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Activity of eleven enzymes in nymphs of *Lipaphis erysimi* as affected by 2,4-D (herbicide) treatment

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Abstract

The activity of eleven enzymes involved in development, ageing and in metabolism of xenobiotics in insects, was investigated under the influence of 2,4-D (2,4-Dichlorophenoxyacetic acid), in the mustard aphid, *Lipaphis erysimi* (Kaltenbach) (Rhynchota Aphididae), after treating the 2nd instar nymphs for 13, 25 and 37 h. Studies of two hydrolases and one group transfer enzyme revealed significant effects of 2,4-D on the activity of these enzymes. The activity of esterases and glutathione S-transferases increased and that of ATPase decreased with the treatment. Observations on the activity of eight oxidoreductases revealed a significant induction in superoxide dismutase and catalase activity and no effect on the activity of NADH dehydrogenase, NADH oxidase, glutathione reductase and glutathione peroxidase, and a significant reduction in the activity of O-demethylase and succinate dehydrogenase. It was concluded that the increase in activity of esterases, glutathione S-transferases, superoxide dismutase and catalase might be due to their involvement in the metabolism and degradation of 2,4-D.

Key words: Mustard aphid, *Lipaphis erysimi*, 2,4-D, Hydrolases, Transferases, Oxido-reductases.

Introduction

The use of herbicides to control weeds is as important and as old as the use of pesticides to control the insect pests. The mode of activity of a number of herbicides is in pattern with plant growth regulators and thus they are selective in their action. In the last few decades, some reports have appeared regarding the influence of plant growth regulators (both natural and synthetic analogs) exercising a development regulatory influence on the phytophagous insects (Visscher, 1982; Bur, 1985).

The herbicide 2,4-D (2,4-Dichlorophenoxyacetic acid) has been extensively used due to its cheap synthesis and due to its selective kill of the broad leaved weeds without injuring the main crops. As most of the insects depend upon their host plants for nutritional requirements, their development too is bound to be affected by the herbicide. Herbicide 2,4-D has been reported to increase mortality and slow down the development of the European corn borer, *Ostrinia nubilalis* (Hübner) (Oka and Pimentel, 1976). Also, the body weight of the greasy cut worm, *Agrotis ipsilon* (Hufnagel) was found to decline with 2,4-D treatment (El-Ibrashy and Mansour, 1970). In 1987, Singh reported the inability of the nymphs of mango shoot psyllid, *Apsylla cistellata* Buckton to survive in galls on plants treated with 2,4-D. The treatment with 2,4-D disrupted the oviposition behaviour of weevil, *Trichosirocalus horridus* (Panzer) (Stoyer and Kok, 1989). Later on in 1994, Ahmad and Ali found that 2,4-D exercised extreme cytotoxicity to *Culex pipens fatigans* (L.). In 1998, Rup *et al*. observed deleterious influence of 2,4-D on population built up of mustard aphid, *Lipaphis erysimi* (Kaltenbach), which is an economically important pest of *Brassica* crops in India, causing damage up to 87% both in yield and quality of the mustard crops in some years (Sekhon and Bakhetia, 1994).

The present study was envisaged to investigate the influence of 2,4-D on the various enzyme systems involved in growth, development, detoxification and aging in mustard aphid, *L. erysimi*. In this endeavour, 11 enzymes were selected for investigation with the hope that the results will throw some light on the involvement of these enzymes in the metabolism of this chemical in *L. erysimi* and provide some insight into the biochemical adaptations taking place in the insect's body in response to non nutritive chemicals. The selected enzymes belong to three classes of enzymes i.e. hydrolases (esterases and ATPase), transferase (glutathione S-transferases) and oxidoreductases (catalase, superoxide dismutase, NADH dehydrogenase, glutathione reductase, NADH oxidase, O-demethylase, succinate dehydrogenase, and glutathione peroxidase).

Materials and methods

The radish, *Raphanus sativus* (L.) plants were grown in clay-pots in a glass house for maintaining the culture of the mustard aphid, *L. erysimi* under controlled 10L: 14D photoperiod regime. One month old (3-4 leaf stage) plants used for experiments were sprayed with 400 ppm (LC_{51}) of 2,4-D (Rup *et al*., 1998) by using fine spraying hand atomizer. The second instar nymphs (48-52 h old) were released on both the treated and control (sprayed with distilled water) plants for periods of 13, 25 and 37 h. The nymphs were harvested after specified treatment period both from treated and control plants and were assayed for activity of the eleven enzymes. Six replications were taken in each experiment and comparisons were made by analysing the data with SSPS software programme for student's t-test.

