Indian Journal of Applied Entomology **23**(2) : 93-98 (2009)

PHARMACODYNAMICS OF OXYDEMETON-METHYL IN 2nd INSTAR GRUB AND ADULT OF *COCCINELLA SEPTEMPUNCTATA* LINN. (COLEOPTERA : COCCINELLIDAE)

MALIK MUKHTAR AHMAD AND SHEIKH BILAL AHMAD

Division of Entomology, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir, Shalimar, Srinagar – 191 121

ABSTRACT

The fate of oxydemeton-methyl was studied in 2nd instar grub and adult of *Coccinella septempunctata* Linn., following topical application. Rate of penetration was almost uniform in both the stages of insect with 90 per cent of applied dose being penetrated within 120 minutes with half life of 36.26 and 32.47 minutes, respectively. There was no difference in the absorption of oxydemeton methyl in the haemolymph at 30 and 60 minutes post treatment in both the stages of the insect, but 46.0 and 43.0 per cent and 53.0 and 50.0 per cent of the applied dose were recovered in the haemolymph of the grub and adult at 90 and 120 minutes post treatment, respectively. Four per cent of oxydemeton methyl could be found from the brain at 60 minutes after treatment in the grub, while as at the same time interval there was no recovery of oxydemeton methyl in the brain of adult. However, no significant difference was found in the accumulation of oxydemeton methyl in the brain of both the stages of insect with only 5.0 per cent of the applied dose being recovered at 90 minutes after treatment; whereas at 120 minutes post treatment slight difference was noticed in the accumulation of oxydemeton methyl in the brain with 3.0 and 4.0 percent of the applied dose being recovered in grub and adult, respectively. The rate of excretion of oxydemeton methyl followed a similar pattern in both grub and adult excreting 3.0, 16.0, 23.0 and 33.0 per cent of the applied dose in grub and 13.0, 20.0, 25.0, and 34.0 per cent of the applied dose in adult respectively at 30, 60, 90 and 120 minutes with half life of 29.53 and 80.52 minutes in case of grub and adult respectively. Both the penetration and excretion rates exhibited first order kinetics.

Key words: oxydemeton- methyl, *Coccinella septempunctata*, penetration, excretion.

Among the coccinellid predators, *Coccinella septempunctata* Linn. occupies an important position and to a large extent is responsible for limiting aphid population by predation under natural conditions. Its predatory role has been duly established throughout the world (Singh *et al.,* 1994; Gour and Pareek, 2003; Soni *et al.,* 2004). pesticides used in the ecosystem are known to have significant toxic effect on these beneficial insects (Dirimanvov *et al.,* 1980). Pesticides continue to be important tools for management of agricultural pests as they offer an immediate measure to combat pests. In view of this, it becomes essential to look for insecticides which are selectively toxic to pests and not to beneficial insects. Many factors are responsible for this selectively of insecticides such as differential penetration, metabolism and excretion which have been attributed to the differential toxicity of various insecticides to different insect species (Camp and Arthur, 1967; Benezet and Forgash, 1972; Welling *et al.,* 1983; Bull *et al.,* 1989).

Penetration, internal accumulation and excretion of insecticides varies with the insecticide and also with the insect species. Differential penetration, transportation, internal accumulation and excretion have a direct implication on the bio-availability of the insecticide and, therefore, exhibit physiological selectivity. Differential pharmacodynamic behaviour could play an important role for the selection of insecticides for insect pest control programmes in modern agriculture and public health programmes (Vinson and Brazzel, 1966; Sawicki and Lord, 1970). Therefore, the present study was proposed to broaden the basic understanding of the pharmacodynamic behaviour of oxydemeton methyl in lady bird beetles, *Coccinella septempunctata* Linn.

