THE EFFECT OF DIAZINON ON SOME BIOCHEMICAL CHARACTERISTICS OF CHILO SUPPRESSALIS WALKER (LEPIDOPTERA: PYRALIDAE), RICE STRIPED STEM BORER

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ABSTRACT: Rice striped stem borer is a cosmopolitan pest that widely distributed in different regions of northen Iran and spraying with diazinon is the most common procedure to control this pest. We evaluated comparative physiological effects of diazinon spraying on the three populations of Chilo suppresalis collected from Gourabzarmikh (Go), Sheikhmahale (Sh) and Rasht (Ra) in Iran. The determination of physiological effects of diazinon, activity levels of some enzymes such as aspartate aminotransferase, alanin aminotransferase, a-amylase, ATPase and lactate dehydrogenase was evaluated. Results showed that aspartate aminotransferase and alanin aminotransferase didn't have significant difference. Activity level of a-amylase, ATPase, LDH also demonstrated significantly different so that Go population was at a higher activity level of these enzyme than the other two populations. Glucose and protein amount showed a significant difference and their amount in Go population was higher than Sh and Ra populations. It is inferred that diazinon treatment decreased activity levels of several enzymes, ATPases, LDH and a-amylase, that had a significant effect on metabolism of nutrients in insect's body. This parameter could be used instead of mortality percent for efficiency of chemicals that would decrease times of spraying and prevent resistance to insecticides, environmental pollution and non-targent toxicity.

KEY WORDS: rice striped stem borer, diazinon, physiological effects, AST, ALT, a-amylase, LDH, ATPase, Protein, glucose

The rice striped stem borer (*Chilo supprressalis* Walker) is a cosmopolitan and destructive pest in rice fields of the world (Khanjani, 2006). This pest was introduced to Iran in 1973 and has been widely distributed in all rice fields of north of Iran. In north of Iran, this pest have been distributed in all areas and its density is more than economic injury level (EIL) (Dezfoulian & Moustofipoor, 1972). Recently, it has been reported from other provinces of Iran such as Isfahan, Shiraz, Eilam, Khozestan and causes severe damages. The chemical control especially organophosphorus (Ops) compounds has been a common control procedure, although other methods like cultural practices such as ploughing, usage of resistant varieties, weed control as overwintering sites and biological control with *Trichogramma* spp. has been incorporated. In recent years, using resistance varieties and pheromones also has been added to control procedure of this important pest. A study

on 78 different varieties of rice showed that Binam with 15% white head is the most resistant variety. Working on different germplasts of rice showed that Khazar variety was resistant to first generation of rice striped stem borer and susceptible to second generation. In recent years, use of pheromone traps including Z-13, octadecenal, Z-11, hexadecenal and Z-9, hexadecenal showed a useful practice for controlling of this pest in north of Iran (Khanjani, 2006). In other parts of the world, use of chemicals and resistant varieties are the main procedures for controlling of rice striped stem borer (Muralidharan & Pasaalu, 2006).

Diazinon is an organophosphorus (OP) insecticide that has been widely used to control rice striped stem borer in Iran. Certainly, chemicals have different effects on physiology and behavior of insects but no work has been carried out in regards to this aspect in Iran. Saleem & Shakoori, (1987) showed that Pyrethroids sublethal concentrations decreased the gut amylase activity in larvae of the beetle Tribolium castaneum. Mordue & Blackwell (1993) showed that azadirachtin had an adverse effect on physiology of more than 200 insect species. Lee et al. (1994) showed that some IGRs decreased activity level of a-amylase and esterase of treated larvae. Nath (2003) reported that lethal and sublethal doses of ethion and fenitrothion (OP) increased glucose and trehalose in hemolymph of silkworm, but decreased their amounts in fat body. Nathan et al. (2005) showed that several doses of neem limonoids affect the gut physiology of *Cnaphalocrosis medinalis*, the rice leaf folder. Salokhe et al. (2005) demonstrated that treatment of *Tribolium castaneum* with flufenoxuron decreased protein amount in hemolymph. Shekari (2006) showed that different concentration of *Artemisia* extract had a significant effect on ten enzymatic and non-enzymatic of *Galerucella luteola*.

