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EFFECT OF TWO PLANT EXTRACTS ON SOME BIOCHEMICAL PARAMETERS IN THE FRUIT FLY *Ceratitis capitata* Larvae (Weid)

Abbas, Afaf A.
Department of Zoology, Faculty of Science for girls, Al Azhar University, Cairo, Egypt.

ABSTRACT

Half lethal doses (LC_{50}) of crude ethanolic extracts of *Vinca rosea* and *Melia azedarach* leaves (80 and 50ppm, respectively) induced significant biochemical alterations in the second instar Larvae (L_2) of the fruit fly *Ceratitis capitata* under Laboratory conditions. Activities of all enzymes tested (trehalase, amylase, invertase, lactic and isocitric dehydrogenases, alanine and aspartate transaminases) were significantly increased as a result of inclusion of both extracts in the Larval diet, while total protein, lipids, glucose, and glycogen contents were significantly decreased at the same doses. In the meantime, the free amino acid (FAA) content in the treated Larvae showed mild significant increase at these doses compared to the untreated control Larvae. Therefore, the macromolecular abnormalities observed in the second instar Larvae of *C. captitata* can reflect the possible use of the two extracts as natural insecticides with special promising safe control of this insect.

INTRODUCTION

Entomologists are aware of the mounting concern over the environmental pollution by insecticides currently in use beside the resistance of the pests to pesticides. Therefore, the sole reliance on synthetic pesticides will result in complete failure to achieve any goal of self-sufficiency in food supply. During the last decade, and for environmental considerations, the use of plant extracts has emerged as a new trend in the production of naturally occurring insecticides for safe and cheap pest control (Schmutterer *et al*, 1981, Epino and Chang, 1993). Numerous aqueous and organic crude extracts of plants from different families have been screened for pesticidal activity (Schmutterer and Ascher, 1984, Lopez-Olguin *et al*., 2002). The application of these extracts and their active principles especially from leaves and seeds of *Azadirachta indica* (neem) have drawn attention because of their pronounced effects on various developmental stages of many insects (Schmutterer and Ascher. 1987; Potenza *et al*., 1999 and Vinuela *et al*., 2000). Such materials proven biodegradable and less toxic than synthetic insecticides. However, *A. indica* does not grow in Egypt. So, the utilization of *Melia azedarach* extracts alongside other plants as alternatives, has been developed for similar studies in Egypt (Taha, 1989; Taha *et al*., 1989; Ibrahim *et al*., 1992). In fact, the introduction of plant extractives of prospective insecticidal value necessitates intensive, multidirectional studies on different insects. Although much work have been done on their toxicity and insecticidal properties. There are very few reports on their sublethal biochemical effects, and their mode of action. The object of the present study is to disclose the macromolecular abnormalities induced in the second instar larvae of *C. capitata* exposed to sublethal doses of two plant extracts to highlight their possible site(s) of action as natural insecticides of prospective applicability.

MATERIALS AND METHODS

Rearing Of Insect:

The master culture of *C. capitata* was obtained from Fruit Fly Section, Plant Protection Research Institute (PPRI, Cairo, Egypt). The insects were maintained in a temperature-controlled laboratory at 26±2^{0}C with relative humidity of 75±5%. The larvae were kept on a stock diet (Walder. 1989) in muslin-covered jam jars (300 ml). The second instar larvae collected 2.5±0.5 day after egg laying, were used in the present investigations.

Extract Preparation And Procedure Adopted:

Crude ethanolic leaf extracts (CELE) of both test plants were prepared as described by Abdul Kareem (1981). Their LC_{50} values on the L_2 of *C. capitata* were preliminary determined under laboratory conditions according to Maurer (1983). The choice of ethanolic extracts was based on the work of Jacobson *et al.*, (1983), who revealed by HPLC measurements that high levels of the antifeedant azadirachtin and its derivatives are found in ethanolic extracts of neem organs. The LC_{50}s were chosen during this study because the larval mortality was low while their physiological effects are expected to be considerably potent. The artificial larval diet including the CELE of both plants at their LC_{50} was introduced to the 24h-starved larvae. Control larvae were kept under the same conditions however fed with an extract-free diet. The L_2 that died naturally or from treatments during experiments were not included in the analysis.