The activities of these enzymes were estimated by using the various methodologies available in literature after incorporating some tissue dependent changes related to the percentage of the extracts to be prepared and used for estimations. Esterases were extracted and estimated by the method of Katzenellenbogen and Kafatos (1971)

using 0.1 M phosphate buffer (pH 6.5) for preparing the homogenates (1% w/v) and the substrate used for estimation was α-naphthyl acetate. The ATPase activity was measured according to the methodology of Kielley (1955). The nymphs (5% w/v) were homogenised in sodium chloride (0.9%) and Adenosine triphosphate was used as substrate. The methodology given by Chein and Dauterman (1991) was followed for extraction and estimation of glutathione S-transferases and 0.1 M sodium phosphate buffer (pH 7.6) was used for preparing the the homogenate (2% w/v) of nymphs. The estimation was done with CDNB as enzyme substrate.

Catalase was estimated according to the protocol given by Bergmeyer (1974). The nymphs (5% w/v) were homogenized in 0.05 M phosphate buffer (pH 7.0) and the decrease in absorbance caused by the breakdown of H_2O_2 was recorded. The activity of superoxide dismutase was estimated by the methodology of Kono (1978). The homogenate (10% w/v) was prepared in sodium carbonate buffer (50 mM, pH 10.0) and the assay mixture contained buffer, Nitroblue tetrazolium dye, Triton X-100, hydroxylamine hydrochloride and enzyme extract. The NADH dehydrogenase activity was estimated according to the procedure given by King and Howard (1967). The nymphs were homogenized (5% w/v) in phosphate buffer (0.1 M, pH 7.4) and the enzyme activity was determined using Potassium ferricyanide as substrate. The glutathione reductase activity was measured according to the methodology of Carlberg and Mannervik (1975) using 50 mM Potassium phosphate buffer (pH 7.6) for preparation of homogenate (10% w/v). The oxidation of NADPH was spectrophotometrically recorded. The NADH oxidase activity was estimated by the method of Mackler (1967). Sucrose solution (5% w/v) was used in preparation of homogenates and the enzyme activity was determined by recording the decrease in absorbance corresponding to oxidation of NADH by molecular oxygen. The O-demethylase activity was estimated by following the method given by Lee and Scott (1989).The homogenate (10% w/v) of nymphs was prepared using 0.05 M Tris-HCL buffer (pH 7.7) and enzyme activity was measured with Para Nitroanisole as substrate. Succinate dehydrogenase activity was measured by following procedure of King (1967). The buffer used for preparing homogenate (10% w/v) of nymphs was Potassium phosphate (0.1 M, pH 7.0) and the substrate Potassium ferricyanide was used for estimating the enzyme. Glutathione peroxidase activity was measured according to the method standardised by Calzyme laboratories, Inc. Sodium phosphate buffer (0.1 M, pH 7.0) was used for preparing homogenates (5% w/v) and the enzyme activity was measured by recording the decrease in absorbance due to NADPH oxidation.

Results

The treatment of the second instar larvae with 2,4-D induced variable changes in the activity of selected eleven enzymes. Among the hydrolases, esterases which are involved in detoxification of xenobiotics showed a significant increase of 68% in activity compared to control after 37 h of treatment whereas the activity of ATPase got suppressed compared to control and it was reduced to 31% of the control after 37 h of treatment (table 1, figure 1).

The glutathione S-transferases which are the principal detoxification enzymes showed a significant increase in

Table 1. Influence of 2,4-D (400 ppm) on the activity of enzymes in *L. erysimi*.

