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Influence of cytokinine, a plant growth regulator (PGR) on the activity of some enzymes involved in metabolism, in the nymphs of *Lipaphis erysimi* (Kalt.)

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Abstract: *Five enzymes were assessed for their activity under the influence of cytokinine (N^6 Benzyladenine) (400ppm) in the nymphs (48-52 hr old) of mustard aphid, L. erysimi treated for 13, 25 and 37 hr. The activity of Glutathione S - transferases and Esterases increased with treatment as compared to control in the nymphs of the same age group. However the activity of Glutathione reductase was suppressed with treatment. NADH dehydrogenase activity and NADH oxidase activity in the nymphs was not affected much with the treatment. It was inferred that Glutathione S-transferases and Esterases might be involved in the degradation of cytokinine whereas, the other enzymes had no significant role to play in the metabolism of cytokinine.*

Key words: *Lipaphis erysimi, Cytokinine, Glutathione S-transferases, Esterases, NADH dehydrogenase, NADH oxidase, Glutathione reductase.*

Introduction

Insects have dominated the earth for the last 250 million years and they are even today providing the toughest competition to the human beings for resource exploitation. This competition got magnified many folds with the cultivation of agricultural crops and their monocultures in the recent past. A number of environmental problems have cropped up due to the extensive and non judicial use of synthetic organic insecticides in the last few decades. In order to overcome these problems scientists are exploring other eco-friendly products for the management of insect pests.

The plant growth regulators (PGRs) are organic compounds, which promote, inhibit or modify plant physiological processes even in small amounts. Since, PGRs induce alterations in plant growth processes they can cause asynchrony in host plant- insect interactions. The cytokinine (N^6 Benzyl adenine/ 6-Aminopurine) is a PGR which belongs to the kinin group of PGRs. It retards senescence in plants and is involved in the cell division. In addition to this, it has been reported to affect the morphology, development and behaviour of some insect species. (Guerra, 1970; Schaefers and Montogomery, 1973; Rup *et al*., 1998).

Mustard aphid, *Lipaphis erysimi* (Kalt.) is a serious pest of *Brassica* crops in India and other tropical countries. It builds up population very rapidly due to presence of unique features like viviparity and parthenogenesis which helps it to attain the economic threshold levels quickly. It also possesses polyphenism in the form of migratory phase which enables it to migrate to places of favourable climatic conditions to overcome the adverse climatic conditions. It can inflict damage both in quality and quantity and thus affects the crop yield sometimes up to 84% (Singhvi *et al*., 1974; Sekhon and Bakhetia, 1994). Therefore, it was proposed to investigate the influence of cytokinine on the biochemical profile of *L. erysimi* as it has already been observed to affect the growth, development and reproduction of *L. erysimi* with LC_{50} as 380 ppm. (Rup *et al.,* 2000).

Materials and Methods

The culture of *L. erysimi* was maintained on radish plants, *Raphanus sativus* Linn. in glass house under a constant photoperiod regime of 10L:14D and RH 60-70%. The second instar nymphs (48-52 hr old) were released on fresh young leaves of radish sprayed with 400 ppm concentration of cytokinine and distilled water (control). The treatment was given for three time intervals (13, 25 and 37 hr) and then nymphs were collected for analysis. The estimations were done for the activity of five enzymes i.e. Glutathione S-transferases (GSTs) (a group transfer enzyme), Esterases (a hydrolytic enzyme) Glutathione reductase (GR), NADH oxidase and NADH dehydrogenase (oxidoreductases).

The GSTs were extracted and estimated by following the methodology of Chien and Dauterman (1991) after some modifications. Two percent homogenate was prepared in sodium phosphate buffer (0.1M, pH 7.6) and centrifuged at 12,000 rpm for 30 minutes at 4 ºC. The supernatant was used for estimation of enzyme activity. The change in absorbance was recorded at 340 nm on a Systronic/UV-VIS/Dual beam spectrophotometer at intervals of one minute for a total time of 5 minutes.

The esterases were extracted and estimated by the method of Katzenellenbogen and Kafatos (1971). The homogenate (1%w/v) prepared in phosphate buffer (0.1M, pH 6.5) was centrifuged at 10,000 rpm for 20 minutes at 4ºC. The supernatant obtained was used for enzyme estimation at 540 nm.

