four dilution of each entomopathogenic fungi of three different growth phases (21, 28, and 35 days old culture) along with five dilution of Chlorpyriphos, the recommended insecticides were evaluated for different bioefficacy parameters like median lethal concentrations (LC_{50}) and median lethal time (LT_{50}) against the assorted population of worker termites. Four strains of *M. anisopliae*, M.a.-1 (local isolate from Nagpur region), M.a.-2. (Procured from Central Institute for cotton Research, Nagpur), M.a.-3 (Procured from Department of Plant Pathology, College of Agriculture, Nagpur), M.a.- 4(Procured from Indian Agriculture Research Institute (IARI),

New Delhi) and one strain of *Beauveria bassiana* (Procured from Department of Plant Pathology, College of Agriculture, Nagpur) were evaluated at spore load of order 10^5, 10^6,10^7 and 10^8 spores/ml.

Five dilutions of Chlorpyriphos and four dilutions of entomopathogens were prepared by serial dilution method in distilled water. 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 25 worker termites replicated four times was subjected to each concentration of entomopathogens and insecticide. The dilutions were applied to the worker population @ 2 ml/cup with the help of hand atomizer, whereas, the control set was sprayed with same quantity of distilled water to record any natural mortality in worker population.

Termites under evaluation were maintained at 25 ± 2°C temperature and 80-90 per cent relative humidity. The mortality count was recorded at 24 hours interval 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).

RESULTS AND DISCUSSION Concentration mortality regression analysis (LC_{50}) of fungal entomopathogens against assorted population of worker termites in phase I (Table 1) reveals significant superiority of *Beauveria bassiana* strain in terms of lowest LC_{50} value (1.5×10^5 spore/ml). *Metarhizium anisopliae* strain M.a.-1 was the next effective treatment, the best strain among four *Metarhizium* strains used during the experimental period. Rest of the strains had LC_{50} value of 6.6×10^7 spores/ml (M.a. -2), 7.8×10^7 spores/ml (M.a. -4) and 5.9×10^8 spores/ml (M.a. -3) in phase I (21 days old culture), respectively. The *Beauveria bassiana* and *M. anisopliae* strain M.a.-4 inflicted 90 per cent mortality at a concentration of 4.1×10^9 and 1.3×10^{11} spores/ml, respectively.

Phase II (28 days old culture) reflected significant superiority of *B. bassiana* (by non overlap of fiducial limits) in terms of lower spore count (4.2×10^4 spores/ml) to inflict 50 per cent mortality in assorted population of worker termites over other entomopathogenic treatments except *M. anisopliae* (M.a.-4 strain) with LC_{50} value of 8.7×10^4 spores/ml, statistically on par with rest of the entomopathogenic treatments. The other *M. anisopliae* strains succeeding the efficacy trend were M.a. - 1 (9.3×10^5 spores/ml), M.a.-2 (1.4×10^7 spores/ml) and M.a.-3 (6.4×10^7 spores/ml). In phase II the LC_{90} of effective entomopathogenic treatments were 2.0×10^7 spores/ml for *B. bassiana* and 1.1×10^9 spores/ml in case of M.a.-4 strain.

Table 1 : Concentration mortality (LC_{50}) response of *Odontotermes obesus* for phase I, phase II and phase III.

Phase	Treatment	n	LC_{50} (FL)	LC_{90} (FL)	Slope	Heter.	Chi squre
Phase I	M.a.-1	500	1.6×10^7 ($3.0 \times 10^6 - 1.3 \times 10^8$)	4.2×10^{11} ($4.0 \times 10^7 - 6.0 \times 10^{15}$)	0.33 ± 0.1	1.93	3.85
	M.a.-2	500	6.6×10^7 ($1.8 \times 10^7 - 8.9 \times 10^8$)	4.5×10^{11} ($1.6 \times 10^{10} - 6.1 \times 10^{14}$)	0.27 ± 0.5	0.44	0.87
	M.a.-3	500	5.9×10^8 ($5.9 \times 10^7 - 2.3 \times 10^{11}$)	9.7×10^{12} ($3.7 \times 10^{10} - 1.3 \times 10^{15}$)	0.34 ± 0.9	0.15	0.31
	M.a.-4	500	7.8×10^7 ($1.5 \times 10^6 - 3.6 \times 10^7$)	1.3×10^{11} ($6.3 \times 10^9 - 1.7 \times 10^{14}$)	0.29 ± 0.1	0.28	0.56
	B.b.-1	500	1.8×10^5 ($6.0 \times 10^5 - 8.7 \times 10^5$)	4.1×10^9 ($1.6 \times 10^6 - 8.9 \times 10^{11}$)	0.29 ± 0.1	0.15	0.3
	Chlorpyriphos	600	0.009 (0.002 – 0.013)	0.201 (0.03 –13.02)	1.54 ± 0.01	2.2	6.61
Phase II	M.a.-1	500	9.3×10^5 ($1.8 \times 10^5 - 2.5 \times 10^6$)	2.6×10^9 ($2.3 \times 10^8 - 8.1 \times 10^{11}$)	0.42 ± 0.1	0.3	0.59
	M.a.-2	500	1.4×10^7 ($1.2 \times 10^5 - 2.2 \times 10^7$)	1.5×10^{11} ($5.7 \times 10^9 - 6.7 \times 10^{14}$)	0.24 ± 0.1	0.62	1.24
	M.a.-3	500	6.4×10^7 ($1.2 \times 10^6 - 2.8 \times 10^8$)	3.6×10^{12} ($3.9 \times 10^{10} - 1.7 \times 10^{15}$)	0.29 ± 0.1	0.86	1.71
	M.a.-4	500	8.7×10^4 ($4.3 \times 10^4 - 3.8 \times 10^5$)	1.1×10^9 ($2.6 \times 10^8 - 1.4 \times 10^9$)	0.23 ± 0.5	0.08	0.17
	B.b.-1	500	4.2×10^4 ($1.2 \times 10^3 - 3.2 \times 10^5$)	2.0×10^7 ($1.5 \times 10^6 - 4.5 \times 10^7$)	0.48 ± 0.1	1.32	2.63
	Chlorpyriphos	600	0.001 (0.001 – 0.005)	0.49 (0.104 – 1.58)	1.50 ± 0.8	1.23	3.7
Phase III	M.a.-1	500	2.2×10^6 (4.8×10^4-3.6×10^6)	5.7×10^9 ($4.5 \times 10^8 - 2.3 \times 10^{13}$)	0.29 ± 0.01	1.81	3.62
	M.a.-2	500	2.3×10^7 (7.0 x106- 1.2 x108)	1.8×10^{11} (7.0 x106- 1.2 x108)	0.33 ± 0.7	0.22	0.43
	M.a.-3	500	3.4×10^7 ($7.3 \times 10^6 - 3.1 \times 10^8$)	3.7×10^{12} ($4.1 \times 10^{10} - 1.4 \times 10^{15}$)	0.26 ± 0.1	0.39	0.78
	M.a.-4	500	6.2×10^4 ($1.0 \times 10^4 - 3.5 \times 105$)	5.9×10^8 ($1.6 \times 10^5 - 7.9 \times 10^{13}$)	0.26 ± 0.1	0.14	0.29
	B.b.-1	500	3.6×105 ($3.0 \times 10^4 - 1.3 \times 10^6$)	2.4×10^9 ($3.8 \times 10^8 - 1.2 \times 10^{11}$)	0.34 ± 0.1	0.47	0.93
	Chlorpyriphos	600	0.001 (0.0008 – 0.003)	0.142 (0.07 – 3.36)	1.55 ± 0.1	1.74	5.21

n = No. of worker termite treated. LC_{50} = Median lethal concentration. FL = Fiducial limit

P = 0.05 @ 4 df = 9.488 and at 5 df =11.070 and P = 0.1 @ 4 df = 7.779 and at 5 df = 9.236

The Chi square values were compared with table values at respective degree of freedom to check for any real deviation.

Application of M.a.-4 in phase III (35 days old culture) proved to be the most effective strain (6.2×10^4 spores/ml) over *B. bassiana* (3.6×10^5 spores/ml), the most effective strain in phase I and II. Rest of the treatment can be arranged in descending order of their efficacy as M.a.-1 (2.2×10^6 spores/ml), M.a.-2 (2.3×10^7 spores/ml) and M.a. -3 (3.4×10^7 spores/ml). Dominance of *B. bassiana* and *M. anisopliae* M.a.-1 in terms of efficacy to inflict 90 per cent mortality at lower concentration was observed in phase III. The LC_{90} for *M. anisopliae* M.a.- 4 (5.9×10^8 spores/ml) and *B. Bassiana* (2.4×10^5 spores/ml) was much lower as compared to rest of the entomopathogen treatments.

Application of Chlorpyriphos, the standard check registered LC_{50} value of 0.009 per cent, 0.001 per cent and 0.001 per cent in phase I, II and III, respectively. The recommended insecticide was able to inflict 90 per cent mortality in assorted worker population at a concentration of 0.201 per cent, 0.490 per cent and 0.142 per cent in phase I, II and III, respectively. The steep slopes reflect the homogenous response of the workers to the pesticide formulation.

The LC_{50} value of entomopathogenic strains indicates towards a pattern wherein the potency increased from phase I to phase II and then declined in phase III (Gurusubramanian *et al.*, 1999). Same conclusion can be inferred from the data on time mortality response. Inverse correlation between the concentration of the entomopathogens used and time mortality response was also observed indicating support to the former hypothesis. Swaran and Varma, 2003 reported higher infectivity of soil mixed *Metarhizium anisopliae* conidia (LC_{50} 9.07 X 10^6 spores/gm of soil) under laboratory condition.

Attainment of mortality up to 100 per cent in termite population with various strains of *Metarhizium anisopliae* (Wright *et al*., 2002) at lethal concentration like 2 $X10^7$ spores/ml (Ramkrishnan, 1999) was in corroboration with the present findings.

Rosengaus *et al*., 1999 observed that higher spore concentration of *Metarhizium anisopliae* (2.2X 10^8 spores/ml) was more lethal to the termite population whereas significant survival in some of the nymphs was observed for longer duration when subjected to lower (10^6 spores/ml) concentration. Highest infectivity of both the entomopathogens was observed at a temperature regime of 25-30°C (Khan et al., 1993a). *B. bassiana*, the white muscardine fungus was more effective in terms of higher mortality, at lower concentration and in a short span of time (Khan *et al*., 1993b, Wright *et al*., 2000), in line with the present findings.

Data in table 2 reflects the time mortality response of *Odontotermes obesus* in all the three growth phases of entomopathogens. The bioassays with Chlorpyriphos registered lowest time to achieve 50 per cent mortality in assorted population of worker termites. Graded pesticide response registered LT_{50} value of 0.97, 1.5 and 1.1 days for 2 per cent, 1.7, 1.9 and 1.6 days for 0.2 per cent, 2.3, 2.5 and 2.0 days for 0.02 per cent, 3.2, 4.1 and 3.1 days for 0.002 per cent, and 13.5, 13.4 and 12.2 days at a pesticide solution strength of 0.0002 per cent in phase I, II and III, respectively. The recommended dose of 0.02 per cent had the LT_{50} in the range of 2.0 - 2.5 days. The mediun lethal time to register 90 per cent mortality was in the range of 1.6 - 6.4 days (2 per cent), 10.1 - 14.8 days (0.2 per cent), 14.2 - 18.8 days (0.002 per cent), 36.0 - 44.5 days (0.002 per cent) and 41.1 - 49.9 days for 0.0002 per cent in phase I, II and III, respectively.

In case of entomopathogenic evaluation *Beauveria bassiana* took the least time for attainment of 50 per cent mortality in worker population. The LT_{50} values at highest spore load (10^8 spores/ml) in phase I-III were 3.4, 2.8 and 2.8 days, respectively. The LT_{50} at lower spore load (10^5 spores/ml) increased significantly to 6.0, 5.1 and 5.9 days in three phases, respectively. The *Metarhizium anisopliae* strain, M.a. - 4 recorded lowest LT_{50} (4.0 days) in phase - I followed by M.a.-1 (4.4 days), M.a. - 3 (5.6 days) and M.a. - 2 (6.3 days), respectively. The time taken by lowest spore load screened differed statistically with LT_{50} of 11.4 days for M.a.-1, 11.1 days for M.a.-2 and 11.8 days for M.a.-3 as against 11.5 days for M.a.- 4, the most effective treatment in phase I.