Enzymatic activity	Status	0 h (48-52 h)	13 h (61-65 h)	25 h (73-77 h)	37 h (85-89 h)
Esterase (mM/g)	Control	833.66±15.00	442.60±9.51	425.66±12.98	761.62±3.14
(EST)	Treated		542.43±14.30**	457.72±1.62 N.S.	1278.36±82.92**
ATPase (mM/g)	Control	1.04±0.06	4.05±0.07	11.40±0.16	15.35±0.16
	Treated		2.25±0.08**	11.28±0.06 N.S.	4.75±0.31**
Glutathione S-transferase (mM/g)	Control	35.20±0.77	59.48±0.55	34.63±1.28	44.59± 2.03
(GSTs)	Treated		85.82±1.37**	40.95±1.65*	52.08±3.05*
Catalase (M/g)	Control	1.26±0.026	2.37±0.107	2.26±0.076	3.43±0.062
	Treated		3.77±0.033**	2.90±0.042**	4.19±0.020**
Superoxide dismutase (mM/g)	Control	36.28±0.64	27.45±0.38	41.88±1.64	33.14±0.79
(SOD)	Treated		28.11±0.11 N.S.	52.61±0.68**	35.63±0.48*
NADH dehydrogenase (mM/g)	Control	4.32±0.06	6.01±0.17	6.64±0.03	5.89±0.19
(NADH-D)	Treated		6.92±0.08**	7.56±6.70 N.S.	5.68±0.26 N.S.
Glutathione reductase (mM/g)	Control	0.23±0.03	0.11±0.01	0.10±0.01	0.21±0.01
(GLUTA-R)	Treated		0.12±0.01 N.S.	0.11±0.01 N.S.	0.25±0.04 N.S.
NADH oxidase (mM/g)	Control	0.09±0.03	0.12±0.02	0.13±0.02	0.14±0.04
(NADH-O)	Treated		0.12±0.04 N.S.	0.15±0.02 N.S.	0.14±0.02 N.S.
O-demethylase (mM/g)	Control	5.89±0.01	5.17±0.11	5.98±0.03	4.57±0.13
(O-DEM)	Treated		4.87±0.05*	5.74±0.01**	3.81±0.05**
Succinate dehydrogenase (mM/g)	Control	0.72±0.08	0.86±0.04	1.10±0.02	0.94±0.08
(SUC. D)	Treated		0.86±0.11 N.S.	0.85±0.06**	0.79±0.03*
Glutathione peroxidase (mM/g)	Control	0.91±0.11	0.84±0.40	0.93±0.40	0.80±0.50
(GLU-P)	Treated		0.94±0.50 N.S.	0.70±0.40**	0.84±0.01 N.S.

** = Significant at 1%, * = Significant at 5%, N.S. =Non significant.

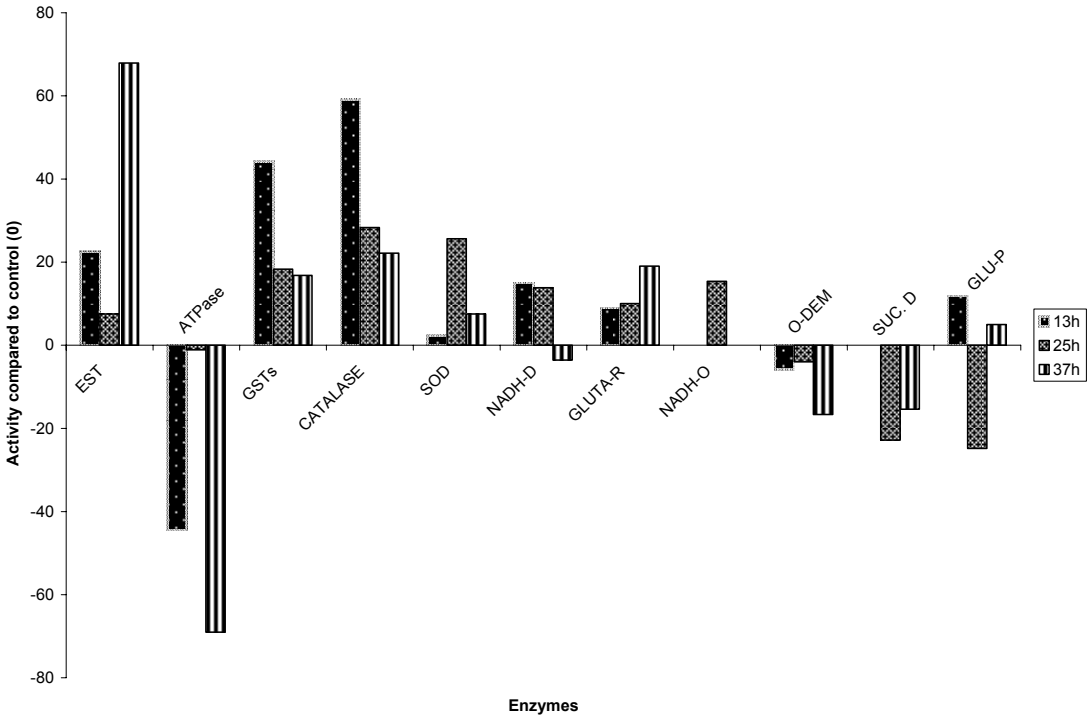

Figure 1. Percentage change in the activity of eleven enzymes as compared to control, at three time intervals under the treatment of 2,4-D, in the nymphs of *L. erysimi* (for abbreviation see table 1).

activity compared to control but the maximum increase was after 13 h of treatment where the increase in activity was 44% as compared to control (table 1, figure 1).

