

SCREENING PHYSIOLOGICAL EFFECT OF *ARTEMISIA HERBA ALBA* AND *ALLIUM SATIVUM* ON *SPODOPTERA LITTORALIS* (BOISD) (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

Artemisia herba alba and *Allium sativum* grow in Sinai peninsula and, Northern area of Egyptian Delta respectively. Both extracts plants were evaluated as anti-insect products in an effort to develop alternative insecticides considering the environment. The effect of *Artemisia* and *Allium* extracts on some Biological aspects as well as some digestive enzymes changes after treatment with those extracts have been studied. The results indicated that treatment the 3rd instar larvae of *Spodoptera littoralis* for 24hours revealed satisfactory effect in larval mortality (50%) and significant prolongation for both larval and pupal durations. This prolongation was accompanied with a decrease in the percentage of pupation and adult emergence. Digestive enzymatic activities were pronounced on estimation of Trehalase, Invertase and Amylase manifested obvious changes in the treated larvae of *Spodoptera littoralis*.

Key words: *Artemisia herba alba*, *Allium sativum*, *Spodoptera littoralis*, Trehalase, Invertase and Amylase.

INTRODUCTION

Considerable progress has been achieved over the last two decades by producing Eco friendly bioinsecticides, However, Bioactive compound synthesis of plant extract leaves (R.Ramanibie *et al.*, 2015). Several plant extracts or isolated active compound have been studied as a potent acute or effective insecticides (Abdel Aziz, 2007). Many plants are capable of synthesizing secondary metabolites with biological properties that are important in the control against insect pests (Mathews, 1993, Enriz *et al.*, 2000, Calderon *et al.*, 2001, Cepedes *et al.*, 2001, Ganzalezcoloma *et al.*, 2002).

Artemisia herba-alba (white wormwood) family Asteraceae is a perennial shrub that grows commonly on the steppes of Northern Africa, Arabian Peninsula, Western Asia and Southwestern Europe. It is widely used in folk medicine for its antiseptic, vermifuge and antispasmodic properties. *Artemisia herba-alba* is a chamaephyte that grows to 20–40 cm (8–16 in). Leaves are strongly aromatic and covered with fine glandular hairs that reflect sunlight giving a grayish aspect to the shrub. The leaves of sterile shoots are grey, petiolate, ovate to orbicular in outline, whereas,

the leaves of flowering stems, more abundant in winter.

Allium sativum, family Alliaceae. Its close relatives include the onion, shallot, leek and, Garlic has been used throughout history for both culinary and medicinal purposes. The garlic plant's bulb is the most commonly used part of the plant which is originated in the Yunnan province of China. In our search for naturally occurring insecticide products the plants have become naturalized including Argentina, Egypt, France, Italy and United States. (Klayman, 1989, 1993), these compounds are phototoxic (Duke *et al.*, 2000) in laboratory assay. For these to be practical and economical the selected species must meet some requirement such as be in cultivatable and having a potent active principle, high stability, and good yield. The plant extracts, and biocides were considered as one of most economically and ecologically suitable element in pest management (Tripathi *et al.*, 2001).

The present study aims to clarify the effects of two extracts of both *Artemisia herba alba* and *Allium sativum* on some biological aspects and digestive enzymes (trehalase, invertase and amylase on *Spodoptera littoralis*).

MATERIALS AND METHODS

The leaves have been dried, each species separately normally in the shade next, they have been ground,

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using electric grinder to be ready for extracting (Table1).

The extracting method:

The organic solvent (ethyl alcohol) has been used in the extracting processes with a rate 3/1 (w/v) by mixing the solvent with 50 gm from the plant powders (*Artemisia herba alba*, and *Allium sativum*) each separately, then the mixture to be left for 24 hours in closed flasks in a place of a shade, away of the light then, the sediments have been separated through its passing in cotton. After that, the extract has been concentrated by the circular vaporizer apparatus then, the extract have been collected each one separately in glass containers and keep in fridge at 10-5 °C as a primary material.

Biological aspects:

Fifty newly moulted 3rd instar larvae of *Spodoptera littoralis* from the stock culture reared in the laboratory under a constant temperature (27±5°C) and 70 ±5 % RH as described by (El Defrawi *et al.*, 1964). Different aqueous concentration (at least five concentrations) for each of *Artemisia* 3%, 1% and *Allium* 5%, 3% were prepared and tested against the newly 3rd instar larvae to determine their biological aspects such as larval, pupal duration, percentages of pupation and adult emergence, using dipping technique, fresh castor oil plant leaves were dipped for about 10 seconds in each concentration for the tested extract. The leaves were left for air dryness and offered to larvae for 24 hours. Three replicates of 50 larvae of each concentration, control consisted of the same number of larvae offered leaves dipped in faucet water and allowed to dry after 24 hours of feeding on treated leaves, the larvae were provided with fresh, clean and untreated leaves of castor –oil plant until pupation. All the tested larvae were starved for a period of 4-6 hours before exposure to treated leaves to ensure rapid effects of extracts.

Estimation of Enzymatic activities:

Haemolymph samples were collected from the late of 4th instar larvae of *Spodoptera littoralis* of both treated and untreated larvae, the haemolymph was collected in cold tube (on ice) coated with crystals of phenylthiourea to prevent melanization, the samples was centrifuged at 2500 r.p.m about 7 minutes at low temperature -4 c to remove the blood cell. After centrifugation the haemolymph was divided into small portions (0.5 ml per each) and stored at - 20 °C until chemical analysis Haemolymph of untreated 4th instar larvae were also prepared by the same method.