In this study, three populations of rice striped stem borer were selected one from Rasht (Ra) where diazinon is applied 3-4 times annually, another from Sheikhmahale (Sh) with 1-2 times spraying and Gourabzarmikh (Go) with no spraying at all. Then, physiological characteristics of these populations were compared. For this purpose, a study was conducted on the activities of different enzymes in all populations of rice striped stem borer. The aspartate aminotransferase (AST, EC 2.6.1.1) and alanin aminotransferase (ALT, EC 2.6.1.2) serve as a strategic link between the carbohydrates and protein metabolism and are known to be altered during various physiological and pathological conditions (Etebari et al., 2005). Alpha amylase is one of the midgut enzymes that is involved in starch and other carbohydrates metabolism. Its activity level is dependent on feeding diet being different. In insects feeding on wool, this enzyme has the lowest amount while in phytophagous insects it is highest, especially in clethrophagous insects (Slansky, 1982; Dow, 1986). Its activity may be affected by chemicals (Saleem & Shakoori, 1987). ATPases are essential for transport of glucose, amino acids and etc. These enzymes are located in the midgut, malpighian tube, muscles and nerve fibers of the lepidopteran insects

(Horie, 1958). Lactate dehydrogenase (LDH, EC 1.1.1.27) is an important glycolytic enzyme being present in virtually all tissues (Kaplan & Pesce, 1996); it is also involved in carbohydrate metabolism and has been used as an indicative criterion of exposure to chemical stress (Wu & Lam, 1997; Diamantino et al., 2001). Although, it is usually used as an index of anaerobic metabolism (Chamberlin & King, 1998). To show correlation between some enzyme activity and non-enzymatic compounds, the amount of glucose and protein was measured.

MATERIALS AND METHODS

Mass culture of rice striped stem borer

To decrease the side effects of laboratory mass culture, 400 pupae of each population was collected from fields and reared on the same variety seedling (Khazar) as sampling sites. Insects were reared based on the method mentioned by Kammano & Sato (1985) in 28±10C, light cycle 16L:8D and RH > 80%. When the larvae grow up to 4th instar larvae, 240 larvae were selected for bioassay experiment and 30 larvae for biochemical analysis (for each sampling sites). To determine of 4th instar larva Dayer's formula described in Majidi et al. (2002) was used.

Preparation of enzyme extract

Midgut of larvae was homogenized in fluid nitrogen and samples of each region were diluted with phosphate buffer in weight to volume proportion and centrifuged for 10 min in 10000 r/min. The supernatant was transferred to new tubes and was preserved at -20 ºC until the onset of the experiments. Thirty larvae from each population in six replicate were provided for biochemical analysis.

Estimation of aspartate (EC 2.6.1.1) and alanin aminotransferases (EC 2.6.1.1)

Alanine aminotrasferase (ALT) and aspartate aminotransferase (AST) were measured using Thomas' (1998) procedure. This assay was done by AST and ALT kit (Biochem Co, Iran). On this basis, solution 1 and 2 were mixed (4:1). Then, samples were added and absorption was read at 340 nm.

Estimation of adenosine triphosphatases

Activity of this enzyme was assayed according to the method described by Shiosaka et al. (1971). The quantity of inorganic phosphorus liberated was assayed based on the method by Fiske & Subbarow (1925). In this method, the protein is precipitated with trichloroacetic acid. The protein free filterate is treated with acid molybdate solution and the phosphoric

acid formed is reduced by the addition of 1-amino-2-naphthol-4-sulphonic acid (ANSA) reagent to produce blue color. The intensity of the color is proportional to the amount of phosphorous present.

Estimation of a-amylase

Alpha amylase was measured using Henry & Chiamari (1960) method. In this method, CNPG3 substrate is used in which 2-chloro-4-nitrophenol has been bound to maltoriose. CNPG3 is hydrolysed by alpha-amylase and its concentration is determined at 405nm.