Among the oxidoreductases, Catalases which are involved in defence of insects against toxic effects produced by generation of free radicals also showed an increase in activity which was highly significant. Maximum increase was observed after 13 h of exposure where it was 59% more than the control (table 1, figure 1). The other defence enzyme i.e. Superoxide dismutase also showed an increase in activity which was significant only after 25 h of treatment. The activity of another oxidoreductase, NADH dehydrogenase increased significantly initially and the increase was approximately 15% compared to that in control. Similarly, the activity of glutathione reductase also increased from 9 to 19%, but was statistically not significant. NADH oxidase activity was not much affected with treatment whereas the activities of O-demethylase and succinate dehydrogenase got suppressed. The maximum suppression was after 37 h in O-demethylase by being 83% of control and after 25 h in succinate dehydrgenase where it was 77% of that in control. The activity of eighth oxidoreductase, glutathione peroxidase showed no specific pattern of change with 2,4-D treatment when compared to control (table 1, figure 1).

Discussion

Contrasting observations on the activity of two hydrolases under the influence of 2,4-D were recorded as the activity of esterases increased whereas that of ATPase decreased significantly as compared to that in untreated nymphs (control) of *L. erysimi* of the same age group. Increase in esterases activity has been perceived in *L. erysimi* with the treatment of chlorogenic acid (Rup *et al*., 1999), in Southern armyworm, *Spodoptera eridania* (Cramer) with 2,4-D and atrazine (Kao *et al*., 1995), in fall army worm, *Spodoptera frugiperda* (Smith) with 6 triazene herbicides (Yu, 2004), in wheat aphid, *Sitobion avenae* (F.) with hydroxamic acid (Loayza *et al*., 2000) and in mosquito, *Aedes* sp. with polyphenols and 7 plant allelochemicals [(-)-B–pinene, B-myrcene, D-limonene, etc.] (David *et al*., 2000; Tilquin *et al.,* 2004) and suggested the involvement of esterases in detoxification process. The role of esterases in insecticidal resistance, in metabolism, in the sequestration of xenobotics, has been well established (Devorshak and Roe, 1999). High levels of esterases found with 2,4-D treatment of *L. erysimi* in the present studies suggest that esterases might also be playing a crucial role in detoxification process of 2,4-D in this insect and helping in development of tolerance.

The ATPase is an energy generating enzyme complex of respiratory chain and any exogenous interference in its synthesis automatically affects the regulatory process of the organism. Inhibition in ATPase activity by an insecticide (allethrin) has been cited by Luo and Bodnaryk (1988) in brain of moth, *Mamestra configurata* Walker in accordance to the present observations. Similarly insecticide like DDT has also been reported to be a potential inhibitor of ATPase acitivity in *Spodoptera littoralis* (Boisduval) and *Apis mellifera* L. (Younis *et al*., 2002). The decrease in ATPase activity in the mustard aphid with 2,4-D could be due to the its interference in the energy generating enzyme complex of the insect.

The activity of only one group of enzyme belonging to

the class, transferases was assessed under the influence of 2,4-D. Glutathione S-transferases are a diverse family of enzymes found ubiquitously in aerobic organisms. They are involved in intracellular transport, biosynthesis of hormones and play a central role in detoxification of endogenous and xenobiotic compounds in insects by acting against oxidative stress (Wadleigh, 1988; Enayati *et al*., 2005). Congruent to the present findings, association of elevation of isoenzymes of glutathione S-transferases with metabolism of herbicides and plant secondary metabolites has been documented by a number of workers. Sivori *et al.* (1997) reported an increase in glutathione S-transferases activity in blood sucking bug, *Triatoma infestans* Klug with 2,4-D, indole 3-acetonitrile and indole 3-carbinol; Yu (2004) in *S. frugiperda* with 6- triazine herbicides, flavones and indole 3- carbinol; Loayza *et al*. (2000) in *S. avenae* with hydroxamic acid; Guillet *et al*. (2000) in *Tettigonia viridissima* L., *Ruspolia nitidula* Scopoli and *Conocephalus discolor* Thunberg with hypericin; Dugravot *et al.* (2004) in *Callosobruchus maculatus* (F.) with DMDS and Francis *et al.* (2005) in aphid, *Myzus persicae* Sulzer with glycosinolates from *Brassica*. Therefore, it could be inferred that in *L. erysimi* also, the glutathione S-transferase's might be actively involved in detoxification of the herbicide, 2,4-D.