Glutathione reductase was estimated according to the method given by Carlberg and Mannervik (1975) after some modifications. The homogenate (10%w/v) was prepared in phosphate buffer (50mM, pH 7.6) and centrifugation was done

Table – 1: The activity profile of five enzymes of *L. erysimi* nymphs after treatment with cytokinin.

Control / treated	Duration of treatment (age of nymphs)	Glutathione S-transferases	Esterases	NADH oxidases	Glutathine reductases	NADH dehydrogenases
Control	0 hr (48-52 hr)	35.33 ± 0.745	416.1 ± 1.184	0.13 ± 0.013	0.0877 ± 0.0051	0.181 ± 0.003
Control	13 hr (61-65 hr)	59.89 ± 0.583	214.75 ± 4.615	0.145 ± 0.012	0.202± 0.0065	0.259± 0.0055
Treated		76.91 ± 1.815	198.18 ± 1.66	0.088 ± 0.004	0.1612 ± 0.0095	0.24 ± 0.008
't' value		**8.92 ****	**3.38****	**4.70****	**5.04****	**1.97 N..S.**
Control	25 hr (73-78 hr)	35.63 ± 1.270	207.25 ± 6.227	0.115 ± 0.010	0.254 ± 0.02	0.287 ± 0.0014
Treated		45.75 ± 0.627	432.74 ± 15.55	0.201 ± 0.012	0.135 ± 0.006	0.288 ± 0.0019
't' value		**7.15****	**13.46****	**5.47****	**0.88 N..S.**	**0.28 N..S.**
Control	37 hr (85-89 hr)	44.57 ± 2.081	370.88 ± 1.375	0.119 ± 0.008	0.261 ± 0.016	0.256 ± 0.0079
Treated		54.082 ± 1.242	937.51 ± 2.432	0.092 ± 0.004	0.075 ± 0.006	0.256 ± 0.0006
't' value		**3.92****	**20.28****	**3.17****	**11.17****	**0.00 N..S.**

** ---- Significant at 1% , N..S. ---- Non-significant

at 12000 rpm for 30 minutes at 4 ºC. The supernatant was used for enzyme assay. The change in absorbance was recorded at a constant temperature of 30±1 ºC at 340 nm after an interval of 1minute for a total of 5 minutes.

NADH oxidase was assayed by the method of Mackler (1967) after some modifications. The homogenate (10%w/v) prepared in sucrose solution (5%w/v) was centrifuged at 12,000 rpm for 30 minutes at 4 ºC. The supernatant obtained was used for enzyme assay. The change in absorbance was recorded at 340 nm at a constant temperature of 38±1 ºC.

NADH dehydrogenase activity was estimated by the procedure given by King and Howard (1967). The homogenate (5%w/v) was prepared in phosphate buffer (0.1M. pH 7.4) and then it was centrifuged at 12,000 rpm for 30 minutes at 4 ºC. The supernatant was used for enzyme assay. The decrease in absorbance was recorded at 420 nm at a constant temperature of 30±1 ºC. The standard curve was prepared using serial dilutions of potassium ferricyanide (5mM).

Six replicates were taken for each experiment and the data was analyzed using students't' test.

Results and Discussion

Five enzyme systems were investigated for their activity under the influence of cytokinine in the nymphs of *L. erysimi* (Table I). Among these the activity of group transfer enzymes, GSTs, which showed a fluctuating pattern during the normal development of *L. erysimi,* showed a similar activity pattern with treatment but with a significant increase at each time interval. Increase in activity of GSTs was observed in grain aphid, *Sitobion avenae* (F.) and the bird cherry oat aphid, *Rhopalosiphum padi* (L.) fed on wheat containing high concentration of allelochemicals such as phenols, hydroxamic acid and indole alkaloids (Leszczynski *et al*., 1989, 1993, 1994; Leszczynski and Dixon, 1990). They suggested the involvement of GSTs in the detoxification of secondary metabolites which might have enabled the two species of aphids to survive on these plants. Similar findings have been reported in the grain aphid by Loayza *et al*. (2000). Allelochemicals such as indole 3-acetonitrile, indole 3-carbinol, flavone, sinigrin and phenylethylisothiocyanates have also been reported to increase the activity of GSTs in the fall armyworm, *Spodoptera frugiperda* (Yu, 1983). Increased levels of GSTs have further been observed in blood sucking bug, *Triatoma infestans* (Klug), Oligophagus black swallowtail, *Papillio polyxenes* (Fab.) cabbage looper *Trichoplusia ni* (Hb.) luna moth, *Actias luna* L. after treatment with allelochemicals (Sivori *et al.*, 1997; Lee, 1991; Lindroth, 1991).