Biochemical Analyses:

About 100 larvae weighing a total of 320-340mg were crushed in cold saline solution using glass potter-Elvehjem homogenizer. The homogenate was centrifuged at 5000xg for 40 min at 4^{0}C. The supernatant thus obtained was used for the determination of LDH (Cabaud and Wroblewski, 1958). ICDH (Bell and Baron, 1960), amylase (Juliano and Verner, 1969) activities. Trehalase and invertase activities were determined according to Ishayaa and Acher(1977), while transaminases (ALT, AST) as given by Reitman and Frankel (1957). All enzymatic activities were assessed using PYE UNICAM ultraviolet SP-1800 spectrophotometer. Total protein content was determined by the method of Lowry *et al*; (1951). Glycogen was estimated via anthrone method of Consolazio and Iacono (1963). While total lipids content was evaluated as described by Henry and Henry (1974). Glucose and FAA contents were determined following the procedures of Hartel *et al.* (1969) and Moore and Stein (1954) respectively.

RESULTS

Effect of CELE On Larval Enzymes:

The diet containing LC_{50} of *V. rosea* extract (80 ppm) when being ingested by L2 of *C. capitata* induced significant increase in the activities of trehalase, amylase, and invertase which amounted to +157%, +65% and +150% respectively ($P<0.001$) with regard to control (Table 1, Fig. 1). These

enzymes displayed relatively high significant activities reaching +60%, +145% and +66%, respectively, in case of inclusion of 50 ppm of *M. azedarach* CELE (Fig.1). Activities of ALT and ICDH were significantly elevated in both treatments (P<0.001), while AST and LDH activities exhibited higher percentage (P<0.01, P<0.001 respectively) by using LC_{50} of *M. azedarach*. (Table 1, Fig. 1).

Table (1): Effect of LC_{50} of crude ethanolic leaf extracts (CELE) on certain enzymatic activities of the second instar larvae of the fruit fly *C. capitata* All values are expressed as Mean±SE.

Treatment	Carbohydrate Hydrolyses (μ g glucose/ min. / mg LBWt)			Dehydrogenases (IU/mg)		Aminotransferases (IU/mg)	
	Trehalase	Amylase	Invertase	LDH	ICDH	ALT	AST
Control	12 6±1.9	19.8±3.0	13.8±2.1	23.6±2.05	45 6±0.11	167.4±4.4	32 7±1 05
V. rosea (80 ppm)	32.4±1.1**	32.7±2.1**	35±3.3**	42.3±4.2*	89.7±1.8**	258±0.38**	39.6±19 1*
M. azedarach (50ppm)	20.15±3.1*	48.5±2.7**	23±2.4*	56.6±7.9*	57.3±1.7*	350.7±0.24*	43.9±1.2*

* P<0.01 Significant.
** P<0.001 Highly significant.

Effect Of CELE On Other Biochemical Components:

A dose level of 80 ppm of *V. rosea* extract exerted significant reduction in the total protein , lipid , glycogen and glucose contents (-24% , -32% , -34% and -32.5% respectively) (Table 2, Fig. 2).
However, this reduction was mostly of highly significant percentages (P<0.001) in case of application of LC_{50} of ***M. azedarach*** CELE. In the same time, the level of larval FAAs have increased 20% and 37% in both treatments compared to the control (Fig. 2).

Table (2): Effect of LC_{50} of crude ethanolic leaf extracts (CELE) on some biochemical parameters of the second instar larvae of the fruit fly *C. capitata*. All values are expressed as Mean±SE

Treatment	Parameters (μ g / mg. Larval B. Wt.)				
	Total Protein	Total Lipids	Glycogen	Glucose	FAAs
Control	363.17±5.6	253.6±11.3	103±11.3	0.16±0.002	0.12±0.003
V. rosea (80 ppm)	211.3±6.41*	172.46±9.2*	67.6±8.12*	0.108±0.003*	0.144±0.005*
M. azedarach (50ppm)	131 4±9 11**	138.98±4.2**	48.12±2 3**	0.084±0.002**	0.165±0.003*

* P<0.01 Significant.
** P<0.001 Highly significant.