MATERIALS AND METHODS

The adult *Coccinella septempunctata* Linn. bettles were collected from mustard, cabbage and wheat crop grown in the fields at Shalimar Campus of SKUAST-K and taken to the Toxicology Laboratory of the Division of Entomology and kept in iron cages (25 × 20 × 20 cm). Flowering shoots of mustard infested with *Lipaphis erysimi* (Kalt.) and *Brevicoryne brassicae* (Linn.) were provided as food for the adults. Leaves of mustard with their petioles dipped in water were provided for egg laying.

Eggs laid on the under surface of leaves were removed from the cages and kept in petriplates. After hatching of eggs, the grubs were provided aphids *viz., Lipaphis erysimi* K. and *Brevicoryne brassicae* L. as food. Active and healthy 2nd instar grubs and adult of uniform size were selected for pharmacodynamic studies.

Analytical grade oxydemeton methyl was supplied by Environmental Protection Agency, North Carolina USA. Acetylthiocholine iodide (ATchI) and dithionitrobenzoic acid (DTNB) were supplied by Sisco Research Laboratories, Pvt. Ltd., Mumbai. All other chemicals used in the study were of highest purity available.

In the present investigations, the brains of bees *Apis mellifera* L. were used as a source of acetyl cholinesterase enzyme (AChE). Honeybees were chilled and the brains were dissected out on ice at 0-4°C. The tissues and other adhesinos of the brain were removed and transferred to glass tissue homogeniser and macerated with 1 ml of 0.1 M cold (0-4°C) phosphate buffer pH 7.55. The macerated material was strained through muslin cloth, collected and diluted 10 fold with buffer and stored at –15°C in stoppered glass vials for further studies.

Enzyme assay was performed by the method of Ellman *et al.* (1961). The reaction was performed in test tubes at 25 ± 1°C in a shaking water bath. The reaction mixture consisted of 6 ml of 0.1 M phosphate buffer pH 8.0 and 60 µl of enzyme solution. The mixture was incubated for five minutes in the water bath with temperature of 25 ± 1°C and then 50 µl of 0.01 M dithionitrobenzoic acid (DTNB) were added to it and then further incubated for five minutes. Then 40 µl of 0.075 M acetylthiocholine iodide (AtchI) was added and absorbance at 415 nm was recorded at zero time on Digital Spectrophotometer model 305 E and the rate of change in optical density with time, (ΔOD) per ten minutes was recorded. The rate of non-enzymatic hydrolysis of acetylthiocholine iodide was found to be 0.011 ± 0.022 units of ΔOD per ten minutes which was subtracted from the enzymatic hydrolysis of acetylthiocholine iodide to get enzymatic activity. This was done in all subsequent observations. In addition to the non-enzymatic hydrolysis of acetylthiocholine iodide, the hydrolysis of acetylthiocholine iodide due to AChE present in *C. septempunctata* brain extract was found to be 0.009 ± 0.018 units of OD per ten minutes which was subtracted from enzymatic hydrolysis of acetylthiocholine iodide to get enzymatic activity when brain extracts were analysed. For studying the per cent inhibition of oxydemeton methyl, the external rinse, brain extract, haemolymph extract and container wash of *C. septempunctata* taken at different time intervals *viz.,* 0, 30, 60, 90 and 120 minutes after the treatment was added to the reaction mixture and the change in absorbance per 10 minutes was recorded. All assays were performed in triplicate and the per cent inhibition was calculated as follows:

$$\textit{Inhibition (\%)} = \frac{\left\{\begin{matrix}\Delta OD/10\\ minutes\\ (No\ pesticide)\end{matrix}\right\} - \left\{\begin{matrix}\Delta OD/10\\ minutes\\ (Pesticide)\end{matrix}\right\}}{\left\{\begin{matrix}\Delta/10\\ minutes\\ (No\ pesticide)\end{matrix}\right\}} \times 100$$

The concentration of the pesticide was determined from a calibration plot which was prepared by adding 2µg to 20µg of analytical grade oxydemeton methyl in the Ellman's reaction mixture and the per cent inhibition recorded for this insecticide.