Table 2 : Time mortality response (LT_{50}) of *Odontotermes obesus* , for phase I, phase II and phase III.

Treatment and Conc.	LT_{50} (Days)	LT_{50} (Days)	Slope	LT_{50} (Days)	LT_{50} (Days)	Slope	LT_{50} (Days)	LT_{50} (Days)	Slope
M.a.-1 10^{02}	11.4 (8.27 – 22.27)	57.2	1.82 ± 0.03	6.0 (5.25 – 6.99)	13.2	2.13 ± 22	6.1 (5.33 – 7.24)	23.7	2.79 ± 35
106	6.4 (5.67 – 7.6)	25.9	2.25 ± 0.24	4.6 (4.05 – 4.99)	11.4	2.33 ± 21	5.0(4.52 – 5.61)	15.8	2.49 ± 29
107	5.3 (4.78 – 6.0)	18.1	2.4 ± 0.23	3.9 (3.54 – 4.31)	11.6	2.37 ± 21	4.9(3.93 – 7.08)	13.6	3.45 ± 28
108	4.4 (4.05 – 4.86)	13.3	2.68 ± 0.23	2.8 (2.47 – 3.05)	9.1	2.42 ± 20	4.0(3.03 – 5.38)	9.3	3.61 ± 26
M.a.-2 10^{7}	11.1 (8.95 – 16.18)	36.8	2.47 ± 0.86	8.6 (7.43 –10.86)	25.1	2.76 ± 54	10.7(7.89 – 20.55)	47.5	1.98 ± 27
106	6.6 (5.2 6 – 10.64)	26.6	2.57 ± 0.31	6.7(5.93 – 7.82)	21.1	2.54 ± 26	8.0(6.80 – 9.64)	25.8	2.47 ± 28
107	7.9 (6.79 – 9.67)	26.2	2.49 ± 0.28	6.1(5.18 – 7.53)	18.7	2.61 ± 25	6.0(5.27 – 6.94)	25.8	2.20 ± 23
108	6.3 (5.63 – 7.40)	21.7	2.39 ± 0.25	4.5(4.29 – 6.62)	13.8	2.99 ± 26	5.2(4.01 – 5.18)	21.1	1.91 ± 20
M.a.-3 10^{7}	11.8 (9.20 – 18.19)	51.4	2.00 ± 0.29	9.3 (7.78 – 2.20)	31.7	2.40 ± 30	9.8(8.07 – 13.32)	36.4	2.26 ± 30
106	8.5 (7.18 – 11.13)	34.7	2.1 ± 0.26	6.5(5.65 – 7.94)	29.2	1.96 ± 22	7.9(6.83 – 10.11)	36.2	2.19 ± 20
107	7.2 (6.26 – 8.89)	28.5	2.15 ± 0.24	5.3(4.77 – 6.10)	19.9	2.24 ± 22	8.2(6.94 – 10.70)	30.7	1.99 ± 24
108	5.6 (4.99 – 6.56)	22.8	2.11 ± 0.22	4.7(4.29 – 5.31)	16.5	2.36 ± 23	4.6(4.03 – 5.23)	21.6	1.89 ± 20
M.a – 4 10^{7}	11.5 (8.94 – 17.38)	53.5	1.91 ± 0.27	7.2(6.32 – 8.55)	22.8	2.55 ± 27	8.4(7.15 – 10.80)	31.4	2.24 ± 27
106	6.7 (5.83 – 8.12)	27.5	2.09 ± 0.24	5.8(4.92 – 7.20)	15	3.08 ± 28	5.9(5.36 – 6.75)	18	2.65 ± 26
107	4.6 (3.8 – 6.9)	17.3	1.1 ± 0.18	4.5(3.80 – 5.81)	10.3	3.57 ± 28	5.4(4.64 – 6.55)	15	2.88 ± 26
108	4.0 (3.62 – 4.90)	15.6	2.17 ± 0.20	3.9(3.35 – 4.57)	9.1	3.53 ± 26	5.2(4.15 – 7.36)	12.2	3.46 ± 29
Bb.-1 10^{7}	6.0 (5.25 – 6.99)	23.7	2.19 ± 0.28	5.1(4.64 – 5.78)	15.6	3.44 ± 28	5.9(5.25 – 6.88)	22	2.24 ± 23
106	4.4 (4.00 – 4.90)	15.2	2.38 ± 0.21	4.0(3.62 – 4.49)	12.1	2.17 ± 20	4.4(4.00 – 4.90)	15.2	2.38 ± 21
107	3.7 (2.73 – 4.85)	12.5	2.25 ± 0.20	3.3(2.99 – 3.67)	12.1	2.28 ± 26	3.4(2.73 – 4.85)	7.9	3.89 ± 26
108	2.4 (2.46 – 3.05)	9.7	2.23 ± 0.20	2.8(2.48 – 3.05)	9.3	2.42 ± 20	2.8(2.18 – 4.72)	7.2	2.92 ± 25
Chlor **2.0** %	0.97 (0.86 – 1.06)	1.6	6.17 ± 0.85	1.5(0.93 – 1.94)	6.4	2.03 ± 19	1.1(0.49 – 1.54)	2.8	3.19 ± 2.8
0.20%	1.7 (1.26 – 2.0)	12.1	1.47 ± 0.18	1.9(1.33 – 2.31)	14.8	1.45 ± 18	1.6(1.25 – 1.93)	10.1	1.60 ± 18
0.02%	2.3 (1.88 – 2.71)	17.5	1.46 ± 0.18	2.5(2.04 – 2.88)	18.8	1.48 ± 18	2.0(1.57 – 2.34)	14.2	1.49 ± 18
0.00%	3.2 (2.62 – 2.88)	39.9	1.17 ± 0.17	4.1(3.40 – 4.99)	44.5	1.23 ± 18	3.1(2.53 – 3.72)	36	1.20 ± 17
0.00%	13.5 (2.26 – 70.64)	41.1	0.66 ± 0.18	13.4(8.04 – 3.26)	49.9	0.73 ± 18	12.2 (7.42 – 49.60)	844.1	0.69 ± 18
Control	22.8 (13.7 – 92.46)	83.8	2.20 ± 0.55	16.8 (11.37 – 31.01)	43.2	3.11 ± 78	17.7 (11.72 – 36.92)	48.2	2.93 ± .73

n = Number of worker termites treated. LT_{50} = Median lethal time. **FL** = Fiducial limit

The Chi-square values (not shown here) were compared with table values at respective degree of freedom to check for any real deviation.

Phase II also had least median lethal time of 2.8 days recorded by *B. bassiana* at highest spore load statistically on par with recommended dose of Chlorpyriphos (0.02 per cent). The lower spore load reflected LT_{50} of 3.3 days (10^7 spores/ml), 4.0 days (10^6 spores/ml) and 5.1 days (10^5 spores/ml), respectively. M.a.-1 at highest spore load 10^8 spores/ml recorded LT_{50} of 2.8 days on par with *B. bassiana* whereas, rest of the *M. anisopliae* treatments had LT_{50} of 3.9 days (M.a. - 4), 4.5 days (M.a.- 2) and 4.7 days (M.a. - 3), statistically inferior over efficacy of *Beauveria bassiana* and M. a.-1 The LT_{50} at lowest *Metarhizium anisopliae* spore load for the treatments were of the order 6.0, 7.0, 8.6 and 9.3 days, respectively.

Efficacy of all the entomopathogens declined in phase III. Statistically there was no difference in efficacy of *Beauveria bassiana* over other entomopathogens treatments except at spore load of 10^5. The LT_{50} of 2.8, 3.4, 4.4 and 5.9 days at spore load of 10^8 -10^5 spore/ml reflected over all superiority of *Beauveria bassiana* as a potent entomopathogens against *Odontotermes obesus*. The *M. anisopliae* strains were also equally potent, especially M.a.-1 and M.a. - 3 with LT_{50} value of 4.0 and 4.6 days, respectively, statistically on par with *Beauveria bassiana*. The other *M. anisopliae* strains M.a. - 2 and M.a. - 4 registered LT_{50} of 5.2 days. The control set had the LT_{50} of 22.8, 16.8 and 17.7 days in phase I, II and III, respectively, indicating natural mortality trend in worker termites.

Though variation in strains of entomopathogren and termite population used reflects different median lethal time, generally 50 per cent mortality was inflicted within 3 - 4.8 days after application of the treatments (Swaran and Varma, 2003, Wright *et al.*, 2000, Rosengaus et *al*., 1999). Efficacy of Chlorpyriphos as a termiticides, for seed treatments or soil application is beyond doubts. (Mishra, 1999, Su and Scheffrahn, 1990,1992 and Su *et al*., 1982. It has almost replaced the persistent organochlorines and cyclodienes (Goyal, 1999 and Khoo and Sherman, 1979). The results of the present laboratory evaluation

of entomopathogens are promising enough to proceed for further trials, a step towards more ecofriendly pest management system.

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T. D. Kakde, R. M. Wadaskar, S. P. Gawande and S. J. Gaikwad
College of Agriculture, Nagpur

Dr. P. D. K. Vidyapeeth
Akola 444 104 (MS), India

Evaluation of Rapeseed-Mustard Genotypes for Resistance to White Rust (*Albugo candida*)

ABSTRACT Out of total 90 genotypes of rapeseed-mustard evaluated, 38 were found free from infection and remaining categorized as moderately resistant, resistant, susceptible and highly susceptible. Both RH-30 and T-59 cultivar grown on large scale in India were found highly susceptible.

KEYWORDS Rapeseed, Genotype, Albugo *candida*, Resistance

INTRODUCTION White rust is a widely distributed disease on a large number of cruciferous crops caused by Albugo candida, an obligate, host-specific pathogen. Rapeseed mustard (*Brassica* spp.) is one of the victims of this disease. The disease, incited by *Albugo candida* (Pers. Ex Lev.) Kuntze is an economically important and endemic one causing considerable losses [up to 47 per cent (Kolte, 1985)]. In view of the above, it is

important to identify the sources of resistance to the disease. The present studies carried out deal with evaluation of rapeseed-mustard genotypes for resistance to *A. candida* under artificial epiphytotic conditions.

MATERIAL AND METHODS Ninety rapeseed-mustard genotypes were screened against *Albugo candida* under artificial inoculation conditions. The entire germplasm belongs to five species of oleiferous *Brassicas, i.e., B. juncea, B. napus, B. carinata, B. campestris* and *Eruca sativa*. Each line of germplasm was sown in two rows of 5m length with checks after every two germplasm lines. All the germplasm lines were artificially inoculated. Observations were recorded on 10 randomly selected plants in each line on 0-5 scale and the screened lines grouped into various categories of disease severity. Percent disease index was calculated using formula Percent disease index = [Sum of individual rating / (No. of leaves assessed x maximum rating)] x100.

RESULTS AND DISCUSSION The tested genotypes gave a wide range of resistant and susceptible reaction to *A. candida*. Data revealed that out of 90 germplasms, 38 were found immune, 22 highly resistant, 17 resistant and remaining susceptible to highly susceptible against *A. candida* (Table 1). Varieties RH-30, T-59, CSR-721, RAURD-106, NDR-871, NDR-8601, and CSR-142 were found highly susceptible. Kolte and Tewari (1980) found YST-6 (yellow sarson), IB-856 (toria) and BS-15 (brown sarson) least susceptible to downy mildew and white rust diseases. Singh and Kolte (1981) reported yellow seeded mustard (*B. juncea*) variety YRT-3 and rapeseed variety TOBIN resistant to white rust pathogen. Saharan *et al.* (1988) reported 25 genotypes of *B. juncea, B. napus, B. carinata* and *B. alba* as resistant. Verma and Petrie (1978) reported that all *B. napus* cultivars examined were immune to *A. candida*. Bhardwaj and Sud (1989) evaluated different lines of four *Brassica* species against *A. candida* and found that all the cultivars of *B. carinata*, 18 of, *B. napus*, 26 of *B. juncea* resistant. Kour (1992) tested many lines of *Brassica* and found none of the mustard lines immune though she reported *B. napus* entries to be free from the disease. In our studies, the entries belonging to *B. napus* and *B. carinata* were found free from infection. The rapeseed variety 'TOBIN' also exhibited resistant reaction. The entries belonging to *Eruca sativa* were categorized as immune, though earlier white rust has been observed on taramira in Rajasthan. This gives an indication of existence of physiologic race(s) of the pathogen. Further studies on variability of the pathogen are needed.