Among the eight oxidoreductases assessed for their activity in nymphs of *L. erysimi*, the treatment with 2,4-D increased the superoxide dismutase activity. Usually superoxide dismutase acts on superoxide radical (O_2^-) and provides the first line of defence against toxicity from free radicals generated during metabolism and helps in development of resistance (Paes *et al*., 2001; Wang *et al*., 2001). Jiang *et al.* (1999) detected the activity of SOD in fourth instar larvae of cutworm, *Spodoptera litura* (F.) and compared the effects of the two photosensitive compounds, α-terthienyl and 1-phenyl-4- (3,4-methylene dioxy) phenyldiaethylene on superoxide dismutase activity under the specified conditions. The increase in the enzyme activity in *L. erysimi* after treatment with 2,4-D indicated a defensive role for this enzyme.

The enzyme catalase is involved in decomposition of hydrogen peroxide and provides the second line of defence against generation of free radicals. A corroboratory increase with the present findings, in the catalase activity was observed by Keywanlee and Berenbaum (1990) in the fruit fly, *Drosophila melanogaster* (Meigen) when fed on plants rich in furanocoumarins and they suggested the involvement of catalase in the detoxification of oxygen radicals generated from furanocoumarins of the host plants. Later on, Figueroa *et al.* (1999) reported a 2-fold increase in catalase activity in *S. avenae* fed on 2 mM DIMBOA (2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one). Loayza *et al.* (2000) hypothesized that catalases played an important role in the detoxification of plant secondary metabolites such as hydroxamic acid in *S. avenae.* Paes *et al.* (2001) concluded that in *Rhodnius prolixus* (Stäl) chemicals which resulted in increase in free radicals were metabolized by catalases. The increase observed in the catalase activity in the present findings in *L. erysimi* could be related to its significant role in the detoxification of free radicals generated by 2,4-D treatment.

The activity of O-demethylase which is an important enzyme of cytochrome P_{450} dependent monooxygenase system (Hodgson, 1985) was significantly reduced in the present case. In 1983, Yu studied the effects of various plant substances and host plants on the O-demethylase activity in the fall armyworm, *S. frugiperda*, maintained on a meridic diet. He also reported that indole-3-carbinol, indole-3-acetonitrile and flavone, the parent substance of all flavonoids caused an increase in microsomal oxidase activity. The relationship among O-demethylase (MFO) and phoxim resistance was studied in cotton bollworm, *Helicoverpa armigera* (Hübner) by Tang *et al.* (2000). The activity of pNA O-demethylase in the resistant strains was 3.4-fold higher than that in the susceptible strain and they concluded that O-demethylase might be playing an important role in development of phoxin resistance in *H. armigera*. It seems that the reduction observed in the activity of O demethylase in *L. erysimi* with 2,4-D could rather be due to the latter's interference in the biosynthetic pathway of this enzyme.

Succinate dehydrogenase belongs to complex-II of respiratory chain and is present in inner mitochondrial membrane. In the present study the activity of succinate dehydrogenase also decreased with 2,4-D treatment. A similar decline in the succinate dehydrogenase activity with dimilin, batex and K-othrine was observed in the bug, *Diplonychus indicus* Venkatesan et Rao (Raja and Venkatesan, 2001). The present study showed that 2,4-D interfered in the respiratory enzyme chain complex to produce its deleterious influence on the insect. The activity of NADH dehydrogenase which is also an inner mitochondrial bound enzyme and catalyzes the transfer of electrons from NADH to ubiquinone was only influenced initially with the treatment of 2,4-D in *L. erysimi*.