Pharmacodynamic studies of oxydemeton methyl were carried out in 2nd instar grub and adult by starving them for 4 hours before treatment so as to clear the alimentary canal of its content and to bring insects into an uniform metabolic state. The experiments were conducted under laboratory conditions at 25 ± 1°C. Penetration studies of all pesticides were carried out by 'wash off technique' of Lewis (1980) in which 10 µg of oxydemeton methyl dissolved in 1 µl acetone was topically applied on abdomen of the grub and on the pronotum of adult with the help of Arnold automatic micro applicator (Burkard Instruments Company, England) using a glass tuberculin syringe fitted with a 30 gauge hypodermic needle. Controls were simultaneously run in which 1 µl of acetone containing no pesticide were used. After solvent had evaporated the treated insects of same age and size were placed in thoroughly cleaned petridishes containing aphids as food and held at 25 ± 1°C in B.O.D. incubator for further processing. For unabsorbed oxydemeton methyl the treated grubs and adult were rinsed with distilled acetone at different time intervals after treatment. All the rinses were combined together and held at 0-4°C in the refrigerator.

After the removal of external insecticide by washing with acetone the presence of insecticide in the excreta were quantitatively estimated by washing the containers 2-3 times at different time intervals after treatment with distilled acetone. The rinses were pooled together and kept in the refrigerator at 0-4°C.

Similarly, haemolymph was also taken out at different time intervals after treatment. The insects were picked in such a way that the thumb was placed against the ventral surface of the insect's abdomen and the forefinger against the dorsal abdominal surface. Transverse cuts were then made completely through the ventral side. Peristaltic pressure was then applied to the insect's body by means of thumb and forefinger. Blood flowed from the cuts.

Mouthparts and anus were plugged with paraffin so that escape of blood could be prevented. The abdominal cavity was flushed with acetone. The sample filtered through muslin cloth was collected in Erlenmeyer's flask and diluted with acetone and stored at –15°C for further analysis.

After haemolymph was taken out at different time intervals, the brain of the insects were dissected out at different time interval after the treatment and macerated with 1 ml of acetone. The material was filtered through muslin cloth and filtrate was collected and stored at –15°C for further analysis. The penetration and excretion kinetics (k) were calculated by the equation.

$k = 2.303/t \log co/c$ and half life was calculated by

$\log 1/1/2 = 0.693 /$

Pesticide penetration, interval accumulation and rate of excretion were quantitatively estimated by already described enzyme inhibition technique of Ellman *et al.* (1961). For this purpose acetone was evaporated from all samples and the residues were diluted in 5 ml of 0.1 M phosphate buffer pH 8.0. For the final assay 1 ml of aqueous solution was taken in the Ellman's reaction mixture and the per cent inhibition was calculated for each sample which was extrapolated from the calibration plot.

RESULTS AND DISCUSSION

Fate of oxydemeton methyl in 2nd instar grubs of *C. septempunctata*

The pharmacodynamic data on oxydemeton methyl in 2nd instar grub of *C. septempunctata* at different time intervals following topical application (Table-1) revealed that external rinse contained 93 per cent of the applied dose indicating that at 0 minutes only 7 per cent of the applied dose might have penetrated into the body of the grub. While at 30, 60 and 90 minutes post treatment, the amount of oxydemeton methyl in the external rinse decreased to 53, 33 and 13 per cent suggesting that 47, 67 and 87 per cent of the applied dose had penetrated into the body of the grub respectively. The rate of penetration increased with the passage of time and was maximum at 120 minutes post treatment when only 10 per cent was recovered in the external rinse and rest 90 per cent of applied does had moved into the body of the grub. The half-life for the penetration of oxydemeton methyl was 36.26 minutes.