Table 1: Screening of germplasm against *A. candida* causing white rust of rapeseed-mustard

Brassica species	Immune	Highly Resistant	Resistant	Susceptible	Highly susceptible
B. juncea	DIR-1507, DIR-519, PWR-9205, ISH-7-3-2, JMM-WR-3938, PWR-9201, JMM-WR-937, JMM-WR-9319, PWR-9314, DIR-573, SKM-91-40, DIR-1001, DIR-1001, DLM-43, DWRR-15, DLM-44, RH-8546	RSK-10, RW-8726, RAURD-1009, SKM-90-13, DIR-247, PR-9021, NDIC-90-2, SKM-90-4, CSR-448, RSK-33, JMM-WR-9339, PR-8998, DOMO (Y), DWRR-78-82, SKM-91-171	NDR-873, CS-52, RH-8689, NDR-872, DOMO-4, RSK-69, CSR-416, RSK-73, RAURD-102, PHR-1, RSK-64, PUSA BOLD, RH-8688	SKM-92-96, RAURD-1002, RAURD-101, RAURD-109, RWARB-3	RH- 30,T-59, CSR-721, RAURD-106, NDR-871, NDR-8601, CSR-142
B. napus	GSB-101, GSL-706, MIDAS, HNS-8, GSL-1, EC-174237,	HNS-4, TOWER-60	-	-	-
B. carinata	DIR-1510, HC-2, DIR-1522,	HC-9001	-	-	-
B. campestris Var. Toria	KTC-3	PT-303	-	-	-
B. campestris Var Sarson	SSK-92-16, NDY-5-2, PYS-841, SSK-6, SSK-13, SSK-11, SSK-1,	EC-129126-1,EC-29121	YST-151,PHS-42,	-	-
B. campestris Var Dichotoma	KSB-3, BSH-1, KSB-81	-	TOBIN	-	-
Eruca sativa	RTM-1263,	RTM-1471	RTM-314	TM 27	-

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R.L.Meena, K.L.Jain, and P. Rawal
Department of Plant Pathology
Rajasthan College of Agriculture
Maharana Pratap University of Agriculture and Technology
Udaipur 313001, Rajasthan, India

*Address for correspondence: C/o Sh B.N. Pansari, Village and Post: Shivdaspura, Tehsil: Chaksu, Dist: Jaipur (Rajasthan) PIN: 303903 INDIA

Relative Toxicity of Nine Insecticides to *Bracon Kirkpatricki* and *Polistes Olivaceus*

Nine commonly used insecticides viz. cypermethrin, endosulfan, carbaryl, dimethoate, phosalone, parathion, monocrotophos, trichlorphos and chlorpyriphos were evaluated for their relative toxicity to the natural enemies *Bracon kirkpatricki* and *Polistes olivaceus*, under the laboratory conditions. Insecticides were tested at field recommended dose according to the standard method for testing side effects of pesticides on natural enemies of insect pests. The purpose of this study was to determine if any of the insecticides

exhibited low toxicity to the natural enemies tested. Endosulfan, and phosalone were generally least toxic to both the natural enemies than were the other compounds, although the degree of this difference was quite variable. Parathion and chlorpyriphos were slightly harmful to the parasitoid as well to the predator. Trichlorphos was found to be highly toxic to *Bracon kirkpatricki* but was moderately harmful to *Polistes olivaceus*. Cypermethrin and dimethoate were slightly harmful to *B. kirkpatricki* but harmless to *Polistes olivaceus*. Carbaryl and monocrotophos exhibited the greatest toxicity to the larval parasitoid *Bracon kirkpatricki* as well as to the larval predator *Polistes olivaceus*. Thus, a potential approach for true compatibility of pesticides and biological control agents with broad implications has been demonstrated.

Conserving natural enemies can provide economic benefit to growers, as natural enemies help to reduce pest populations. Studies on natural enemies of insect pests of chickpea have identified key species and outlined the role played by them in pest population dynamics (Verma et. al.,1995). However, their full potentiality to provide the control of pests in nature needs to be exploited particularly when integrated with the use of limited need based insecticidal applications. Testing of pesticides on natural enemies is important before field application and their selectivity depending upon the abundance or inundative release of natural enemies is imperative for conserving bio-agents and for maintaining a healthy agro-eco system (Brahman 1988). The larval predator *Polistes olivaceus* plays a prominent role in biological control of the *Heliothis* species (Lingren et al.1968). *Bracon kirkpatricki* is an important larval parasitoid of lepidopteran pests (Bell and Whitcomb 1962). These key natural enemies can be important in suppressing insect pest populations and thus their conservation is a valuable integrated pest management (IPM) approach in crops. Among the larval parasitoids *Bracon kirkpatricki* is very common. *Bracon kirkpatricki* Wilkinson, an exotic larval parasite of gram caterpillar was also found to exercise satisfactory control of bollworms (Raodeo et al.,1983). Divakar and Pawar (1982,1987) also reported the efficacy of *Bracon spp*. in biocontrol of crop pests in India. Information on the relative toxicity of different pesticides against a range of natural enemies is available from a variety of sources including the long-term IOBC-WPRS working group research programme. The increasing indiscriminate use of pesticides adversely affects such potential natural enemies (Jones et al., 1998). Therefore, selectivity of pesticides is important in Integrated Pest Management. To manage the pest effectively research efforts have been made during last two decades under All India Coordinated Pulses Improvement Project (AICPIP). This research was conducted to evaluate the toxicity of the nine insecticides to the predominant species of natural enemies (noted above) using the laboratory initial toxicity test.

The following technical grade insecticides were used in laboratory initial toxicity test: cypermethrin, Endosulfan, carbaryl, dimethoate, phosalone, parathion, monocrotophos, trichlorphos and chlorpyriphos. Insecticides were purchased from M/S Lupin Agro-chemicals (India) Ltd Bombay. Samples of *Bracon kirkpatricki* and *Polistes olivaceus* for experiment purposes were obtained from Sisco Research Laboratory, Mumbai, India and M\S Biocontrol Research Lab., Banglore, India respectively. *H. armigera* insects were collected from all chickpea growing areas of Bilaspur, Chhattisgarh and were used in present study. The larval parasitoid *Bracon kirkpatricki* and the larval predator *Polistes olivaceus* were regularly reared in the laboratory using the pod borer *Heliothis armigera* as host. Insecticides were tested at field recommended dose according to the standard method for testing side effects of pesticides on natural enemies suggested by Hassan et, al., (1985). While mortality of the parasitoid 48 hrs after treatment was considered for grading the pesticides, the efficiency index based on the oviposition of surviving females was also taken into consideration in case of the predator.

The insecticides exhibited a range of toxicity to the natural enemies screened after 48 hrs of exposure (Table 1). Results presented in table1 indicated that endosulfan and phosalone are least toxic to both the natural enemies. Phosalone and endosulfan both had very little toxicity to both of the species tested, indicating that these products could likely be used with very little impact on natural enemy populations in the field. The present observations on the effect of phosalone and endosulfan are in agreement with those of Reddy et al., (1998) on *Bracon kirkpatricki* and *C. carnea*, Krishnamorthy, (1985) on C. scelestes, Ruberson, et al., (1994) on *Microplitis croceipes* and Lewis (1972) on *Cardiochiles nigriceps*. In earlier evaluations endosulfan was found to be safe to *B. hebetor* and *B. brovicornis* (Sharma and Sarup, 1982) but was reported to be toxic to *B. kirkpatricki* (Hamilton and Attia, 1976, Mani and Nagarkatti 1982). It is apparent from the table1 that insecticides viz parathion and chlorpyriphos, were found to be slightly harmful to the parasitoid as well as to the predator in the present study. Cypermethrin and dimethoate were slightly harmful to *B. kirkpatricki* but harmless to *Polistes olivaceus*. Trichlorphos was highly toxic to *Bracon kirkpatricki* but was moderately harmful to *Polistes olivaceus*. Carbaryl and monocrotophos were found to be most toxic to both the natural enemies. These results are in agreement with earlier finding of Dulmage et al.,(1998) who also reported highly toxicity of Carbaryl and monocrotophos to *Chrysoperla carnea*. Previous laboratory and field research studies have

shown that major lepidopteran pests are currently being controlled by the application of broad-spectrum insecticides such as monocrotophos, endosulfan or carbaryl four times at weekly intervals during the growing season. However, these broad-spectrum materials are highly toxic to insect natural enemies (Hamilton and Attia, 1976). The results obtained in the present study showed that all the nine insecticides were not equally effective to both the bio-agents. Reduction in pest population and increase in yield following inundative release of *Clubiona sp*. has been reported (Ridgway et. al., 1970). The activity of natural enemies is often hampered by the high insecticidal pressure throughout the crop growth and indiscriminate use of pesticides adversely affects such potential natural enemies (Armstrong et al., 1996). Pesticides because of their selectivity are well suited to being key components in an agro-ecosystem, because they lack direct activity on natural enemies (Rote, et. al., 1981). A major problem with pesticides, even modern selective bioinsecticides, is that they can cause disruptions to the natural enemy complex by removing the food/host resource required by parasitoids and predators. Scientists have suggested that sub-lethal or slow-killing doses could potentially provide immediate control of crop damage by a pest while stimulating the buildup of its natural enemies. The availability of insecticides that are less toxic to insect natural enemies will permit growers to conserve natural enemies and limit problems with secondary pests.

Based on the above observations, it may be concluded that the endosulfan and phosalone are not harmful to both the bio-agents tested in the present evaluation.

Table1.Relative toxicity of nine insecticides to *Bracon kirkpatricki* and *Polistes olivaceus*

S. No.	Insecticides	Concentration tested	Effect on *Bracon kirkpatricki*		Effect on *Polistes olivaceus*	
			Mortality	Evaluation category	Efficiency index	Evaluation category
1	Cypermethrin	0.01%	65.9	2	25.7	1
2	Endosulfan	0.07%	20.7	1	11.7	1
3	Carbaryl	0.15%	100	4	100	4
4	Dimethoate	0.02%	60.5	2	47	1
5	Phosalone	0.05%	23.9	1	38.6	1
6	Parathion	0.10%	53.5	2	69.9	2
7	Monocrotophos	0.04%	100	4	99.9	4
8	Trichlorphos	0.05%	96.7	4	88.5	3
9	Chlorpyriphos	0.05%	72.4	2	55.8	2

** Efficiency index (E) is calculated as per the formula E=100%-(100-M) x R
where M is larval mortality and R=Egg laying in comparison to contro *Evaluation category:
1=Harmless (less than 50%) 2=Slightly harmful (50-79%). 3=Moderately harmful (80-90%).
4=Harmful (above 90%).

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I. Chaturvedi
Department of Zoology, C.M.D. Post Graduate Colleg
Bilaspur 495001, [C.G.]

Significance of LT_{50} and PT in determining relative residual toxicity of insecticides on jute against *Tribolium castaneum*

ABSTRACT The persistence of insecticides, viz., malathion (EC), dichlorvos (EC), cypermethrin (EC), deltamethrin (EC&WP) and bifenthrin (EC&WP) sprayed on jute surface was studied upto six months based on their relative residual toxicity (LT_{50}) values and persistent toxicity (PT) against adults of susceptible and malathion resistant strains of rust red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). The relationship has been worked out between the relative efficacies of synthetic pyrethroids taking into consideration the two criteria i.e., LT_{50} and PT values at concentration of 10 and 30 mg a.i.m^{-2}, respectively. The relationship predicted that deltamethrin EC showed maximum persistence on jute surface when LT_{50} and PT values were considered against susceptible as well as malathion resistant strains of *T. castaneum*.

INTRODUCTION The continuous use of organophosphates leads to the resistance problem in most of the stored grain insects against commonly used insecticides. Focus has been shifted to the more promising synthetic pyrethroids viz, deltamethrin, cypermethrin, bifenthrin etc, which are fast emerging as potent chemicals. Apart from fumigation insect infestations are controlled by treating storage surfaces with a persistent insecticide. The residual toxicity of insecticides can be studied on the basis of two criteria i.e. PT and LT_{50} values. The former can be determined by a criterion developed by Saini (1959) and later elaborated by various workers (Pradhan 1967; Sarup *et al*., 1970). Considering the two criteria, the present contribution reports the results of residual toxicity of different insecticides used in storage on jute surface at various doses.