Estimation of lactate dehydrogenase (EC 1.1.1.27)

For evaluating lactate dehydrogenase (LDH), the King (1965) method was used. To standardize volumes, 0.2 ml NAD+ solution was added to the test tubes and 0.2 ml of water was added to control test tubes, each containing 1 ml of the buffered substrate. 0.01 ml of the sample was also added to the test tubes. Test tube samples were incubated for exactly 15 min at 37 0C and then arrested by adding 1 ml of color reagent (2,4-dinitrophenyl hydrazine) to each tube and the incubation continued for an additional 15 min. after the contents were cooled to room temperature, 10 ml of 0.4N NAOH was added to each tube to make the solutions strongly alkaline. At exactly 60 s after the addition of alkali to each tube, the intensity of color was measured at 440 nm.

Estimation of glucose and protein

Protein was measured based on Biuret's method and by utilizing a total protein assay kit (Biochem Co, Iran). In this method proteins made a complex purplish blue with an alkaline copper solution, which with its absorption value at 540 nm has a direct relation to the amount of whole body protein. Glucose was analyzed as described by Siegert (1987).

Statistical analysis

Biochemical extraction data were analyzed using SAS software and Tukey's studentized range (HSD) test in a complete randomized design (SAS, 1997).

RESULTS

Figure 1 shows activity level of ALT and AST in three populations of rice striped stem borer. ALT activity in Go, Sh and Ra was 121.66 µmol/mg/h, 118.71 µmol/mg/h and 116.12 µmol/mg/h, respectively. AST activity was 115.22 µmol/mg/h, 113.62 µmol/mg/h and 11.89 µmol/mg/h in Go, Sh and Ra populations, respectively. This data didn't show significant difference between different populations. ATPase activity level showed significant different between Go, Sh and Ra populations and was 51.11 µmol/mg/h in Go population, 39.32 µmol/mg/h in Sh population and 26.58 µmol/mg/h in Ra population (Figure 2). Activity level of a-amylase was measured 11.58 µmol/mg/h, 8.96 µmol/mg/h, 6.32 µmol/mg/h in Go, Sh and Ra populations, respectively and showed a significant difference (Figure 2). Lactate dehydrogenase had a greater activity level in Go population (50.05 µmol/mg/h) than other populations (32.45 µmol/mg/h in Sh and 17.09 µmol/mg/h in Ra) and showed a significant difference between these populations (Figure 2). The amount of two non-enzymatic compounds also showed a significant difference in different populations of C. suppressalis (Figure 3). The amount of protein in Go population was 2.52 mg/dl, 1.81 mg/dl in Sh population and 0.75 mg/dl in Ra population. The amount of glucose was 18.5 mg/dl in Go population, 14.8 mg/dl in Sh population and 8.5 mg/dl in Ra population.

DISCUSSION

Different factors may affect the enzymatic and non-enzymatic compound of insect body such as weather conditions, feeding diet, chemicals and etc. The aim of this study was to show diazinon spraying on physiological characteristics of rice striped stem borer. In these two sites climatic factors such as temperature, relative humidity and photoperiod are similar and about feeding habit, it can be said that in these sites Khazar and Hashemi varieties have been cultivated alternatively for more than 10 years. Several researches have showed that chemicals could affect biochemical characterizations of insects. Diazinon as an insecticide widely used in Iran, is not exceptional and these results confirm other research. But, there is not any report about rice striped stem borer resistance to diazinon in paddy fields of Iran. Al-Hosseini et al., (1998) showed that percentage of diazinon mortality in Ramsar and Tonekabon populations (places with a 100 Km distance from Rasht) is 67% in recommended dose. Four years later, it was presented that diazinon had an acceptable mortality in Rasht, Lahidjan and Somehe Sara populations of rice striped stem borer and there wasn't a significant difference in percent mortality of diazinon (Saeb, 2002).

In this study, the activity level of two aminotransferases, AST and ALT, were evaluated. Our results showed that AST and ALT activity levels didn't have significant differences in Go, Sh and Ra. These enzymes can be correlated with some resistant mechanisms to pesticides. Ender et al.