The enzyme glutathione peroxidase which plays an important role in defence mechanisms of animals against oxidative damage by catalyzing the reduction of a variety of hydroperoxides, using glutathione as a reducing substrate, was not influenced with treatment of 2,4-D. However, Lee (1991) reported an inhibition in glutathione peroxidase activity under the influence of two plant phytotoxins (xanthotoxins and harmine) in *Trichoplusia ni* (Hübner) and *Papilio polyxenes* F.. Similarly, Leszczynski *et al.* (1993) reported a decrease in the glutathione peroxidase activity in *S. avenae* when fed on wheat cultivar containing high concentration of cereal allelochemicals (phenolic compounds and hydroxamic acid). On the other hand, induction of glutathione peroxidase reported in *A. mellifera* with exposure to flumethrin was correlated to elevated oxidative stresses by Neilson *et al*., (2000).

The activities of glutathione reductase, and NADH oxidase, were also not significantly influenced with the treatment of 2,4-D. An induction in glutathione reductase activity has been reported in *T. infestans* as enzymatic defence against reactive oxygen species which were generated due to flavonoids like quercetin, myricetin, quercetagetin and delphinidin (Sivori *et al*., 1997). Later on Guillet *et al.* (2000) confirmed that glutathione reductase is involved in insects like *T. viridissima*, *R. nitidula*, and *C. discolor* in order to attenuate the oxidative stress

caused by photosensitisation of hypericin.

The increase in the activities of catalase, esterases, glutathione S-transferases, and superoxide dismutase and decrease in the activities of ATPase, succinate dehydrogenase and O-demethylase under the influence of 2,4-D might be the underlying cause at molecular level for the reduction in the percentage emergence and then on population built up of *L. erysimi* observed earlier by Rup *et al.* (1998, 2002).

Conclusions

It can be concluded from the present studies that among the eleven enzymes investigated for their activity in the nymphs of *L. erysimi* after treatment with 2,4-D, the enzymes catalase, esterases, glutathione S-transferases, and superoxide dismutase might be involved in the metabolism of 2,4-D or were helping in reducing the oxidative stress caused by the treatment and the increase in their activities was in response to feedback mechanisms. Since the enzymes catalases, esterases and glutathione S-transferases constitute super-families of enzymes, therefore these enzymes need to be further explored for the identification of the specific enzyme involved in the possible metabolism of 2,4-D. Further 2,4-D treatment reduced the activity of ATPase, succinate dehydrogenase and O-demethylase indicating its interference in the metabolic pathways involving synthesis of these enzymes. The remaining four enzymes were not much affected indicating their non involvement in the metabolism or detoxification of 2,4-D.

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References

AHMAD S., ALI M. N., 1994.- Cytotoxic assay of 2, 4-dichlorophenoxyacetic acid (2, 4-D) by mitotic index profiles in *Culex pipiens fatigans*.- *Mitteilungen der Schweizerischen Entomologischen Gesellschaft*, 67: 169-175.

BERGMEYER H. U., 1974.- *Methods of enzyme analysis*.- Academic Press, New York, USA.

BUR M., 1985.- Influence of plant growth hormones on development and reproduction of aphids (Homoptera: Aphidinea: Aphididae).- *Entomologia Generalis*, 10: 183-200.

CARLBERG I., MANNERVIK B., 1975.- Purification and characterization of the flavoenzyme glutathione reductase from rat liver.- *Journal of Biological Chemistry*, 250: 5475-5480.

CHEIN C., DAUTERMAN W. C., 1991.- Studies on glutathione S-transferases in *Helicoverpa* (=*Heliothis*) *zea*.- *Insect Biochemistry*, 21: 857-864.

DAVID J. P., REY D., CUANY A., AMICHOT M., MEYRAN J. C., 2000.- Comparative ability to detoxify alder leaf litter in field larval mosquito collections.- *Archives of Insect Biochemistry and Physiology*, 44: 143-150.

DEVORSHAK C., ROE R. M., 1999.- The role of esterases in insecticide resistance.- *Reviews in Toxicology*, 2: 501-537.

DUGRAVOT S., THIBOUT E., ABO-GHALIA A., HUIGNARD J., 2004.- How a specialist and a non-specialist insect cope with dimethyl disulfide produced by *Allium porrum*.- *Entomologia Experimentalis et applicata*, 113: 173-179.

EL-IBRASHY M. T., MANSOUR M. H., 1970.- Hormonal action of certain biologically active compounds in *Agrotis ypsilon* larvae.- *Experientia*, 26: 1095-1096.

ENAYATI A. A., RANSON H., HEMINGWAY J., 2005.- Insect Glutathione transferases and insecticide resistance.- *Insect Molecular Biology*, 14: 3-8.