MATERIALS AND METHODS The laboratory susceptible strain (S) and a malathion resistant x129 (MR) of *Tribolium castaneum* were used. Tests were performed with Malathion 50EC, Dichlorvos 76EC, Deltamethrin 2.8 EC and 2.5 WP, Bifenthrin 10EC and 10WP and Cypermethrin 25EC. All formulations sprayed using atomizer on jute surface of one m^{-2} area at five doses (100, 150, 250, 350 & 450 and 10, 30 50, 70 & 100mg a.i.m^{-2} for organophosphates and synthetic pyrethroids respectively). On sprayed surface adults were released at every 48 hr interval and one month interval for residual toxicity and persistent toxicity respectively upto a period of six months or until negligible mortality was observed.

RESULTS AND DISCUSSION The persistent toxicity of various insecticides at different doses against susceptible and resistant strain are given in Table 1.The results showed that LT_{50} value of malathion at doses of 100 and 150 mg a.i.m^{-2} for S strain and at 100, 150 and 250 mg a.i.m^{-2} for MR strain could not be calculated as mortality at these doses was less than 50 per cent (2 days after spray). Similarly, the LT_{50} value of various insecticides with doses (mg a.i.m^{-2}) in parenthesis were not calculated as the mortality was more than 50 per cent upto last day of observation (180th day) i.e., dichlorvos (450); deltamethrin EC (30, 50, 70 and 100); bifenthrin EC (50, 70 and 100); cypermethrin (70 and 100); deltamethrin WP (30, 50, 70 and 100) and bifenthrin WP (50, 70 and 100) in susceptible strain and deltamethrin EC (100); deltamethrin WP (70 and 100) in malathion resistant strain. Thus it was clear that the above-mentioned insecticides at their corresponding doses (of which LT_{50} could not be calculated due to high mortality) were showing more residual toxicity than the insecticides of which LT_{50} were calculated.

Table 1: Order of persistent toxicity of various insecticides on jute against *T. castaneum*

Insecticides	Doses	Susceptible strain		Malathion resistant strain	
	(mg a.i.m^{-2})	PT	OPT	PT	OPT
	100	549.0	28	66.6	33
	150	1249.5	27	66.6	33
Malathion	250	1701.0	26	73.4	32
	350	10458.0	20	100.0	31
	450	12834.0	16	133.4	30
	100	4200.0	25	2799.0	27
	150	4465.8	24	2850.0	26
Dichlorvos	250	6878.4	23	3000.0	25
	350	8397.6	21	4332.0	21
	450	18000.0	1	6903.0	15
	10	10890.0	19	4968.0	20
	30	16110.0	9	9684.0	10
Deltamethrin (EC)	50	17748.0	3	13374.0	4
	70	18000.0	1	14940.0	2
	100	18000.0	1	16290.0	1
	10	10890.0	19	3120.0	24
	30	14580.0	13	6255.0	17
Bifenthrin (EC)	50	16884.0	7	9594.0	11
	70	16974.0	6	11988.0	7
	100	17820.0	2	13284.0	5
	10	8226.0	23	2700.0	28
	30	11736.0	18	3825.0	23
Cypermethrin	50	13878.0	15	5585.0	19
	70	15876.0	10	8388.0	13
	100	17266.0	5	9252.0	12
	10	11736.0	18	5652.0	18
	30	15154.0	12	7704.0	14
Deltamethrin (WP)	50	16632.0	8	10800.0	8
	70	17478.0	4	12168.0	6
	100	17748.0	3	13878.0	3
	10	8226.0	22	1503.0	29
	30	12762.0	17	4284.0	22
Bifenthrin (WP)	50	14148.0	14	6426.0	16
	70	15768.0	11	9684.0	10
	100	16632.0	8	10458.0	9

PT = Persistent toxicity [Product of P (period) and T (average toxicity)]

OPT = Order of persistent toxicity

The value of relative residual toxicity of different insecticides was calculated by taking LT_{50} value of dichlorvos at recommended dose (150 mg a.i.m^{-2}) as unity for both strains (Table 2). Since the LT_{50} value of organophosphates in S and MR strain and synthetic pyrethroids in S strain at recommended dose could not be calculated, so the residual toxicity of these insecticides can be predicted based only on PT values. When the order of efficacy of different synthetic pyrethroids based on two criteria i.e., LT_{50} and PT values were compared at 10 mg a.i.m^{-2} dose in S strain and at 30 mg a.i.m^{-2} in MR strain, it was not similar. The descending order of relative efficacy, based on LT_{50} and PT value which is in parenthesis was deltamethrin (EC) 1.00 (1.00); deltamethrin (WP) 0.99 (1.07); bifenthrin (EC) 0.92 (1.00); bifenthrin (WP) 0.91 (0.75) and cypermethrin 0.72 (0.75) in S strain (Table-3) and deltamethrin (EC) 1.00 (1.00); bifenthrin (EC) 0.98 (0.64); deltamethrin (WP) 0.69 (0.79); cypermethrin 0.59 (0.39) and bifenthrin (WP) 0.16 (0.44) in R strain (Table 4). Deltamethrin (WP) persisted for longer period compared to other synthetic pyrethroids whereas deltamethrin (EC) showed highest LT_{50} compared to others in S strain. The order of other synthetic pyrethroids also changed according to these two criteria's. Similarly in 'R' strain, the deltamethrin (EC) was most toxic (according to both LT_{50} and PT), but the order of efficacy of other synthetic pyrethroids varied. Based on 't' test, there were statistically no significant differences in the LT_{50} values of bifenthrin EC and bifenthrin WP at 10 mg a.i.m-2 in S strain however, significant differences in the LT_{50} values were observed in case of deltamethrin (EC) and deltamethrin (WP) at 10 mg a.i.m^{-2} in S strain and deltamethrin EC and bifenthrin EC at 30 mg a.i.m^{-2} in MR strain at 50 per cent level (Table 5). Thus, the relationship between the relative efficacy of synthetic pyrethroids based on LT_{50} and PT values were worked out for S and R strain and depicted graphically (Fig a & b). The correlation between the LT_{50} and PT was worked out as y = 0.984x (at 10 mg a.i. m^{-2} for S strain) and y = 1.039x (at 30 mg a.i.m^{-2} for MR strain).

Table 2: Relative efficacy of various insecticides at five different doses

Insecticides	Doses	Susceptible			Malathion resistant strain		
	(mg a.i. m^{-2})	LT_{50}	RRT	ORRT	LT_{50}	RRT	ORRT
	100	#	#	#	#	#	#
	150	#	#	#	#	#	#
	250	25.04	0.33	16	#	#	#
	350	131.84	1.72	7	3.27	0.07	29
Malathion	450	137.6	1.79	4	4.49	0.09	28
	100	69.38	0.9	15	42.48	0.88	24
	150	76.79	1	14	48.28	1	22
	250	84.96	1.11	13	57.42	1.19	20
	350	87.14	1.13	12	58.08	1.2	19
Dichlorvos	450	*	*	*	81.72	1.69	12
	10	135.1	1.76	5	62.94	1.3	17
	30	*	*	*	103.21	2.14	8
	50	*	*	*	154.22	3.19	4
	70	*	*	*	174.1	3.61	1
Deltamethrin (EC)	100	*	*	*	*	*	*
	10	123.78	1.61	9	44.56	0.92	23
	30	174.53	2.27	2	101.08	2.09	9
	50	*	*	*	108.35	2.24	7
	70	*	*	*	150.3	3.11	5
Bifenthrin (EC)	100	*	*	*	158.81	3.29	3
	10	97.91	1.27	11	22.41	0.46	25
	30	130.87	1.7	8	60.81	1.26	18
	50	172.6	2.25	3	81.38	1.68	13
	70	*	*	*	97.08	2.01	11
Cypermethrin	100	*	*	*	112.82	2.34	6
	10	134.22	1.75	6	69.06	1.43	16
	30	*	*	*	71.69	1.48	15
	50	*	*	*	99.96	2.07	10
	70	*	*	*	*	*	*
Deltamethrin (WP)	100	*	*	*	*	*	*
	10	123.11	1.6	10	12.58	0.26	27
	30	178.03	2.32	1	16.77	0.35	26
	50	*	*	*	51.38	1.06	21
	70	*	*	*	75.34	1.56	14
Bifenthrin (WP)	100	*	*	*	161.55	3.35	2

LT_{50} – Residual time (days) required to give 50 per cent mortality

* LT_{50} was not calculated, as mortality was more than 50 per cent at 180th day

LT_{50} was not calculated, as mortality was less than 50 per cent even two days after spray; RRT – Relative residual toxicity; ORRT – Order of relative residual toxicity

Table-3: Relationship between the relative efficacy of synthetic pyrethroids at dose (10mg a.i.m^{-2}) taking into consideration the two criteria i.e., LT_{50} and PT values for susceptible strain

Insecticide	LT_{50}	y	ORE	PT	x	ORE
Deltamethrin (EC)	135.1	1.00	1	10890	1.00	2
Bifenthrin (EC)	123.8	0.92	3	10890	1.00	2
Cypermethrin	97.9	0.72	5	8226	0.75	3
Deltamethrin (WP)	134.2	0.99	2	11736	1.07	1
Bifenthrin (WP)	123.1	0.91	4	8226	0.75	3

Table 4: Relationship between the relative efficacy of synthetic pyrethroids at recommended dose (30 mg a.i.m^{-2}) taking into consideration the two criteria i.e., LT_{50} and PT values for malathion resistant strain

Insecticide	LT_{50}	y	ORE	PT	x	ORE
Deltamethrin (EC)	103.21	1	1	9684	1	1
Bifenthrin (EC)	101.08	0.98	2	6255	0.64	3
Cypermethrin	60.81	0.59	4	3825	0.39	5
Deltamethrin (WP)	71.69	0.69	3	7704	0.79	2
Bifenthrin (WP)	16.77	0.16	5	4284	0.44	4

x = Relative efficacy based on PT values

y = Relative efficacy based on LT_{50} values

ORE = Order of relative efficacy

LT_{50} = Residual time (days) required to give 50 per cent mortality

PT = Persistent toxicity [Product of P (period) and T (toxicity)]

Table 5: 't' values for testing the differences between log LT_{50} values of those insecticides where the LT_{50} values were close

Insecticides	Log LT_{50} ± SEM.	LT_{50}	't' values
Susceptible strain			
Deltamethrin (EC)	2.133 ± 0.00125	135.1	2.702*
Deltamethrin (WP)	2.128 ± 0.00137	134.22	
Bifenthrin (EC)	2.092 ± 0.00136	123.78	1.129
Bifenthrin (WP)	2.089 ± 0.00199	123.11	
Malathion resistant strain			
Deltamethrin (EC)	2.013 ±0.00204	103.21	2.29*
Bifenthrin (EC)	2.004 ± 0.00338	101.08	
*Significant at 5.0 per cent level			

Fig a: Relationship between the relative efficacy of different synthetic pyrethroids at 10 mg a.i.m-2 (S strain) based on two criteria i.e., LT50 and PT values

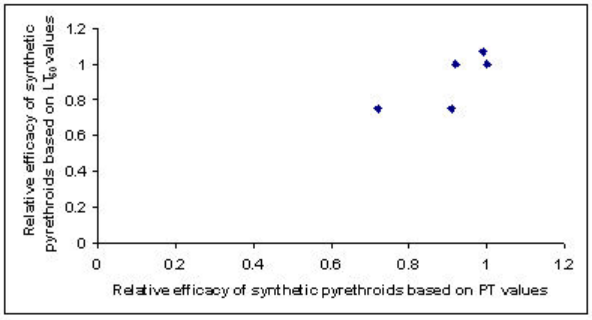
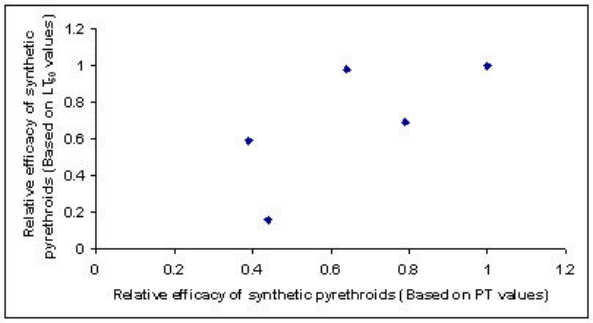


Fig b: Relationship between the relative efficacy of different synthetic pyrethroids at 30 mg a.i.m-2 (MR strain) based on two criteria i.e., LT50 and PT values



In spite of this relationship, the PT value based on the percent mortality has its own importance because it takes into account the duration of effectiveness of a particular insecticide over the entire period until it shows negligible mortality. It is very easy to calculate and thus, can serve as a ready reckoner for quick selection of persistent insecticides, but an exact idea of the relative residual toxicity of different insecticides can be obtained only by calculating LT_{50} values. This, of course, is quite time consuming and also creates little complication when there are different peaks stretched over a longer duration. In cases where there is a rapid fall in percentage mortality, the values of LT_{50} could not be calculated. This relationship, however, can be conveniently used for predicting relative residual toxicity based on LT_{50} from the PT values indicating the persistent toxicity (Sarup *et al*., 1970). Thus it was clear from the investigations that deltamethrin persisted for longer duration showing maximum toxicity followed by bifenthrin and cypermethrin to both susceptible and malathion resistant strain.