On the other hand, Rup *et al.* (2000) had earlier perceived a decrease in the GSTs activity in *L. erysimi* after treatment with kinetin, another kinin hormone. Inhibitory effects of some plant secondary compounds such as phenols, flavonoids, isothiocyanates on Glutathione S-transferase activity have also been reported in *T. ni*, *P. polyxenes*, *T. infestans* and *S. frugiperda* (Lee, 1991; Sivori *et al*., 1997; Yu, 1983; Yu and Abo-elghar, 2000; Wood *et al*., 1990). However, Yu (1983) further made the observation that the plant growth regulatory compounds, gossypol, GA_3, stigmasterol, sitosterol and β carotene had no effect on Glutathione S-transferase activity in *S. frugiperda*. Similar findings have been reported by Sivori *et al*. (1997) in *T. infestans* after treatment with flavone. It could be inferred that GSTs were selectively involved in the metabolism of some xenobiotics in specific insects only.

The activity of esterases, a hydrolytic enzyme fluctuated between 214 to 430 µm during the development but it started to increase significantly after 13 hr of exposure to the cytokinine. The enzyme activity was two and a half times more compared to control after 37 hr of the exposure (Table 1). Earlier studies made by Rup *et al*. (1999) with other PGRs such as GA_3, Chlorogenic acid and Abscissin had also showed an increase in esterases activity in *L. erysimi*. But, suppression in enzyme activity was observed when nymphs were treated with Indole butyric acid (IBA), Alar B-9 and Maleic hydrazide. Rup and Kaur (1993) had also reported elevated levels of esterases in the larvae of banana fruit fly, *Zaprionus paravittiger* (Godbole and Vaidya) treated with IAA and IBA, but GA_3 treatment

suppressed the enzyme activity. An increase in esterase activity has also been reported in the grain aphid, *S. avenae* reared on wheat cultivars containing the plant secondary compound, Hydroxamic acid (Loayza *et al*., 2000). A high midgut esterase activity has also been reported by Lindroth (1991) in eastern tiger swallowtails, *Papilio glaucus canadensis* Rothschild and Jordan, fed on plants containing Salicin derived phenolic glycosides as compared to *Papilio glaucus glaucus*.L.

Nevertheless, Nizam *et al.* (1986) had perceived suppression in cholinesterase activity in nymphs and adults of cockroach, *Blatella germanica* (L.) treated with the plant secondary compound, reserpine. Another plant compound, cytosine has also been reported to inhibit the esterase and carboxylesterase activity in the diamond back moth, *Plutella xyllostella* L. (Wanchun *et al*., 1999). Similar observations were made by Smirle *et al*. (1996) in the larvae of the oblique banded leafroller, *Choristoneura rosaceana* (L.) fed on artificial diet containing various doses of neem oil.

Among the three oxidoreductases investigated, the activity of Glutathione reductase increased during the normal development of *L. erysimi*, but it was significantly depressed with the treatment and maximum depression was after 37h of treatment where the activity was even less than one-third of that in the normal insect. Similar findings have been reported by Rup *et al.* (2000) when the nymphs of *L. erysimi* were treated with kinetin. The Glutathione reductase levels were also found to be depressed in the black swallowtail butterfly, *P. polyxenes* following quercetin (a flavonoid) feeding (Pristos *et al.,* 1988).

In case of the other oxidoreductases, NADH dehydrogenase activity showed no correlation with development and it was not influenced at all with application of cytokinine as the activity pattern remained similar to that of the control. On the other hand the activity of NADH oxidase showed fluctuations during development in the control as well as in the treatments. Nevertheless, an increase in NADH dehydrogenase activity has been reported in the nymphs of *L. erysimi* after treatment with another Kinin, Kinetin.The present findings indicate that these enzymes might not be involved in aphids biochemical adaptation towards cytokinine.

It is concluded from the present investigation that Glutathione S-transferases and esterases whose activity increased after treatment might be involved in the metabolism of cytokinine. The suppression in the activity of Glutathione reductase indicates that while the enzyme is not involved in degradation of cytokinine, the metabolic pathway leading to synthesis of the enzyme might be somehow affected with treatment. The activity of other two enzymes NADH dehydrogenase and NADH oxidase was not affected with treatment indicating that these enzymes have no significant role to play in the metabolism of the cytokinine in *L. erysimi*.

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