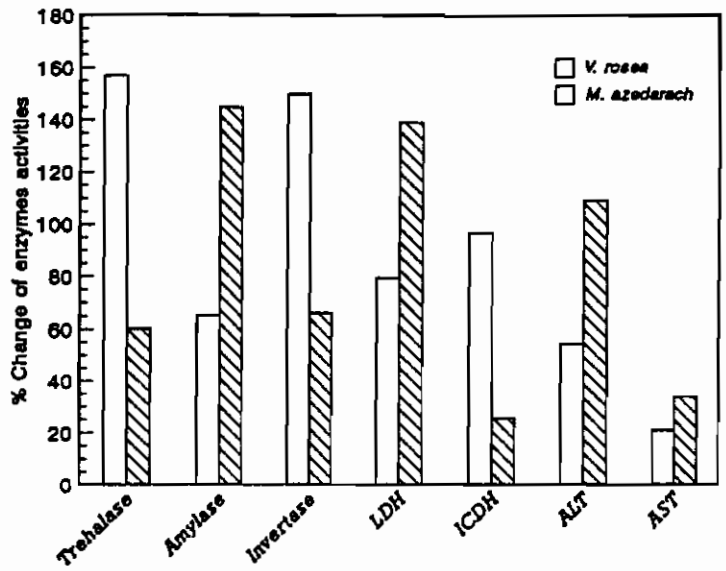

Fig. (1): Percentage change of some enzymatic activities of the second instar larvae of *C. capitata* treated with LC_{50} of crude ethanolic leaf extracts of V. *rosea* and *M.azedarach*.

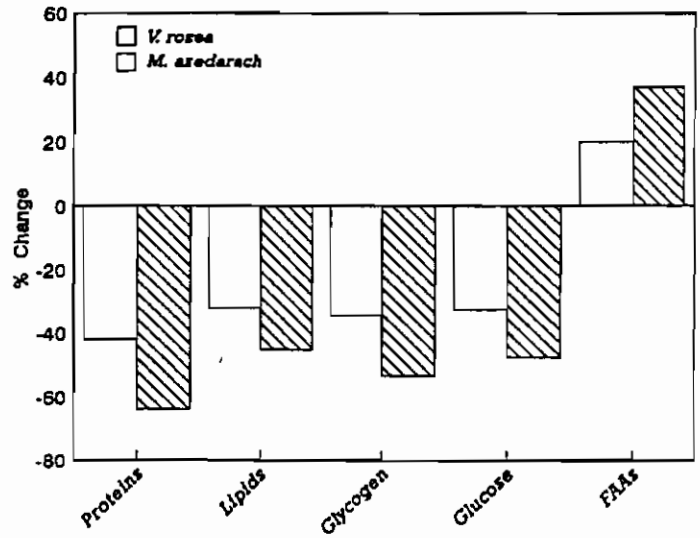

Fig. (2): Percentage change of some biochemical parameters of the second instar larvae of *C. capitata* treated with LC_{50} of crude ethanolic leaf extracts of V. *rosea* and *M. azedarach*

DISCUSSION

The biological effects of crude methanolic extracts from neem seeds on *C. capitata* larvae fed with an artificial medium , were investigated at concentrations of 15-30 ppm (Steffens and Schmutterer, 1982). Deleterious effects of this extract were also recorded in *C. capitata* even at lower concentration (<_2-3 ppm) (Boller *et al*, 1981). To reveal the possible mechanisms through which the active ingredients in CELE of *V. rosea* and *M. azedarach* can act, this work has been conducted.