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D. S. Reddy and C. Srivastava
Division of Entomology
Indian Agricultural Research Institute, New Delhi - 110012

Woolly aphid resistant cultivar: Need of the hour for sugarcane growers in India

Sugarcane woolly aphid *Ceratovacuna lanigera* Zehnter hitherto recorded as minor pest has recently proved to be potential sucking pest and appeared in epidemic form in Karnataka and Maharashtra on Sugarcane (Kulkarni *et al.* 2003). The pest created a big havoc recently in all the sugarcane-growing belts of Maharashtra, Karnataka, Tamil Nadu and slowly moving towards the central states of India, really becoming a nightmare for cane farmers day by day. Due to heavy menace of woolly aphid, which sucks the sap from lower surface of the leaf resulting in yellow colour of the cane, honeydew secreation in turn hindering photosynthetic activity. Area under sugarcane is being reduced drastically and has been replaced by soybean and sorghum in parts of Karnataka due to the heavy loss.

Despite wealth of research since two years for the management of SWA, the task still remained a challenge. Some of the *Ad hoc* recommendations include paired row planting, minimal use of fertilizers and irrigations, spraying common insecticides like Malathion 50EC, Endosulfan 35 EC, DDVP 76EC,

Acephate, Dimethoate30EC, Monocrotophos 36 SL, Chlorpyriphos 20EC and granular application of phorate etc., encouragement of natural bioagents *viz.*, *Micromus*, *Dipha*, syrphids and coccinellids by avoiding chemical spray looking to their density. But due to one or the other reason, all of the recommended *Ad hoc* strategies have their own lacunas like, minimal use of fertilizers and irrigations will reduce the yield, spraying of chemicals in sugarcane ecosystem is very difficult after certain stage as one cannot enter into the thick canopy of sugarcane. Researchers have tried several systemic and contact insecticides from different chemical groups. Though a few products have been identified as effective, all such products cannot be recommended to farmer either due to their high mammalian toxicity or harmful effects on the natural enemies in the sugarcane ecosystem (Shankar and Shitole, 2004). Paired row planting, even if it permits certain chance for spraying, yield may be low when compared to unpaired row planting due to reduced plant population, collection and release of bioagents is very laborious which one cannot afford. Even under natural condition where, bioagents can breed in plenty, their number is not sufficient to keep pace with that of SWA, which is viviparous by reproduction, continuously laying individuals without any resting stage in its life cycle. Moreover, bioagents like, *Micromus* and *Dipha* not only undergo a resting stage called pupa but also prone to many hyperparasites and highly sensitive to common pesticides. Further no standardized mass multiplication technique is available hitherto for large-scale production on synthetic/semisynthetic diet for inundative release.

No particular variety seems to be tolerant to this pest and pest was reported in epidemic form on commonly cultivated CO cultivers (Shankar and Shitole, 2004). Attempts made to identify resistant sources by Pan *et al*. (1984) resulted in identification of the variety ROC 1 to offer resistance to some extent. Similarly Lingappa *et al*. (2003) reported that varities with acute leaf (narrow and erect types) viz., COM-88121, CO-86032,CO-89010 were less colonized by woolly aphid than with broader and droopy and they opined that genotypes expressing any type of resistance mechanism could be the good source for breeding programmes. Under such situations, where none of the component works better to give effective control for SWA, host plant resistance i.e., sugarcane cultivars with inbuilt antibiosis/antixenosis/tolerance mechanism in combination with other IPM tactics especially habitat management and bioagents can come to farmers rescue. It's the need of the hour that future research should focus in searching a agronomically good cultivar with woolly aphid resistant trait and mass produce the seed material in large scale through micropropogation/tissueculture to supply the resistant clones to the farmers in short period which will definitely work along with bioagents and other principle components of IPM to suppress the menace to replace the CO- varities which are highly vulnerable to woolly aphid.

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U. Nazneen* and Vijaykumar**
*Karnataka University, Dharwad
**UAS, Dharwad
For Correspondence: findnaz@rediffmail.com

Fungicide Resistance

Occurrence of resistance to QoI, DMI, and MBC fungicides in *Podosphaera xanthii* in 2004 and implication for controlling cucurbit powdery mildew

ABSTRACT Monitoring of resistance in commercial plantings of summer squash and pumpkin revealed that strains of the powdery mildew fungus with resistance to QoI fungicides, with moderate resistance to DMI fungicides and/or with resistance to MBC fungicides were common before these fungicides were applied to cucurbit crops on Long Island, NY, in 2004. The situation may be similar elsewhere in the US. Managing fungicide resistance is complicated by the ease with which this pathogen is dispersed long distances in a season and the fact that most QoI resistance strains are also moderately resistance to DMIs. Based on results from monitoring resistance, the recommended fungicide program for managing powdery mildew in cucurbits and fungicide resistance entails applying the DMI fungicide Procure at a high

labeled rate in alternation with Pristine, which contains the new systemic active ingredient boscalid, and tank-mixing both of these high-risk fungicides with protectant fungicides.

INTRODUCTION Application of fungicides continues to be the principal practice for managing powdery mildew in cucurbit crops, but successful control is challenged by development of resistance to key fungicides (McGrath 2001). While there are varieties with genetic resistant to this disease, an integrated program is recommended to reduce selection pressure for pathogen strains able to overcome the genetic resistance in the plant as well as fungicide resistance. Powdery mildew is the most common disease occurring every year throughout the US. The pathogen develops best on the lower surface (underside) of leaves, thus a successful management program necessitates controlling the pathogen on the lower as well as the upper surface. It is difficult to directly deliver fungicide to the lower surface, even with new nozzle types and air assist sprayers. Consequently, an important component of fungicide programs has been fungicides able to move to the lower leaf surface. Most of these fungicides are systemic (e.g. Topsin M, Nova) or have translaminar activity (e.g. Flint, Amistar, Quadris). Some, notably the new fungicide Quintec, have high volatility enabling redistribution from upper to lower leaf surfaces.

Unfortunately, these fungicides effective on lower leaf surfaces have been prone to resistance development due to their single-site mode of action. Additionally, the cucurbit powdery mildew fungus has demonstrated ability to evolve new strains resistant to these fungicides. Presence of resistant strains has been associated with control failure. With some fungicides, including MBC (methyl benzimidazole carbamate) fungicides aka benzimidazoles (e.g. Topsin M) and QoI (quinone outside inhibiting) fungicides aka strobilurins (e.g. Flint, Cabrio, Amistar), this change renders the pathogen strain completely resistant to the fungicide (qualitative resistance). With other fungicides, including the DMI (demethylation inhibiting) fungicides (Bayleton, Nova, and Procure), pathogen strains exhibit a range in fungicide sensitivity depending on the number of genetic changes they possess that affect the fungicide's ability to function (quantitative resistance).

In 2002, resistance to QoI fungicides was detected on Long Island, NY, and several other sites in the US for the first time (McGrath and Shishkoff 2003). In 2003, QoI resistant strains were shown to be present at a low level at mildew onset, their frequency increased greatly during the season, efficacy was affected, and they occurred in pumpkin crops not treated with QoIs (McGrath 2004). Strains with moderate resistance to DMI fungicides were also present. This information on resistance occurrence, combined with demonstrated superior control with a new fungicide, Quintec, provided justification for granting registration of this new fungicide in NY as an emergency exemption under Section 18 of FIFRA in 2004 (McGrath 2005).

To use fungicides at-risk for resistance wisely, growers need to know the proportion of the pathogen population that is resistant before the first application and how much the population changes with use. The goal of this project was to determine the proportion of resistant strains at the start of powdery mildew development in 2004 on Long Island before QoI or DMI fungicides were used on cucurbit crops and to examine the impact of applying these fungicides on frequency of resistance.

MATERIALS AND METHODS Fungicide resistance was monitored on Long Island during the 2004 growing season using the seedling bioassay that was used in 2003 (McGrath 2004). Squash seedlings were treated with fungicide (Flint, Nova, Topsin M), then placed with non-treated seedlings in a production field with powdery mildew for 4 hours to overnight. Two concentrations of Nova were used for the second bioassay because DMI resistance is quantitative. Isolates able to tolerate 20 ppm Nova are considered to have a moderate level of DMI resistance. The higher concentrations (40 and 80 ppm) have been tolerated by few isolates tested previously. The seedlings were kept in a greenhouse until symptoms of powdery mildew were visible, which took at least one week. Then severity (percent tissue with symptoms) was visually estimated for each leaf. Frequency of resistant pathogen strains in a field was estimated by calculating the ratio of severity on fungicide-treated plants relative to non-treated plants for each group, then determining the field average.

RESULTS AND DISCUSSION For the first assay, seedlings were placed on 29 Jul in spring plantings of squash and in pumpkin fields that had not been sprayed yet with fungicides at high-risk for resistance development (Figures 1 and 2). Resistance to QoIs was found, often at a high level, in all 8 fields (Table 1; Figures 3 and 4). Moderate resistance to DMIs and resistance to benzimidazoles was also common in all fields. In the 7 commercial production fields, an average of 44% of the pathogen population (range of 15% to 84%) was resistant to QoI fungicides, 66% (32-91%) were moderately resistant to DMIs, 40% (1-64%) were resistant to both QoIs and DMIs, and 69% (31-91%) were resistant to benzimidazoles. Powdery mildew was evidently at too low a level in the research field at LIHREC to obtain sufficient infection of seedlings to obtain a definitive estimate of the frequency of resistance; however, resistance to all 3 classes of fungicides was detected.

Table 1. Proportion of cucurbit powdery mildew fungal population estimated to be moderately insensitive to DMI fungicides and proportion resistant to QoI or MBC fungicides based on results from a fungicide sensitivity seedling bioassay.

		Resistant isolates (%) on July 29, 2004			
Site	Crop	QoI	DMI	QoI + DMI	MBC
1	Pumpkin	39	20	1	36
2	Summer squash	91	57	59	91
2	Pumpkin	56	26	34	84
3	Summer squash	88	78	64	75
4	Summer squash	87	84	53	90
5	Pumpkin	66	15	24	73
6	Pumpkin	32	31	50	31

Figure 1. Seedlings for a powdery mildew fungicide sensitivity assay in an early spring-planted summer squash crop on 29 July 2004.

Figure 2. Seedlings for a powdery mildew fungicide sensitivity assay in a main-season pumpkin crop on 29 July 2004.

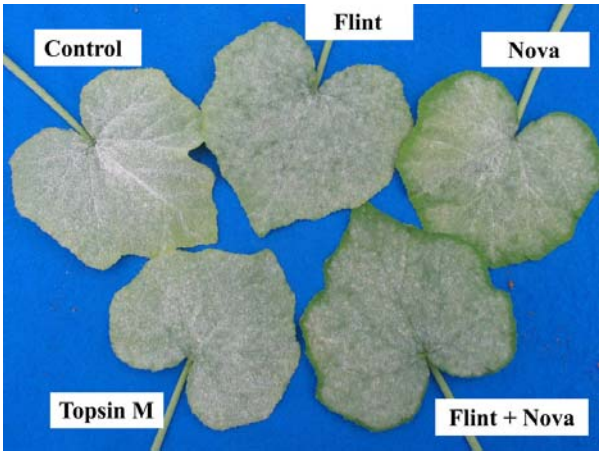

Figure 3. Leaves on 7 August 2004 from seedlings that were in an early spring-planted summer squash crop 9 days earlier. Based on powdery mildew severity on leaves treated with the QoI Flint, the DMI Nova, and the MBC Topsin M relative to the non-treated control leaf, strains of the pathogen resistant to these 3 fungicide groups were present at a high frequency in the field the seedlings were placed in.