(2005) reported that the diet with high level of methyl parathion significantly increased the activities of ALT and AST in greater wax moth, *Galleria mellonella* L. larvae. But, the activity levels of aminotransferases were decreased by low level of this insecticide. Ethyl parathion in diet significantly increased ALT activity at low level. Shekari (2006) demonstrated that activity levels of these enzyme decreased in *Galerucella luteola* larvae treated with *Artemisa annua*, but increased after 48 h and reached to similar amount of control. This mechanism might have occurred in rice striped stem borer larvae treated with diazinon in Sh and Ra populations.

ATPases are membrane bound enzymes. The role of membrane lipids and their micro-environmental changes at physical and chemical levels may be responsible for the differential response observed at the level of ATPases activity in these populations. Membrane ATPases assist transport, reabsorption of metabolites and nutrients. These enzymes are secondary careers of ions and non-electrolytes (Lechleitner et al., 1988; Fogg et al., 1991). Blasiak (1994) represented that parathion and methylparation inhibited the activity ATPases. Babu et al. (1996) showed that the ATPase activity in *Helicoverpa armigera* was significantly decreased because of toxic effects of chemicals. Nathan et al. (2005) showed that ATPase activity level in *Cnaphalcrocis medinalis* significantly decreased after treatment with azadirachtin. In Ra and Sh populations where diazinon spraying is applied 1-4 times annually compared with Go population that don't use any insecticides, activity level of ATPase significantly decreased. It could be concluded that diazinon is effective on ATPases and disturbs absorption of metabolites and nutrients.

Lactate dehydrogenase (LDH) is an important glycolytic enzyme being present virtually in all tissues (Kaplan & Pesce, 1996); it is also involved in carbohydrate metabolism and has been used as an indicative criterion of exposure to chemical stress (Wu & Lam, 1997; Diamantino et al., 2001) and it is used as an index of anaerobic metabolism (Chamberlin & King, 1998). Activity level of lactate dehydrogenase in *Culex* after treatment with DDT, malathion and cyfluthrin decreased 58.88%, 33.33% and 66.66% respectively (Arshad et al., 2002). Nathan et al. (2005) showed that feeding of *Spodoptera litura* on *Ricinus communis* L. treated with azadirachtin and nucleopolyhedrovirus decreases the amount of this enzyme in midgut that demonstrates low nutritional efficiency of the larvae. Similar results were also observed on effectiveness of *Melia azedarach* on rice leaffolder (Nathan, 2006). Hence, using chemicals may decrease activity level of LDH. In this study, activity level of LDH in Ra and Sh population was significantly lower than Go population that it could be due to diazinon application in these two sites.

In this study, activity level of a-amylase had a significant difference between different populations and activity level of this enzyme was higher in Go population. Saleem & Shakoori, (1987) showed that Pyrethroids sublethal concentrations decrease gut amylase activity in larvae of the

beetle Tribolium castaneum. Lee et al. (1994) showed that some IGRs decreased activity level of a-amylase and esterase of treated larvae. Shekari (2006) reported that activity level of this enzyme decreased due to treatment of *G. luteola* with *A. annua* extracts.

In the present study the amount of two non-enzymatic compound was also measured. The aim of this study was to show correlation of these compounds with some enzymatic components. The amount of these compounds has a significant correlation with activity levels of ATPase, alpha-amylase and lactatedehydrogenase. In Ra and Sh populations the activity level of these enzymes was lower than Go population and there was similar condition of glucose and protein amounts. As it has been, weather and feeding condition in these populations are similar shown and lower amount of these non-enzymatic compounds could be attributes to use of diazinon in Ra and Sh populations. Etebari (2006) showed that many insecticides decreased feeding efficiency and protein amount of an insect's body. The amount of protein in *Tribolium castaneum* decreased due on application of sublethal concentrations of flufenoxuron (Salokhe et al., 2005). Schmidt et al. (1998) showed that treatment of *Spodoptera littoralis* and *Agrotis ipsilon* with azadirachtin decreased protein of hemolymph. This could be due to the break down of protein into amino acids, so with the entrance of these amino acids to TCA cycle as a keto acid, they will help to supply energy for the insect. So, protein depletion in tissues may constitute a physiological mechanism and might play a role in compensatory mechanisms under insecticidal stress to provide intermediates to the krebs cycle by retaining free amino acid content in hemolymph (Nath et al., 1997). Shekari (2006) also showed similar results on *G. luteola* in amount of glucose. Radhakrishna & Devi (1992) showed that treatment of silkworm larvae with organophosphorus decreased the amount of glucose. Nath (2003) represented that lethal and sublethal doses of ethion and fenitrothion decreased the amount of glucose and trehalose of silkworm.