FIGUEROA C. C., KOEING C., ARAYA C., SANTOS M. J., NIEMEYER H. M., 1999.- Effect of DIMBOA, a hydroxamic acid from cereals, on peroxisomal and mitochondrial enzymes from aphids; evidence for the presence of peroxisomes in aphids.- *Journal of Chemical Ecology*, 25: 2465-2475.

FRANCIS F., VANHAELEN N., HAUBRUGE E., 2005.- Glutathione S-tranferases in the adaptation to plant secondary metabolites in the *Myzus persicae* aphid.- *Archives of Insect Biochemistry and Physiology*, 58, 166-174.

GUILLET G., PODESZFINSKI C., REGNAULT R. C., ARNASON J. T., PHILOGENE B. J. R., 2000.- Behavioural and Biochemical adaptations of generalist and specialist herbivorous insects feeding on *Hypericum perforatum* (Guttiferae).- *Environmental Entomology*, 29: 135-139.

HODGSON E., 1985.- Microsomal Mono-Oxygenases, pp. 225-321. In: *Comprehensive Insect Physiology, Biochemistry and Pharmacology* Vol. 11 (KERKUT G. A., GILBERT L. I., Eds).- Pergamon Press, New York, USA.

JIANG Z., XU H., WAN S., CHIU S. F., ZHAO S., SU S., SHANG Z. C., 1999.- Comparative effect of two photosensitive compounds on Superoxide Dismutase activity in *Spodoptera litura* larvae.- *Entomologia Sinica*, 6: 253-258.

KAO L. M., WILKINSON C. F., BRATTSTEN L. B., 1995.- In-vivo effects of 2, 4-D and Atrazine on Cytochrome P-450 and insecticide toxicity in Southern armyworm (*Spodoptera eridania*) larvae.- *Journal of Pesticide Science*, 20: 331-334.

KATZENELLENBOGEN B., KAFATOS F. C., 1971.- General esterases of silk worm moth moulting fluid: Preliminary characterization.- *Journal of Insect Physiology*, 17: 1139-1151.

KEYWANLEE K., BERENBAUM M. R., 1990.- Defence of parsnip webworm against photo-toxic furanocoumarins: role of antioxidant enzymes.- *Journal of Chemical Ecology*, 16: 2451-2460.

KIELLEY W. W., 1955.- Mg^{2+} activated muscle ATPases.- *Methods in Enzymology*, 2: 588-591.

KING T. E., 1967.- Preparation of Succinate dehydrogenase and reconstitution of succinate oxidase.- *Methods in Enzymology*, 10: 322-331.

KING T. E., HOWARD R. L., 1967.- Preparation and properties of soluble NADH dehydrogenases from cardiac muscle.- *Methods in Enzymology*, 10: 275-294.

KONO Y., 1978.- Generation of superoxide radical during auto oxidation of hydroxyl amine and an assay for Superoxide dismutase.- *Archives of Biochemistry and Biophysics*, 186: 189-195.

LEE K., 1991.- Glutathione-S-transferases activities in phytophagus insects: Induction and inhibition by plant phytotoxins and phenols.- *Insect Biochemistry*, 21: 353-361.

LEE S. S. T., SCOTT J. G., 1989.- Microsomal cytochrome P_{450} monoxygenase in the house fly, *Musca domestica*: Biochemical changes associated with pyrethroid resistance and Phenobarbital induction.- *Pesticide Biochemistry and Physiology*, 35: 1-10.

LESZCZYNSKI B., URBANSKA A., MATOK H., DIXON A. F. G., 1993.- Detoxifying enzymes of the grain aphid.- *Bulletin OILB/srop*, 16: 165-172.

LOAYZA-MURO R., FIGUEROA C. C., NIEMEYER H. M., 2000.- Effect of two wheat cultivars differing in hydroxamic acid concentration on detoxification metabolism in the aphid *Sitobion avenea*.- *Journal of Chemical Ecology*, 26: 2725-2736.

LUO M., BODNARYK R. P., 1988.- High affinity (Ca^{++} / Mg^{++}) ATPase in adult brain synaptosomes of the bertha armyworm, *Mamestra configurata* Wlk.- *Insect Biochemistry*, 18: 87-91.

NIELSON S. A., BRODSGAARD C. J., HANSON H., 2000.- Effects on detoxification enzymes in different life stages of honey bee (*Apis mellifera* L., Hymenoptera: Apidae) treated with synthetic pyrethroid (flumethrin).- *Alternatives to Laboratory Animals*, 28: 437-443.