Absorption of insecticide in the haemolymph was found directly dependent on the rate of penetration as is evident from the data that at 0 minutes no absorption of insecticide was observed in haemolymph, whereas at 30, 60, 90 and 120 minutes post treatment 33.0, 43.0, 46.0 and 43.0 per cent of the applied dose was recovered from the haemolymph. The initial increase in rate of absorption of insecticide followed by decrease in the haemolymmph reflects that excretion removes substantial amount of insecticide from the haemolymph. This was also evident from the accumulation of insecticide in the brain as at 0 and 30 minutes post treatment no accumulation of insecticide was observed in the brain whereas at 60 minutes post treatment 4.0 per cent of the applied dose got accumulated in brain which increased to 5.0 per cent by 90 minutes post treatment and then again decreased to 3.0 per cent by 120 minutes, suggesting that accumulation of the insecticide in the target organ depends on the concentration in the haemolymph which exhibits first order kinetics.

Rate of excretion is directly related to the penetration rate as is evident from the data that at 0 minutes post treatment no excretion of insecticide took place from the body of the grub. Whereas, at 30 minutes post treatment only 3 per cent had been excreted which increased to 16 per cent at 60 minutes post treatment. This increase in excretion rate continued at 90 and 120 minutes post treatment excreting 23.0 and 33.0 per cent of the applied dose which clearly indicated that rate of excretion is

Table 1: Penetration, absorption in haemolymph, accumulation in brain and excretion of oxydemeton methyl (applied topically 10µg/insect) by 2nd instar grubs of *Coccinella septempunctata* Linn.

% of applied dose (± SD)*

Time after treatment (min.)	External rinse	Penetration	Absorption in haemolymph	Accumulation in brain	Excretion	Unextractable
0	93.0	7.0 (0.0030)	—	—	—	7.0 (0.021)
30	53.0	47.0 (0.0087)	33.0 (0.0035)	—	3.0 (0.0064)	11.0 (0.031)
60	33.0	67.0 (0.0059)	43.0 (0.0010)	4.0 (0.104)	16.0 (0.0097)	4.0 (0.037)
90	13.0	87.0 (0.0030)	46.0 (0.0050)	5.0 (0.0055)	23.0 (0.0107)	13.0 (0.018)
120	10.0	90.0 (0.0026)	43.0 (0.0053)	3.0 (0.0065)	33.0 (0.0038)	11.0 (0.027)
Half life (min.)	—	36.26	—	—	29.53	—

* Data represents averages of three replicates

dependent on the amount present in haemolymph thereby again exhibiting first order kinetics. The half life for the excretion of oxydemeton methyl was 29.53 minutes.

Fate of oxydemeton methyl in adult of *C. septempunctata*

The data on rate of penetration of oxydemeton methyl in adult of *C. septempunctata* at different time intervals following topical application (Table-2) revealed that at 0 minutes post treatment, external rinse contained 98 per cent indicating 2 per cent of the applied dose might have penetrated into the body of the adult, however, at 30 minutes post treatment 50 per cent was recovered from external rinse indicating rest 50 per cent of the applied dose got penetrated into the body of the adult. With the passage of time the amount of insecticide in the external rinse decreased and penetration rate increased, with 60, 90 and 120 minutes post treatment the amount of insecticides in the external rinse decreased to 34.0, 13.0 and 10.0 per cent, establishing that the percentage that got penetrated into the body of adult increased to 66.0, 87.0 and 90.0 respectively of the applied dose. The half life for the penetration of oxydemeton methyl in the adult was 32.47 minutes. There was no absorption of insecticide in haemolymph at 0 minutes which increased with the time reaching to 34.0, 43.0, 53.0 and 50.0 per cent of the applied dose by 30, 60, 90 and 120 minutes post treatment respectively, thereby suggesting that the build up of toxicant in haemolymph is directly related with penetration rate. Accumulation of toxicant in brain is proportional to the amount present in the haemolymph as is evident that at 0, 30 and 60 minutes post treatment there were no traces of insecticide in the brain while as at 90 minutes post treatment 5.0 per cent of the applied dose was recovered from brain which slightly decreased to 4.0 per cent at 120 minutes post treatment.