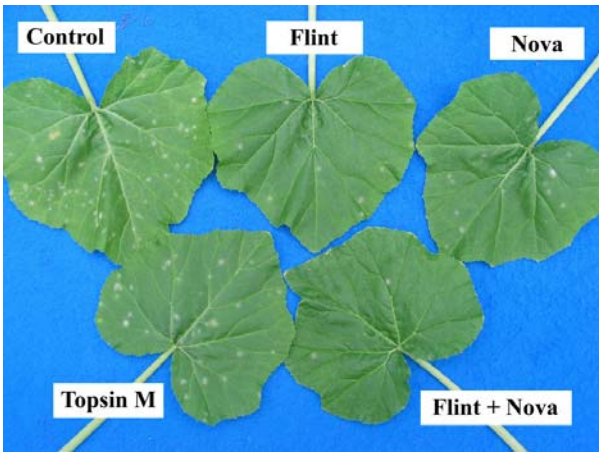

Figure 4. Leaves on 7 August 2004 from seedlings that were in a main-season pumpkin crop 9 days earlier. Based on powdery mildew severity on leaves treated with the QoI Flint, the DMI Nova, and the MBC Topsin M relative to the non-treated control leaf, strains of the pathogen resistant to these 3 fungicide groups were present at a moderate to high frequency in the field the seedlings were placed in.

The assay was conducted in pumpkin fields on 20-21 Aug. Strains able to tolerate a high concentration of Nova (80 ppm) were detected in 1 field. Resistance to QoIs and MBCs and moderate resistance to DMIs were common.

In conclusion, strains of the powdery mildew fungus with resistance to QoI fungicides, with moderate resistance to DMI fungicides and/or with resistance to MBC fungicides were common before these fungicides were applied to cucurbit crops on Long Island, NY, in 2004. Resistance to MBC fungicides has evidently persisted in the pathogen population despite their reduced use. The situation may be similar elsewhere in the US considering this pathogen moves long distances each year (eg throughout the eastern US) and resistance to QoI,

MBC, and DMI fungicides have previously been documented throughout most of the US.

Efficacy of fungicide programs with QoI fungicides appeared to be affected by resistance in a fungicide evaluation experiment conducted on Long Island in 2004 (McGrath 2005). Treatments were applied weekly to pumpkin beginning at the action threshold of 1 of 50 older leaves with symptoms (11, 18, and 24 Aug; and 1, 7 and 14 Sep) using a tractor-mounted boom sprayer (85 gpa; 100 psi). Powdery mildew control on lower leaf surfaces at the 13 Sep assessment was 69% with the DMI fungicide Flint tank-mixed with sulfur and applied in alternation with Procure (8 oz/A) plus sulfur while it was 93% where a similar program was used with Quintec substituted for Flint and 98% where Quintec was applied alone on the same dates. Good control was also obtained when the other new fungicide, Pristine, was used in place of Flint: 87% with Pristine plus sulfur applied in alternation with Nova (5 oz/A) plus sulfur. Based on AUDPC (area under disease progress curve) values, which summarize severity over the entire season, control on the lower surface of leaves was significantly more effective when block applications of Procure and Quintec were used than when a strict alternation was used (88% versus 98%). The block application schedule was Procure + sulfur (week 1, 4, 5), Quintec + S (week 2,3), and sulfur alone (week 6). Fungicide resistance was monitored in some treatments by testing individual isolates using a leaf disk bioassay. Of the 14 isolates tested from nontreated pumpkins on 9 Aug before treatments were started, 14% were resistant to QoI fungicides and 79% were moderately resistant to DMIs. On 23 Aug, frequency of QoI-resistant strains was 20% in plots receiving the block alternation schedule of Procure and Quintec while it was 44% in nontreated plots, 45% where Procure and Quintec were alternated weekly, and 71% where Procure was alternated with Flint. Frequency of isolates moderately resistant to DMIs for these treatments was 80%, 69%, 73%, and 94%, respectively. On 23 Sep, frequency of QoI-resistant strains was 43%, 44%, 60%, and 80%, respectively, and frequency of moderately DMI-resistant strains was 93%, 94%, 90%, and 100%. Most QoI-resistant strains (92%) were also moderately resistant to DMIs. Four of the 123 isolates tested tolerated Nova at 50 ppm ai.

The fungicide program currently recommended for managing powdery mildew in cucurbits and resistance entails alternating among high-risk fungicides with different modes of action and mixing these with protectant fungicides. Neither QoI nor MBC fungicides are recommended based on results from 2004 and from previous years. Since pathogen strains have been detected that are able to tolerate moderately high concentrations of DMI fungicides, these fungicides should be used at a high label rate. The DMI fungicide Procure is labeled up to 8 oz/A, which is equivalent to twice the amount of active ingredient as in the DMI fungicide Nova at its highest labeled rate of 5 oz/A. Recommendations for managing the quantitative resistance to this fungicide group include using the highest labeled rate to avoid selection of isolates able to tolerate lower rates. Nova at 5 oz/A has continued to provide good suppression of powdery mildew, which hopefully indicates that strains able to tolerate this concentration do not exist yet. The only other active ingredient registered for cucurbit powdery mildew that is able to move to the lower surface of leaves is boscalid in the product Pristine, which also contains the QoI pyraclostrobin.

As a result of QoI and DMI resistance, the cost of controlling powdery mildew with fungicides has doubled. If the cucurbit powdery mildew fungus was fully sensitive to fungicides and there was no risk of resistance, it would be possible to manage powdery mildew and obtain control of several other diseases by alternating between Nova at the lowest labeled rate of 2.5 oz/A, costing about $9.86/A, and Cabrio at 12-14 oz/A, costing about $19.13-$22.31. Due to resistance, Nova needs to be applied at 5 oz/A ($19.71/A) and using Procure at 6-8 oz/A is preferred ($19.50-$26/A). Also Pristine ($28.49-$42.16) needs to be substituted for Cabrio. In addition, a protectant fungicide is needed at each application to further manage resistance and minimize the impact on control should resistance develop. A highly effective inexpensive product is sulfur (Microthiol Disperss at 4 lb/A costs about $2.64/A). The cost of a fungicide program for controlling powdery mildew if resistance was not an issue is $97/A for 3 sprays of Nova (2.5 oz/A) alternated with 3 of Cabrio (14 oz/A). And the cost of a similar program for managing powdery mildew where QoI and DMI resistance occurs is $193/A for 3 sprays of Procure (8 oz/A) alternated with 3 of Pristine (14.5 oz/A), plus Microthiol Disperss (4 lb/A) included with each application.

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M. T. McGrath
Department of Plant Pathology, Cornell University
Long Island Horticultural Research and Extension Center
3059 Sound Avenue, Riverhead, New York 11901-1098

Research in Resistance Management

Random Amplified Polymorphic DNA Markers for Demitan and Talstar Resistance Detection in Spider Mite, *Tetranychus urticae* koch (Acarina, Tetranychidae)

INTRODUCTION The spider mite *Tetranychus urticae* - one of the major phytophagous pests, alterating cultivated plants. Among the host plants of this pest there are various vegetables and decorative glass-house cultures, fruit-trees, grape and cotton-plants. High fecundity, exceptional ecological plasticity of mite and its capability to give more than one generation during the season makes it an extraordinary harmfull object. On this reason mite capable at short periods to form the high-level acaricides resistance that vastly reduces efficiency of the chemical method of plant protection (Smirnova, 1968, Smirnova *et all*. 1972, Kornilov *et all.* 1975). In connection with aforesaid extremely actual is searching for simple and reliable methods, allowing reveal the trend to resistance forming in natural populations of the pest.

The biochemical mechanisms of resistance more varied and hang from chemical nature of toxicant. However, in any event, change of sensitivity is followed by the change of pest populations genetic structure.

In this work is shown the intercoupling between resistance forming in common spider mite to two acaricides of different chemical nature, demitan and talstar, and changes in the resistant individuals genome, as well as possibility of the detection these typical changes by means of amplification of the free sequences DNA in the polymerase chain reaction (RAPD-PCR). This method, for instance, is successfully used with morphometric methods for comparison between morphological different strains of spider mite (Hance *et all*. 1998) and is the most perspective for complex genome estimation inwardly and between populations, including for resistance study.

MATERIALS AND METHODS

Rearing conditions

The breeding and selection spider mite were conducted in laboratory of ecotoxicology of VIZR. Two strains of common spider mite *Tetranichus urticae* Koch were used for study, resistant to demitan and talstar (RD and RT, accordingly), as well as sensitive checking strain (SS). The material for under investigation strains was received from production glasshouses of the Leningrad area.

The families of the mite were kept is insulated one from another. For this were used first leaves of the young beans plants decomposable on humid cotton wool in glass Petry dishes under constant temperature +25° C and photoperiodic 18:6 h L:D

Used acaricides

Demitan - 20% suspension concentrate. The active substance is phenasahine (4-tret-butilfenethylhinazolin-4-il). Demitan - contact acaricide, acting on movable stages of the mite and possessing by expressed ovicide effect.

Talstar - 10 % emulsive concentrate. The active material - bifentryne (2-methyl-/1,1-bifenil/-3-il)-methyl-3(2-hlor-3,3,3-tri-fluoro-1-propenil)-2,2-dimethyl cyclopropanecarboxylate). Talstar - contact insectoacaricide with broad spectrum of the action.

Methods of the treatment and selection of resistant individuals

Treatment with acaricides was realized by method of the submersion of leaf bits from beans with mite in solution of the preparation with discriminative concentrations (0.0036% on acting material for demitan and 0.002% for talstar). The account of mortality was conducted through day after treatment by demitan and on the third day after treatment by talstar.

Constant family selection in RD and RT strains was winnowed In the course of breeding. After treatment with discriminative concentration females from the families in which was noted minimum of mortality, were transferred for the further breeding separately. The offspring of each female was tested and analyzed individually. Analogically was winnowed selection of susceptible strains (SS), with that only difference that for the further breeding were selected females from families, in which was noted maximum of mortality.

DNA extraction and PCR reaction

DNA extraction and PCR-amplification were performed in the laboratory of adaptive biochemistry of insects, Institute of Biochemistry and Genetics of Russian academy of sciences, Ufa. Total DNA extraction carried out in 1.5 ml tubes by described by Chomezynski *et all.* (1987) with some modifications.

Pulled mites (30 females from one clone) were homogenized in 400 µl of extraction buffer, containing 4 M guanidinum thiocyanate, 25 mM sodium citrate, 100 mM 2-mercaptoethanol and 0.5% sodium sarkosyl, TRIS-HCl-buffer pH 8.0 up to 0.1 M. Samples were incubated at 0° Cfor 20 min, vortexed energically for 15 min with 400 µl phenol, pH 8.0/400 µl chloroform: isoamil alcohol (24:1 v/v) and cenrifugated for 15 min at 10000 g. The supernatant (400 µl) was transferred to a new tube, vortexed again for 5 min with 200 µl chloroform and cenrifugated for 5 min. After centrifugation, 400 µl of the supernatant was transferred to a new tube and DNA was precipitated with 400 µl cold (-20° C) 96% EtOH for 24 h. Following the cenrifugation for 20 min the precipitate was washed with 200 µl 70 % EtOH, dried and dissolved in 50 µl of bidistilled water. DNA samples stored at -20° C.

Polymerase chain reaction (PCR) were carried out in a total volume of 30 µl, containing 2 µl of mite DNA solution. The reaction buffer consists of 10 pM of primer (Sintol, Russia), 250 mkM each of dNTP, (Fermentas, Lithua), 1*Taq buffer (10 mM TRIS-HCl, pH 8.8, 50 mM KCl, 2.5 mM MgCl) and 2.5 U Taq polymerase (Silex, Russia). Reaction mix was topped with a drop of sterile mineral oil. PCR amplification of RAPD markers was performed in thermal cycler "Cycloterm" (Russia) under the following cycle conditions: five cycles of 94° C - 1 min for denaturing the DNA, 34° C- 2 min for annealing and 72° C- 2 min for elongation; 25 cycles of 94° C- 1 min, 42° C- 2 min, 72° C- 2 min. An additional 7 min at 72° C was allowed for last strand elongation.

After amplification, 10 µl product was separated by electrophoreses in 1.5% agarose gels (18*10 cm) in 1xTAE buffer for 1 h at 70 mA. Gels were stained with ethidium bromide and the DNA was visualized by fluorescence UV-light (312 nm) transilluminator TM-36 and photographed.