Elevated activities of carbohydrate hydrolyzing enzymes resulted from treatments with both CELEs are comparable to the exerted reduction in glycogen content and hence suggest utilization of more carbohydrate by L2 of *C. capitata* for energy demands. Results of the present study are in line with that reported by Ishayaa and Swiriski (1970) and Ramadan *et al.*, (1985), who found similar variable levels of increased activities of invertase, amylase and trehalase in the Florida red scale and *Spodoptera littoralis* larvae reared on different diets fortified with neem extracts. However, Abdul Kareem (1981), recorded an inhibition of amylase and protease activity in *S. litura* at 0.5% of neem seed kernle extract, but strange enough, this activity was increased at higher dose (2.0%). The raised activity of LDH probably indicates either larval damage or higher production and accumulation of lactic acid in tissues. Furthermore, reduced levels of glycogen and glucose after treatment with CELEs, presumably suggest the energy supply mechanism via glycolsis was switched on, and increased tremendously to cope with the stressed conditions. However, this mechanism seem to be shifted to higher lactate production, which could be lethal for survival of larvae when certain threshold is reached (Mushtaq and Shakoori , 1985). The mild increase in the activity of aminotransferases (ALT, AST) after ingestion of both extracts was accompanied by elevation of FAA content in larval tissues. This presumably reveal that there was an interconversion of AAs through transamination against both CELEs to route proteins to the main catabolic pathway, as suggested by Mushtaq and Shakoori, 1987). Elevated activities of LDH, ICDH. ALT and AST are either due to (1) their higher concentrations resulting from decreased larval body weig ht,(2) increased synthesis of these particular enzymes to defend against stress conditions or (3) increase the source of energy production via breakdown of energy-rich nucleotides and amino acids (Mushtaq and Shakoori, 1987). Drastic metabolic disorders including disturbances in protein and lipid contents, are expected physiological consequences of the stimulated enzymatic activity. In the present study, the pronounced biochemical alterations induced by CELE of *V. rosea* could be mainly attributed to the interference of some biologically active indol alkaloids (Rizk and El-Nowaihi, 1989), with the larval physiology. Quite similar disturbances in glycogen, total protein and lipids contents were recorded in L2 of *S. littoralis* treated with acetonic *V. rosea* extracts (Taha *et al.*, 1989).

In addition, *M. azedarach* leaves are reported to contain more than 20 biologically active principles belonging to tetranortriterpenoids (Kraus *et al.*, 1986; Rizk and El-Nowaihi, 1989), the most potent of which is azadirachtin. This compound was intensively

investigated chemically and biologically (Bidmon, 1986; Broughton *et al.* ,1986; Rembold *et al.* , 1986; Miller and Chamberlian. 1989). It was believed to be the cause of many disruptive effects in various insects. Moreover, azadirachtin might interfere with some transmitters involved in the regulation, biosynthesis and/or release of ecdysone hormones (Siddiqui *et al.*, 1992 and Vinuela *et al.*, 2000). This could be a result of (a)-diminishing the rate of formation of molting hormone, (b)-lowering rate of its metabolism or (c)-decreasing the rate of hydroxylation of ecdysones (Rembold *et al.*, 1986). This in turn would permanently induce consequent disturbances in the expressivity of some genes which are responsible for biosynthesis of the considered enzymes, and hence other variations in the non enzymatic investigated parameters. The previously mentioned biochemical events, as we thought, are the first step in the mode of action of these extracts on the most sensitive stage (L_2) of *C. capitata*.

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and came in the second class. The highest activity of predation was recorded during winter in both years of study. The average percentages of mortality caused by predators all over the year represented by 9.45 and 12.91 % during the first and second year. In respect to unknown mortality factors, it contributed with the low percentages of the average mortality during both years of study (9.40 and 5.38 %).

DISCUSSION

The San Jose scale, *Q. perniciosus* was found on plum trees at Mansoura district, Dakhlia Governorate as first record in Egypt. It recorded 3-4 periods of seasonal abundance during both years of study. Similar results were recorded by Navrozidis *et al.* (1996). The highest abundance was recorded during July and April in the first and second years of study. Also, studies carried out on the SJS biology in Central Macedonia (Paloukis, 1984; Katsoyanndas and Argyriou, 1985), have shown that during mid June to July there are high populations of mature scales.

In the present study, *A. diaspidis* was the most common parasitoid on *Q. perniciosus* and represented the majority of the total number of emerging parasitoids at Mansoura district. Similar results were recorded in Georgian (USSR) by Popva (1976), in California (Gulmahamed and DeBach, 1978) and in Chile Gonzalez (1982). It recorded 3-4 peaks of seasonal abundance during both years. These results approximately are come in the same line of Jahn and Polesny (2002).