The rate of excretion increased from 13.0 per cent at 30 minutes to 20.0, 25.0 and 34 per cent at 60, 90 and 120 minutes post treatment respectively showing that the excretion was dependent on the amount of toxicant present in haemolymph. The half-life for the excretion of oxydemeton methyl was 80.52 minutes.

The two stages of the insect used in the present investigations showed by and large similar pharmacodynamic behaviour, (Fig. -1 and Fig. -2). Within 30 minutes post treatment 47.0 and 50.0 per cent of oxydemeton methyl had penetrated the 2nd instar grub and adult of *Coccinella septempunctata* respectively, while at the same time interval absorption in haemolymph had reached to 33.0 and 34 per cent respectively in the grub and adult. However, the excretion rate was markedly different as is evident from the fact that only 3.0 per cent of oxydemeton methyl had been excreted in grubs and 13 percent in adult at the same time interval. Earlier studies (Bull *et al.,* 1987) suggested that high level of toxicity of malathion applied topically to *Microplitis croceipes* (Cresson) was most likely related to extremely rapid penetration of the compound. At 60 minutes post treatment the penetration rate was similar in both the stages with 67.0 and 66.0 per cent of oxydemeton methyl being penetrated in grubs and adult, while absorption reached to 43.0 per cent in both the stages at the same time intervals. While there was no presence of oxydemeton methyl in the brain of adult, 4.0 per cent was found in brain of grubs at 60 minutes post treatment, but, at this time the excretion had increased to 16.0 and 20.0 per cent in grubs and adult, respectively. At 90 minutes post treatment the penetration rate was uniform in both the stages of insect with 87.0 per cent penetration, while absorption in the haemolymph varied being 46.0 and 53.0 per cent in grubs and adults and accumulation in brain remained uniform with 5.0 per cent in both the stages of the insect at the same time interval. However, excretion rates did not differ significantly at this time interval as 23.0 and 25.0 per cent had been excreted by the grub and adult respectively.

Table 2: Penetration, absorption in haemolymph, accumulation in brain and excretion of oxydemeton methyl (applied topically 10µg/insect) by adults of *Coccinella septempunctata* Linn.

% of applied dose (± SD)*

Time after treatment (min.)	External rinse	Penetration	Absorption in haemolymph	Accumulation in brain	Excretion	Unextractable
0	98.0	2.0 (0.0072)	—	—	—	2.0 (0.029)
30	50.0	50.0 (0.0049)	34.0 (0.0050)	—	13.0 (0.0082)	3.0 (0.033)
60	34.0	66.0 (0.0069)	43.0 (0.0030)	—	20.0 (0.0081)	3.0 (0.041)
90	13.0	87.0 (0.0080)	53.0 (0.2044)	5.0 (0.0077)	25.0 (0.0026)	4.0 (0.025)
120	10.0	90.0 (0.0059)	50.0 (0.1998)	4.0 (0.0060)	34.0 (0.0072)	2.0 (0.033)
Half life (min.)	—	32.47	—	—	80.52	—