Data analyses

Amplification products were scored as discrete and binary stares (present /absent or 1/0) for each individual samples. A data matrix of "individuals´bands" containing the band scoring information was calculated using the simple matching coefficient described by Sokal et all. (1958) and were given by:

$$\frac{(a+d)}{(a+b+c+d)}$$

where *a* is the presence of the marker in both bands, *d* - is absence in both bands and *b* and *c* are markers present in one and absent in the other bands (Hance *et all.* 1998). This dataset was used to calculate the genetic diversity among individual trees using the Statistica (v.5.0) software package. The clustering was performed on Ward's method, as a distance measure was used Euclidean distances.

Coefficient biodiversity between strain was calculated by Shannon-Weaver index (Chalmers *et all*. 1992):

$$H_0 = -\sum p_i * \ln p_i$$

where Pi -frequency of i-allele in strain.

RESULTS AND DISCUSSION

Toxicological features of investigated strains

In spite of strictly individual nature of the breeding during 20 generations, in strains detected the genetic heterogeneity (Fig. 1). In the course of family testing there periodically proceeds the isolation of susceptible families from resistant strains and resistant families - from susceptible ones. From tables is seen that practically in each generation of the selection in resistant strains are present the families with level of mortality 30-60% or below. One of the spider mites biological particularities is the male emergence from nonfertilized eggs and, accordingly, their haploidy, as well as dominant nature of the resistance sign inheritance (Helle, 1962, Schulten *et all*. 1968). In consequence if in strains are present the females, heterozygous on sign of resistance, then half a males in their offspring will carry the dominant allele, but another half - the recessive one. Under sibmating condition (crossbreeding of individuals from offspring of one female) this promotes the conservation an heterozygous females in strains and, consequently, periodic appearance of susceptible individuals in resistant females offspring.

We analyzed RAPDs in search of molecular-genetic marker, allowing differentiate these strains of the mites. Performed screening of several primers has allowed to choose one oligonucleotide sequence (5'-GTGCTCGGC-3'), suitable for revealing the difference between mite individuals of different strains (Fig.2).

On the first stage we had chose 3 RAPD-fragments, allowed separate the DNA samples from mites of the different strains. The fragments A are present only in resistant individuals genomes. This fragments allow to separate the susceptible strain from resistant ones and differ the resistant strains one from another. The fragment B allows to reveal SS-strains.

On the second stage we performed cluster analysis for revealing possible heterogeneity of these strains on molecular-genetic level. On figure 3 is presented dendrogram of genetic relations for 6 analyzed DNA samples. On given dendrogram is seen clear isolation of cluster, including only susceptible strain (SS1-SS5) samples. Two other clusters contain as samples from resistant to demitan strain (RD1-RD4), as from resistant to talstar strain (RT1-RT7). This, on our glance, speaks about the genetic heterogeneity of investigated strain as evidenced by isolation of

susceptible families from resistant strains and resistant families - from susceptible ones. The genetic distances on the dendrogram between families in each cluster also speak of heterogeneity of considered strains.

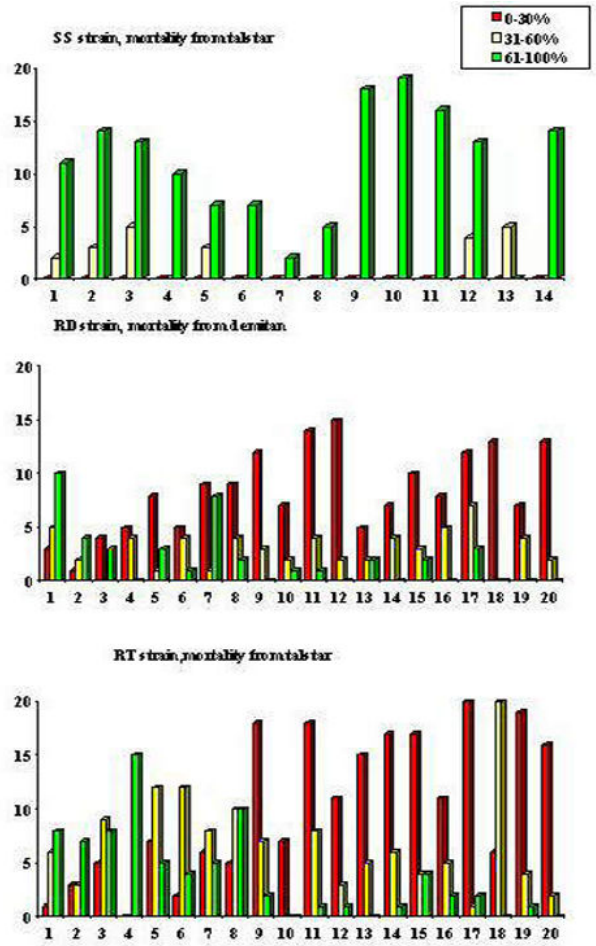


Fig.1: Susceptibility to selectants in *T. urticae* strails. Axis X – selecting generation, axis Y – persentage mortality, %.

RAPD-PCR results

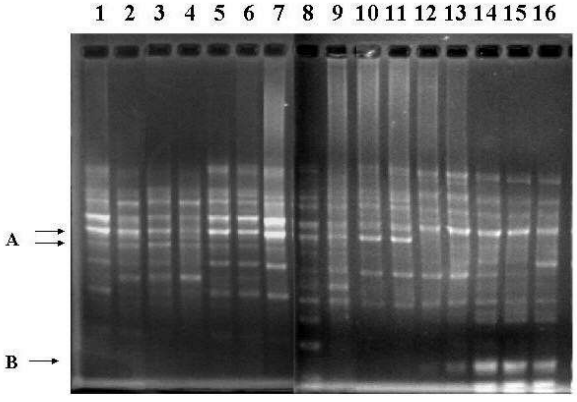
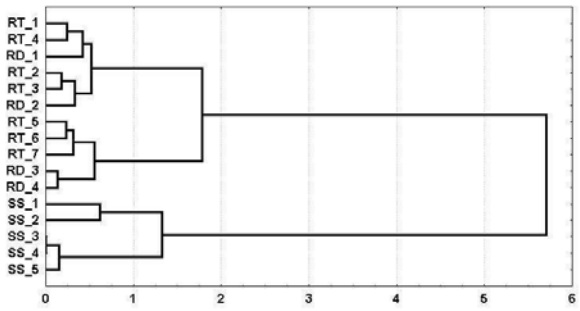


Fig. 2: RAPD banding profile of common *T. urticae*. Lanes 1-7 – talstar-resistant strain, lanes 8-11 – demitan-resiant strain and lanes 12-16 – susceptible strain. A – resistance-marking fragments, B – susceptibility-marking fragment (see text for details).

Fig.3: Trees of the 16 DNA-samples of *T. urticae*. RT - talstar-resistant strain, RD - demitan-resistant strain, SS - susceptible strain.



Such heterogeneity in selected strains speaks about insufficiency of the use for checking in the course of selection only toxicological method.

The reduction of biodiversity level in resistant strains (comparatively susceptible one) is, in our opinion, the result of acaricides selection (Fig.4). Herewith in the course of selection the elimination of individuals with susceptible genotypes occurs that brings about reduction of the genetic diversity in populations.

Under artificial populations control it is necessary to know that any one of them has its biological optimum of biodiversity, established throughout the evolution. The mentioned optimum is limited with frames of minimum and maximum biodiversity level which are revealed in the course of perennial observations. Without knowledge of the optimum biodiversity borders in the populations there is impossible to realize some work with any population, whether reproduction or reduction of its individuals number. In particular, the biodiversity level reduction in spider mite populations (where for control of its number acaricides are using) is a signal of the resistance development.

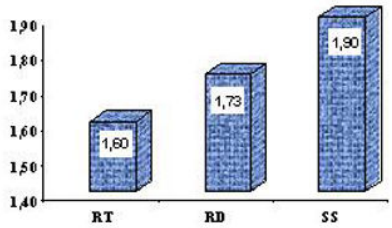


Fig. 4: The biodiversity level in of *T. urticae* strains. RT – talstar-resistant strain, RD – demitan-resistant strain, SS – susceptible strain. Axix Y - coefficient biodiversity.

CONCLUSIONS In spite of uneven nature of the resistance forming in the laboratory reared common spider mite, we had received two strains with resistance factor 38,4 (RD) and 54,5 (RT). The DNA analysis of mites from these strains has shown that simultaneously with resistance forming qualitative and/or quantitative changes of the object's genome occured, well

distinguished on RAPD-spectrum.

In our study analysis was conducted on one mite strain of each variant only i.e. on one SS, RD or RT genotype. The confirmation of the data obtained on the other strains, wich are resistant to insecticides mentioned, will allow to perform the molecular-biological analysis for other strains or populations of the mite, and at finding of similar marker bands to draw a conclusion about the resistance of strain or about resistant genotypes presence in analysed population.

This method of the DNA investigation can be used and at analysis of any other mite strain. When use the other primers and on the other strains line and species the other marker bands can be received, but under corresponding confirmation of obtained results all of these can be recommended for practical use.

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M.B.Udalov*, A.V.Poskryakov*, G.V.Benkovskaya*, I.A.Tulaeva**, M.K.Barinov**, and A.G.Nikolenko*
*Institute of Biochemistry and Genetics, USC RAS, 450054, Russia, Bashkortostan, Ufa, prospekt Oktyabrya, bild 69
e-mail: udalov-m@yandex.ru, For Correspondence
**All-Russia Institute of Plant Protection, 196608, Russia, St. Petersburg-Pushkin, 3 Podbelsky shosse, e-mail: barimaksim@yandex.ru

Resistance Management News

Companies and researchers join to keep glyphosate resistance rare in Australian cropping

The new national Glyphosate Sustainability Working Group is an industry-wide effort to promote the sustainable use of the most widely used herbicide in Australian agriculture. To encourage awareness and accurate reporting of the number and background of glyphosate resistant weed populations in Australia, the group has established the Australian Glyphosate Resistance Register. Managed by Dr. Chris Preston, the register provides an up to date record of confirmed glyphosate resistant populations, with classifications including region and land use. The Australian Glyphosate Resistance Register is now included on the GSWG website: www.weeds.crc.org.au/glyphosate. Also available is a risk management guide for keeping glyphosate resistance rare in Australian cropping and recommendations on what to do if glyphosate resistance is suspected . The national Glyphosate Sustainability Working Group is a collaborative initiative involving the CRC for Australian Weed Management, Monsanto, Syngenta, Nufarm, Western Australian Herbicide Resistance Initiative (University of Western Australia), University of Adelaide, Charles Sturt University, University of Melbourne, Queensland Department of Primary Industries & Fisheries, Department of Agriculture Western Australia, New South Wales Department of Primary Industries, CRT/Town &Country, AVCARE and the Grains Research and Development Corporation. The aimes of the group include:

- Increase the sustainability of glyphosate usage through the development and delivery of clear and consistent information, based on industry consensus.
- Increase collaborations and consistency among the glyphosate research and extension activities of key research, extension and industry groups.
- Contribute to the development of Research, Development and Extension initiatives aimed at improving the management of glyphosate.

For more information see: www.weeds.crc.org.au/glyphosate or contact Dr. Rick Llewellyn (Chairman, GSWG).

Integrated Pest Management Network

15 March 2005 Programs, activities, or enterprises worldwide involved with, linked to, or interested in IPM and management/control of:

INSECT, WEED, PATHOGEN, NEMATODE, or VERTEBRATE

crop pests and invasives, as well as reduced-risk pest management, usually find that every monthly issue of IPMnet NEWS--a free, non-commercial, globally recognized electronic newsletter that delivers current IPM and pest management information--offers useful details, e-mail contacts, insights, and active links to other informative sites.

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IPMnet is an electronic (only) Integrated Pest Management infor- mation resource specifically focused on contemporary, economic, envi- ronmentally aware approaches to managing/controlling weed, pathogen, insect, nematode, and vertebrate pests in crops and amenity plantings, as well as preventing or containing invasive crop-related species.

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- on the World Wide Web in either HTML or PDF format, but without complex graphics requiring bandwidth and lengthy download time that ties up computers.

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- research
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- policy
- impact/socio-economics.