The present findings clearly depict the effects of diazinon application on physiological characteristics of rice striped stem borer in north of Iran. It has been shown by various authors including the present one, that chemicals have a significant effect on the physiological characteristics of insects. These aspects could be considered for future study rather than taking into consideration only mortality for efficiency of chemicals. Hence, the times of spraying for prevention of resistance, environmental pollution and non-target toxicity could be considered.

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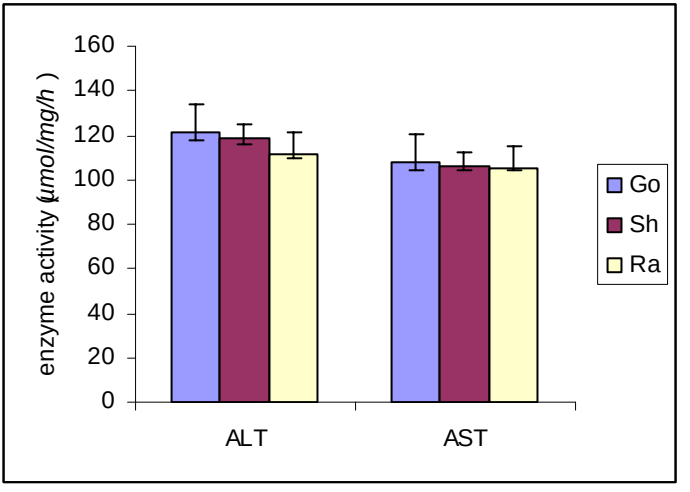

Figure 1. Aminotransferases activity in fourth instar larvae of *Chilo suppressalis* from Gourabzarmikh (Go), Sheikhmahale (Sh) and Rasht (Ra) populations , Tukey test (p <0.05).

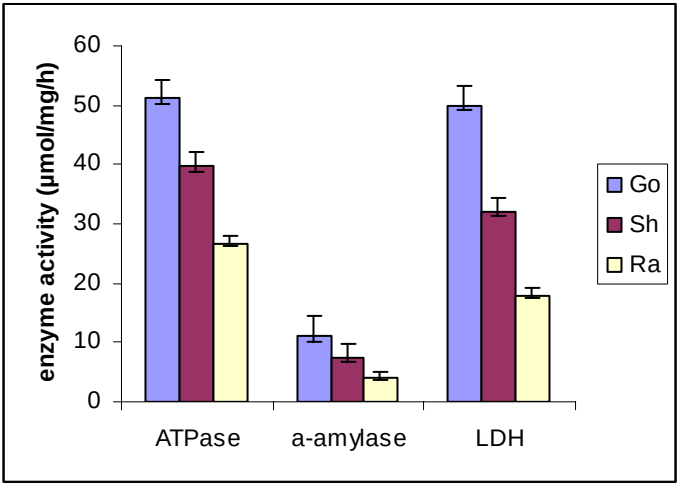

Figure 2. Activity of ATPase, *a*-amylase and lactate dehydrogenase in fourth instar larvae of *Chilo suppressalis* from Gourabzarmikh (Go), Sheikhmahale (Sh) and Rasht (Ra) populations, Tukey test (p <0.05).

Figure 3. Amount of non-enzymatic compounds in fourth instar larvae of *Chilo suppressalis* from Gourabzarmikh (Go), Sheikhmahale (Sh) and Rasht (Ra) populations, Tukey test (p <0.05).