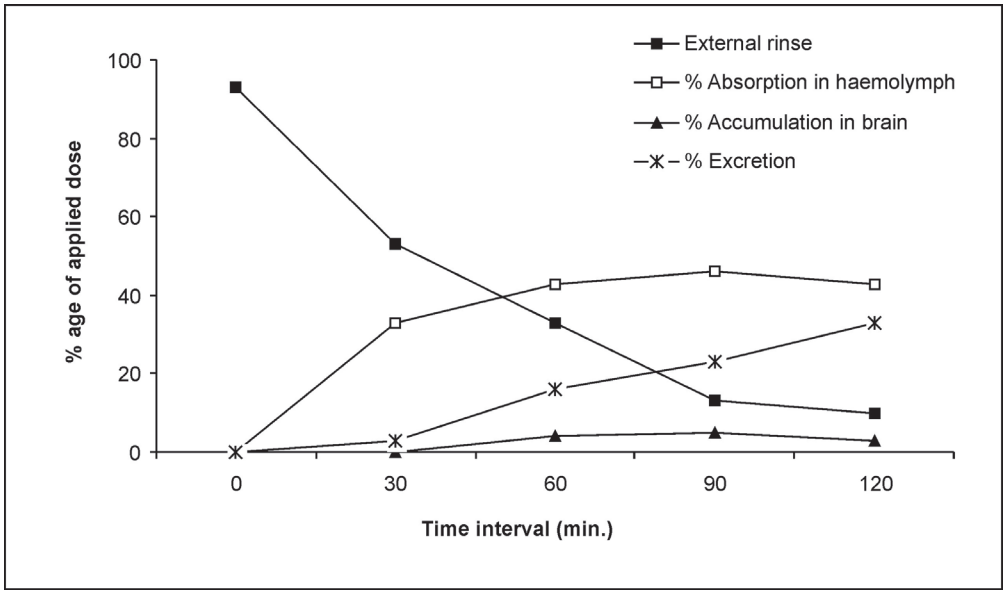

Fig. 1: Pharmacodynamics of oxydemeton methyl in 2nd instar grub of *Coccinella septempunctata* at different time intervals

Other studies (Frances *et al.,* 1973) have also shown that carbaryl, malathion, endrin and DDT penetrated more rapidly and reached higher concentration in interval tissues of 4th instar larvae of susceptible strain of Tobacco bud worm. Similar results were also observed by Benezet and Forgash (1972) who reported higher concentration of topically applied malathion was observed in midgut, muscle tissue, salivary glands and brain of the susceptible strain of housefly and malathion being distributed by the haemolymph. At 120 minutes post treatment 90.0 per cent of oxydemeton methyl had penetrated in both the stages of the insect, while absorption in haemolymph and accumulation in brain declined to 43.0 and 3.0 per cent in grubs and 50.0 and 4.0 per cent in case of adult at the same time interval. However, the excretion rate at 120 minutes post treatment increased to 33.0 and 34.0 per cent in grubs and adult respectively. Penetration and excretion processes exhibited first order kinetics in both the stages of insect. Similar findings (Bull *et al.,* 1989) on malathion and carbaryl have demonstrated that after topical application to adults of *Blattella germanica* Linn rates of absorption and excretion in susceptible and resistant

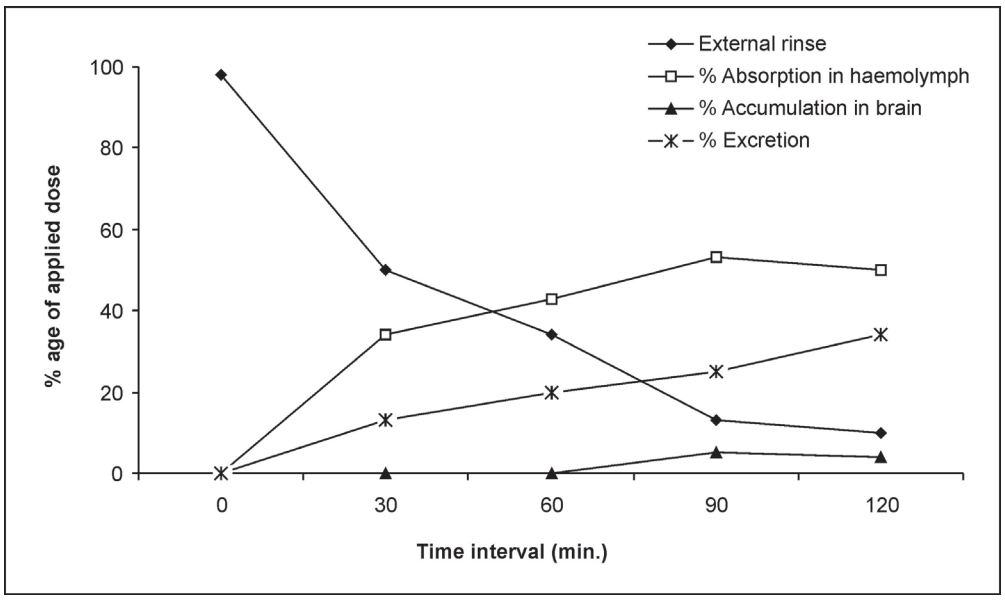

Fig. 2: Pharmacodynamics of oxydemeton methyl in adults of *Coccinella septempunctata* at different time intervals