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Cordially, --IPMnet Staff

Abstracts

Insecticide Resistance in Obliquebanded Leafroller, 1997-2004

ABSTRACT Insecticide resistance in various field-collected populations of bliquebanded leafroller (OBLR) was surveyed in 1997, 2001 and 2004. A susceptible laboratory colony was evaluated for tolerance to azinphosmethyl, screened for resistance to all three insecticides, azinphosmethyl, spinosad and methoxyfenozide. Resistance ratios were determined by comparing the baseline LC_{50} of the susceptible colony with LC_{50} value of each insecticide for each field population. Each field population was then analyzed for azinphosmethyl mediated cross-resistance to either spinosad or methoxyfenozide. Nearly every population of OBLR collected in the field since 1997 had some level of resistance to azinphosmethyl with the exception of one population collected from a non-commercially managed orchard. The average LC_{50} from the laboratory colony was 7.0 ppm (range 3.1-12.2 ppm). LC_{50}s from 24 commercially managed orchards ranged from 30.7-290.5 ppm. Interestingly, the insect growth regulator methoxyfenozide had fairly well correlated cross-resistance to azinphosmethyl prior to widespread use of this insecticide chemistry. The average LC_{50} from the laboratory colony was 1.44 ppm (range 0.45-2.86), but in 1997, 2001 and 2004 field collected populations had LC_{50} values ranging from 5.7-11.8 (2 orchards), 9.7-28.1 (5 orchards) and 2.93-11.0 (6 orchards), respectively. Although cross-resistance was noted initially with methoxyfenozide, it did not appear that resistance levels have changed significantly within geographic regions between 1997-2004. Initial spinosad bioassays were conducted in 2001. The average LC_{50} from the laboratory colony was 0.3 ppm (range 0.13-0.50). In 2001, it was apparent that azinphosmethyl mediated cross-resistance was not a concern as LC_{50} values from azinphosmethyl resistant populations ranged from 0.19-0.62 (8 orchards). However, in 2004 LC_{50} values from field-collected populations ranged from 0.46-1.82 (10 orchards). This represented a significant shift in the susceptibility of some field-collected populations, and it appeared that those populations of OBLR have become more tolerant to spinosad since its introduction.

KEYWORDS insecticide, resistance, cross-resistance, obliquebanded leafroller, *Choristoneura rosaceana*, Tortricidae, Washington, apple, azinphosmethyl, spinosad, methoxyfenozide.

J. Brunner, M. Doerr, and J. Dunley
Washington State University
Tree Fruit Research and Extension Center
Wenatchee, WA

Management of Organophosphate-Resistant Codling Moth in the Field

ABSTRACT A survey of organophosphate resistance was conducted in 2003 to determine levels of azinphosmethyl resistance in codling moth throughout Washington State. Resistance was examined by topically treating field-collected males (captured in pheromone traps) with several concentrations of a selected insecticide. Insecticides examined including azinphosmethyl, esfenvalerate, chlorpyrifos, and acetamiprid. Levels of azinphosmethyl resistance were found to be low to moderate in most fruit-growing areas of the state. However, a population near Manson, WA, was particularly high (8-10 fold resistance). In

2004, field efficacy trials were conducted in Manson to determine the abilities of several insecticides to control azinphosmethyl-resistance codling moth. Several codling moth control programs were applied to large plots and fruit was evaluated for infestation following each generation. Treatments including azinphosmethyl (2 applications per generation at 3 lb), acetamiprid (2 apps/gen at 3.4 oz), thiacloprid (2 apps/gen at 6 oz), chlorpyrifos (2 apps/gen at 2 lb), esfenvalerate (2 apps/gen at 14.5 oz), spinosad (2 apps 1st gen, 3 apps 2nd gen, all at 6 oz), and a novaluron/acetamiprid tank-mix (1 app/generation, novaluron at 40 oz, acetamiprid at 3.4 oz). Codling moth pressure was moderate in this orchard in 2004, and azinphosmethyl resistance was determined to be 5-fold. All programs maintained damage at harvest at less than 0.3%. It appears that, at this point in time, potential cross-resistance between azinphosmethyl and acetamiprid (or thiacloprid) does not impact codling moth control.

KEYWORDS codling moth, *Cydia pomonella*, resistance management, organophosphate resistance.

J.E. Dunley, T.M. Madsen, and B.M. Greenfield
Washington State University
Tree Fruit Research and Extension Center
Wenatchee, WA

Resistance to Sex Pheromone Mating Disruption by Codling Moth: Is There a Trend Toward More Mating?

ABSTRACT The mating status of female codling moths within apple orchards treated with sex pheromones for mating disruption has been monitored since 1992 in Moxee and since 2000 in Brewster. These orchards have been treated with sex pheromones every year since 1991. Populations have been monitored with the use of passive interception traps, light traps, and sticky traps baited with the pear ester. Orchards have been treated with various rates of hand-applied dispensers, widely spaced clusters of dispensers or aerosol emitters, and microencapsulated formulations. Data are summarized for both the first and second moth flights. Prior to 2000, the proportion of mated females during the first flight in Moxee was <0.6 and during the second flight was <0.8. Since 2000 mating rates have increased to >0.85 and 0.95 in the two flight periods, respectively. Similarly, the proportion of mated females in Brewster in 2000 was 0.7 and 0.8 for the two flights. Over the past four years there has been a positive trend in the proportion of mated females in these orchards and during 2004 >0.96 females were mated in both flights. In 2002 I evaluated the mating success of tethered females from the Moxee orchards, from another orchard that has never been treated with sex pheromone, and from a laboratory colony in screened field cages with releases of males. No difference in mating was found among these populations. However, differences in the behaviors of females among populations were not addressed in these studies. Several potential mechanisms associated with mating success in codling moth will be discussed.

KEYWORDS *Cydia pomonella*, apple, pear ester, monitoring, sex pheromone

A. Knight
USDA, ARS, YARL, Wapato, WA

Codling Moth Control in Oregon's Hood River Valley: Is it Resistance, Poor Timing, or Less Effective Control Programs?

ABSTRACT Pear and apple orchards in Oregon's Hood River Valley have experienced increasing amounts of codling moth damage in the last two seasons. Working with concerned growers and fieldmen, experiments were conducted to determine if resistance to organophosphates (OP) was evident in the valley. Topical bioassays were conducted on male moths collected with pheromone traps in problem orchards where resistance was a concern. Four out of five orchards surveyed exhibited an increase in resistance to azinphosmethyl compared to a susceptible population. Other insecticides such as phosmet and the neonicotinyls will be examined in 2005 for cross-resistance. Potential cross-resistance to insecticides that do not have adulticidal activity, such as the insect growth regulators, will be examined by treating reactivated diapausing larvae. Growers' spray records and pack-out reports were also collected to determine if increased amounts of codling moth damage were a result of poor management practices, incorrect timing of sprays or ineffective control programs. A codling moth egg survey was conducted to help validate the current phenology model and to correlate trap catches to egg laying. Changes in flight patterns within the first codling moth generation have been observed for the past few years. Population density maps were also created using GIS and extensive trapping data from fieldmen to indicate "hot-spots" throughout the valley. Future plans are to create real-time maps to indicate when populations are increasing in a given area and

how they change numerically from year to year under different management programs.

KEYWORDS codling moth, apple, pear, organophosphates, resistance, azinphosmethyl, bioassay, phenology

A. Walston and H. Riedl
Oregon State University Mid-Columbia
Agricultural Research and Extension Center
Hood River, OR

Induction, characterization and genetic analysis of tricyclazole-resistance in *Magnaporthe grisea*

ABSTRACT Tricyclazole is one of the rare modern fungicides, which had been applied in agricultural practices for many years without resistance emerging. Even laboratory resistance has not been reported. Here, we report the induction, characterization and genetic analysis of tricyclazole-resistant mutants of *Magnaporthe grisea*. The minimum inhibitory concentration of hyphal melanization (MIC-H) was adopted to detect the sensitivity of one hundred and twenty-nine *M. grisea* isolates, which were collected from Guangdong, Guangxi, Anhui and Jiangsu Provinces of China in 2000, to inhibitor tricyclazole. Results showed that the mean value of MIC-H was 0.2 μg/ml and no isolate with a MIC-H =1μg/ml was detected. Thus, 1μg/ml was chosen as a distinctive dose to recover resistant mutants by ultraviolet (UV) radiation. Only three resistant mutants generated from TH16 (with MIC-H 0.1μg/ml) were obtained and numbered R1,R2 and R3, representing a very low frequency that could not be correctly calculated.

All the three mutants could grow and produce melanin at 1μg/ml normally .These three mutants were tested on the rice seedlings treated by a series of concentrations of tricyclazole. The medium effective concentration (EC50) of the chemical to control blast disease caused by them was 43.3μg/ml, 47.5μg/ml and 38.2μg/ml respectively, while the EC50 of their origin wild type isolate TH16 was only 4.0μg/ml.

Meanwhile, fitness decrease was observed for all the three mutants, their sporulation ability and pathogenicity were weakened. Four crosses between S×S and S×R1,R2,R3 were set to determine the inheritance mode of resistance during the process of sexual recombination. By analyzing the sensitivity of hybrid F1 progeny to tricyclazole, phenotype segregation in progeny clearly fit a 1:1 ratio of both parents of S×R, and no segregation was found in the crosses of S×S.

Fungal genomic DNA extracted from mycelia through a CTAB/ NaCl method was used for PCR of 1,3,6,8-tetrahydroxynaphthalene reductase (4HNR) and 1,3,8-trihydroxynaphthalene reductase (3HNR) with primers M4-1 (5'-CGGACTTGTTGGTCTTTGTGC-3', 5'- CCTCGGACGGCTTGCTGAT-3'), M4-2 (5'-CCTCCGCAGACATCACCAGC-3', 5'-CGAGAACCCGACTTGACCACTA-3'), M3-1 (5'-GTGCGTTTCTCACCTTCAGC-3', 5'-CGGTCATCCGATTCCCATA-3'), M3-2 (5'-GTGCGTTTCTCACCTTCAGC-3', 5'-CCACCAGGAGCAACCACATTA-3'), M3-3 (5'-TGTCTCGCTGGCACCCT-3', 5'-GGCACGGCACCTCTGTA-3'), respectively. Whole genes of both 4NHR (accession number bankit669199) and 3HHNR (bankit669203) were got. However, none nucleotide difference in sequences was found between resistant mutants and their original wild type isolate. So it can be concluded that tricyclazole-resistance in M. grisea was conferred by a one loci mutation in a single Mendelian gene other than 4HNR or 3HNR genes.

KEYWORDS *Magnaporthe grisea*, melanin biosynthesis, tricyclazole-resistance, fitness, genetics, reductase genes

C. Zhang and M. Zhou
College of Plant Protection
Nanjing Agriculture University
Nanjing 210095, China

Symposia

QoI Fungicide Resistance: Current Status and Management Strategies

Symposium to be held during the annual meeting of the American Phytopathological Society, July 31 - Aug 3, 2005 in Austin, Texas

QoI fungicides (e.g., strobilurins) represent the most important group of new plant pharmaceuticals to become available over the past two decades. This symposium will examine the mechanisms by which fungi develop resistance to these compounds; the current resistance status and resistance-management strategies for specific horticultural, ornamental, and agronomic crops; and the agrichemical industry's

response to this challenge.

Presentations

Overview: QoI resistance mechanisms and occurrences. H. Ypema, BASF Corp., Durham, NC

Commodity case study: Cereals. J. Lucas, Rothamsted Research, Harpenden, UK

Commodity case study: Turf. F. Wong, University of California, Riverside, CA

Commodity case study: Apples. W. Koeller, Cornell University, Geneva, NY

Commodity case study: Grapes. W. Wilcox, Cornell University, Geneva, NY

Commodity case study: Cucurbits. M. McGrath, Cornell University, Riverhead, NY

Agrichemical industry: Management strategy and responses. R. Kaiser, Bayer Crop Science, Research Triangle Park, NC

For more information on this symposium and meeting, and to register at a $25 discount, go to http://www.apsnet.org/ after April 1 and before June 1. Select 'Annual Meeting' under 'Meetings' in the Main Menu on the left.

Announcements and Submission Deadlines

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

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

The Newsletter is a resource to many around the globe. It is also a wonderful and effective way to enhance the flow of ideas and stimulate communication among global colleagues. We appreciate your efforts to support the newsletter and we look forward to your continued contributions.

The next two **submission deadlines** are:

Monday, September 19th, 2005
Monday, March 20th, 2006

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

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