The parasitoids, *A. diaspidis* and *E. citrina* were exhibited low efficiency on *Q. Perniciosus* populations. In addition the synchronization between the host and both parasitoids was obviously weak during the period of study. According to Abdel- Kareim and Kozar (1987), *Epidiaspis leprii* Sign. which introduced to Hungary and there was no effective parasitoid or predator. The synchronization of the parasitoid with the host population is pad. The native scale insect species have effective specialized parasites with good synchronization, therefore, the role of insect parasitoids was very low and the synchronization between the parasitoid and host population was not good. Also, the armored scale *Fiorina externa* Ferris and *Tsugaspidiotus tsugae* (Marlatt) (Hemiptera: Diaspididae) of hemlock are a good example. Clearly, *E. citrina* plays a major role in the regulation of native population of both scales in Japan. Even though *E. citrina* is already well established in the northeastern United States, *F. externa* populations increase to such high densities that frequently injure and kill their new hosts, yet is ineffective in regulation exotic populations of these same scales (McClure, 1978, 1986 and 1988). The author hypothesize that these differences are due to a lack of synchrony in the life cycles of parasitoid and host in the United States.

Generally, it can be concluded that, there was no specific and effective parasitoid for *Q. perniciosus* which introduced to Egypt for the first time. So, many studies must be carried out in the future on the relationship between the insect and parasitoid populations, mass rearing and release techniques to improvement efficiency of above parasitoids under field conditions.

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دور الطفيليات في تنظيم تعدادات حشرة سان جوزيه القشرية (رتبة نصفية الأجنحة: دياسبيديدى) *Quadraspidiotus perniciosus* (Comstock) كافة دخيلة جديدة إلى مصر.

عبد الستار إبراهيم عبدالكريم و عادل حسن عبد السلام و نجدى فاروق عبد الباقى و محمد حسن محمد بيومى

قسم الحشرات الاقتصادية- كلية الزراعة-جامعة المنصورة- المنصورة- مصر

أجريت الدراسة على أشجار البرقوق الموجودة في المزرعة البحثية الخاصة بكلية الزراعة- جامعة المنصورة خلال موسمي الدراسة ٢٠٠١/٢٠٠٢ و ٢٠٠٢/٢٠٠٣. تم تسجيل حشرة سان جوزيه القشرية *Quadraspidiotus perniciosus* (Comstock) لأول مرة في مصر. كما وجد اثنين من طفيليات عائلة افليتيدى مرتبطة بتعداد هذه الحشرة هي *Aphytis diaspidis* DeBach و *Encarsia citrina* (Craw) خلال موسمي الدراسة ولوحظ ان طفيل الـ *A. diaspidis* كان أكثر الطفيليات تواجدا على تعداد الحشرة وكان تعداده يمثل العالبية العظمى من مجموع الطفيليات الخارجة خلال موسمي الدراسة. ويتضح من النتائج المتحصل عليها ان الطفيل الداخلى *E. citrina* سجل تاعداد قليلة جدا خلال موسمين الدراسة بينما سجلت الاطوار غير الكاملة لطفيل *A. diaspidis* ثلاث ذروات خلال الاسبوع الاول من مايو والثالث من اكتوبر والثانى من ديسمبر لعام 2001. بينما سجلت اربع ذروات فى السنة الثانية خلال الاسبوع الرابع من مارس والاول من سبتمبر لعام ٢٠٠٢ والاسبوع الاول من يناير والاول من مارس ٢٠٠٣.

ويتضح من النتائج المتحصل عليها ان متوسط نسب الموت التى تحدث بواسطة الطفيليات خلال السنه كلها كانت ١٧,٠٥ و ١٨,١٩ % بينما التى تحدث بواسطة المفترسات كانت ٩,٤٥ و ١٢,٩١ % بينما تلك التى تحدث بواسطة عوامل الموت الغير معروفة كانت ٩,٤٠ و ٥,٣٨ % خلال السنة الاولى والثانية على التوالى.