OKA I. N., PIMENTEL D., 1976.- Herbicide (2, 4-D) increases insect and pathogen pests on corn.- *Science*, 193: 239-240.

PAES M. C., OLIVERIA M. B., OLIVERIA P. L., 2001.- Hydrogen peroxide detoxification in the midget of the blood sucking insect, *Rhodinius prolixus*.- *Archives of Insect Biochemistry and Physiology*, 48: 63-71.

RAJA N., VENKATESAN P., 2001.- Effect of Pesticide on Acetylcholinestrase (Ache), Succinic dehydrogenase (SDH) and Lactate dehydrogense (LDH) on enzyme activity of bug, *Diplnychus indicus* Venk & Rao (Heteroptera: Belastomatidae).- *Journal of Entomological Research*, 25: 107-111.

RUP P. J., SOHAL S. K., KAUR P., KAUR S., DHILLON M. K., KUMARI J., DHINGRA P., 1998.- Population studies on the mustard aphid, *Lipaphis erysimi* (Kalt.) in response to 14 plant growth regulators.- *Journal of Aphidology*, 12: 67-73.

RUP P. J., SOHAL S. K., DHILLON M. K., SOHI R., GURM S. K., SANDHU N., DHINGRA P., WADHWA S. K., KAUR G., 1999.- Esterases activity in *Lipaphis erysimi* (Kalt.) in response to seven PGRs.- *Journal of Applied Zoological Research*, 10: 94-97.

RUP P. J., SOHAL S. K., KAUR, G., DHILLON M. K., 2002.- Influence of allelochemicals and plant growth regulators on emergence and development of the mustard aphid, *Lipaphis erysimi* (Kalt.).- *Allelopathy Journal*, 10:53-58.

SEKHON B. S., BAKHETIA D. R. C., 1994.- Relationship between population of mustard aphid*, Lipaphis erysimi* (Kalt.) and yield losses in *Brassica napus*.- *Journal of Insect Science*, 7: 67-69.

SINGH G., 1987.- Effect of plant growth regulators on the shoot gall formation in mango caused by *Apsylla cistellata* Buckton (Psyllidae: Homoptera) in India.- *Tropical Pest Management*, 33: 176-179.

SIVORI J. L., CASABE N., ZERBA E. N., WOOD E. J., 1997.- Induction of glutathione-S-transferase activity in *Triatoma infestans*.- *Memorias do Instituto Oswaldo Cruz*, 92: 797-802.

STOYER T. L., KOK L. T., 1989.- Oviposition by *Trichosirocalus horridus*, a biological control agent for *Carduus* thistles, on plant treated with low dosages of 2, 4-diclorophenoxyacetic acid.- *Environmental Entomology*, 18: 715-718.

TANG F., YUE Y., HUA R., 2000.-The relationships among MFO, Glutathione S-transferases and Phoxim resistance in *Helicoverpa armigera*.- *Pesticide Biochemistry and Physiology*, 68: 96-101.

TILQUIN M., MEYRAN J. C., MARIGO G., 2004.- Comparative capability to detoxify vegetable allelochemical by larval mosquitoes.- *Journal of Chemical Ecology*, 30: 1381-91.

VISSCHER S. N., 1982.- Plant growth hormones affect grasshopper growth and reproduction, pp. 57-62. In: *Proceedings of the fifth International Symposium on Insect Plant Relationships* (VISSCHER J. H., MINKS A. K., Eds).- Centre for Agricultural Publishing and Documentations, Wageningen, Netherlands.

WADLEIGH R. W., 1988.- Metabolism of an organothiocyanate allelochemical by glutathione S-transferase in three lepidopterous insects.- *Journal of Economic Entomology*, 81: 776-780.

YOUNIS H. M., ABO-EL-SAAD M. M., RAZIK R. K. A., ABO-SEDA A., 2002.- Resolving the DDT target protein in insects as a subunit of the ATP synthetase.- *Biotechnology and Applied Biochemistry*, 3: 9-17.

YU S. J., 1983.- Induction of detoxifying enzymes by allelochemicals and host plants in fall armyworm (*Spodoptera frugiperda*).- *Pesticide Biochemistry and Physiology*, 19: 330-336.

YU S. J., 2004.- Induction of detoxification of enzymes by triazine herbicides in the fall armyworm *Spodoptera frugiperda* (JE Smith).- *Pesticide Biochemistry and Physiology*, 80: 113-122.

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