strains did not differ significantly as both compounds were extensively metabolised. However differences were observed in the *in vivo* inhibition of AchE after topical application by these compounds suggesting strong resistance to malathion and tolerance to carbaryl in resistant strains.

REFERENCES

Benezet, H.J. and Forgash, A.J. (1972). Penetration and distribution of topically applied malathion in the housefly. *Journal of Economic Entomology,* **65**: 53-57.

Bull, D.L., Pryor, N.W. and King, E.G.Jr. (1987). Pharmacodynamics of different insecticides in *Microphitis croceipes* (Hymenoptera: Braconidae), a parasite of lepiodopteran larvae. *Journal of Economic Entomology,* **80**: 739-749.

Bull, D.L., Wadleigh, R.W. and Patterson, R.S. (1989). Pharmacodynamics of malathion and carbaryl in susceptible and multi-resistant German cockroaches (Dictyoptera: Blattellidae). *Journal of Economic Entomology,* **82**: 1512-1519.

Camp, H.B. and Arthur, B.W. (1967). Absorption and metabolism of carbaryl by several insect species. *Journal of Economic Entomology,* **60**: 803-807.

Dirimanvov, M., Angelova, R. and Babrikova, T. (1980). States of the harmful entomo and acarofauna and predacious species of insects in apple orchards with some plant protection technologies. *Nauchni Trudove, Entomologiya, Mikrobiologiya, Fitopatologiya,* **25**: 15-30.

Ellman, G.L., Courtney, K.D., Andres, V. and Featherstone, R.M. (1961). A new and rapid calorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology,* **7**: 88-95.

Frances, M. Szeiez. Frederick, W. Plapp, Jr. and S., Bradleigh Vinson. (1973). Tobacco Budworm: Penetration of several insecticides into the larva. *Journal of Economic Entomology,* **66**: 19-15.

Gour, I.S. and Pareek, B.L. (2003). Biology and predation potential of *Coccinella septempunctata* Linn. on mustard aphid, *Lipaphis erysimi* (Kalt) in the semi arid region of Rajasthan. *Annals of Biology,* **19**: 225-229.

Lewis, C.T. (1980). The penetration of cuticle by insecticides, pp 367-400. In : *Cuticle Techniques in Orthropodes* (Ed. T.A. Miller). Spring-Verlag, N.Y., Meidelberg, Berlin.

Sawicki, R.M. and Lord, K.A. (1970). Some properties of a mechanism delaying penetration of insecticides into houseflies. *Pesticide Science* **1**: 213-217.

Singh, V.S., Yadav, R.P. and Singh, R. (1994). Post embryonic development, survival rate and predation potential of *Coccinella septempunctata* Linn. in relation to the mustard aphid, *Lipaphis erysimi* Kalt. *Journal of Entomological Research,* **18**: 5-10.

Soni, R., Deol, G.S. and Brar, K.S. (2004). Feeding potential of coccinellids on mustard aphid, *Lipaphis erysimi* (Kalt.) *Insect Environment,* **10**: 15-16.

Vinson, S.B. and Brazzel, J.R. (1966). The penetration and metabolism of ^{14}C-labelled DDT in resistant and susceptible tobacco budworm larvae, *Heliothis virescens* Fabr. *Journal of Insect Physiology*, **59**: 600-604.

Welling, W., DeVries, J.W., Paterson, G.C. and Duffy, M.R. (1983). Toxicodynamics of Malaoxon in houseflies. *Pesticide Biochemistry and Physiology,* **20**: 360-372.