RESULTS

Effect of *Artemisia herba alba* and *Allium sativum* on some Biological Aspects of 3rd instar of *S. littoralis*.

Short- term biological activity:

Data given in Table (2) clearly show that there is a remarkable effect on development of larvae fed on concentrations of *Artemisia*, and *Allium*, which prolonged the larval and pupal duration but in different values due to the type of each plant extract, the tested plant extracts could be arranged descendingly according to both larval and pupal duration as *Artemisia* 3% > *Artemisia* 1% > *Allium* 5% > *Allium* 3% .

long – term biological activity:

In this part of investigation pointed to evaluate the effects of the early 3rd instar larvae of *S. littoralis* which treated with the tested plant extracts on the resultant successive stages including the percentages of both pupation and adult emergence. The latent effects on both pupation and adult emergence percent which resulted from 3rd instar larvae treated with *Artemisia* 3%, *Artemisia* 1%, *allium* 5%, *allium* 3%. The failure of pupation and adult emergence which represented one of the principal indicator of these tested plant extracts latent effects against *S. littoralis* also recorded. The effects of previous extracts extended from the the treated larvae to resultant pupae and adult which presented the delayed effects of such compounds on the successive stages formed through developmental processes.

Effect of the tested plant extracts on the activities of certain specific enzymes (carbohydrases) in the haemolymph of 4th instar larvae of *S. littoralis*:

Carbohydrases enzymes which are commonly found in salivary secretions and regions of the digestive tract of most insect have been investigated by House (1965).

It is well known that trehalase and invertase enzymes were very important in food digestion and energy supply while Amylase was active in the digestive system. These groups of specific enzymes were very important in insect growth and development so that it was of interest to evaluate their activity level in late of 4th instar larvae of *S. littoralis* after they were fed on castor oil leaves treated with *Artemisia* 1%, 3% and *Allium* 3%, *Allium* 5%.

Trehalase: The data presented Table (3) and graphically illustrated in Fig (2) showed that *Artemisia* 3% and *Artemisia* 1% caused a remarkable reduction in this enzyme activity reached (17.8, 8.24) followed by *Allium* 5% where the reduction percent was (3.2). On the contrary, the minimum and slightly reduction of trehalase activity caused by *allium* 3% was only (1.8) as compared to check. Trehalase has the important function for liberating glucose for energy.

Invertase: The data in the same Table and Figure indicated that *Artemisia* 3% and *Artemisia* 1% caused

maximum reduction in this enzyme activity reached (16.6, 7.7) while, the minimum reduction due to *Allium* 5%, 3% were (4.9, 2.3) as compared to control.

Amylase enzyme activity reached to be (19.4% and 7.9%) as check while minimum reduction percent (1.9%) due to *Allium* 3% also *Allium* 5% resulted in a significant reduction in Amylase activity with 2.8% as check.

Amylase: The data in Table (3) and Fig (1) showed that Artemisia 3%, 1% resulted highest reduction in

Tables and figures

Table 1: The plant species used in the study with their basic components and active ingredients.

The part of plant used	Basic components and effective material	Family	Scientific name	Common name	References
Segments'	Volatile oil sulfuric compound + Vitamin+ Horomons+ antirollen	Liliaceace	<i>Allium sativum L</i>	Garlic	(Mohiudden <i>et al.</i> , 1987)
Leaves	1,8 Cineol, alpha, beta thujane + oxygenated monoterpenes	Asteraceae	<i>Artemisia herba alba</i>	Shieh or armoise blanche	(Yashphe <i>et al.</i> , 1987)

Table 2: Effect of *Artemisia herba alba* and *Allium sativum* on larval, pupal durations, pupation and adult emergence percentages of the 3rd instar larvae of *Spodoptera littoralis*.

Compounds	Larval duration	Pupal duration	Pupation %	Adult Emergence(%)
Artemisia 3%	13.6 ± 1.15	12.9 ± 2.89	59.3*	49.3*
Artemisia 1%	13.1±0.81	11.9±1.42	68.5 *	61.3*
<i>Allium</i> 5%	12.2 ± 1.1	11.7±.32	72.8 *	75.6ns
<i>Allium</i> 3%	11.4 ± 2.23	10.8 ±2.5	88.9	80.43*
Check	10.7 ± 0.16	10.0 ±0.1	99.2	98.9

* → significant at P < 0.05

ns → non-significant of P < 0.05

Table 3: Effect of *Artemisia* and *Allium* on the haemolymph carbohydrase activity of the 3rd instar larvae of *Spodoptera littoralis*.

Compound	Mean Carbohydrases activity # (Iu/mi) ± S.E					
	Trehalase	Deviation from check %	Invertase	Deviation from check%	Amylase	Deviation from check %
<i>Artemisia</i> 3%	18.12 ± 1.6**	-17.8	26.12± 0.4 ***	-16.6	46.8 ± 0.4**	- 19.4
<i>Artemisia</i> 1%	19.81± 2.2 **	-8.24	28.8 ± 1.2*	- 7.7	42.5 ± 1.2 *	-7.9
<i>Allium</i> 5%	20.1 ± 0.30	-3.2	30.01 ± 0.68**	- 4.9	51.3 ±0.7 *	- 2.8
<i>Allium</i> 3%	22.4±0.2	-1.8	33.02 ± 0.56*	-2.3	53.2± 0.81	-1.9
Control	22.52±1.10	-	33.62 ±0.70	-	53.99±1.32	-

*: → significant at p < 0.05

**,: → highly significant at p < 0.01

***,: → very highly significant at p < 0.001

Iu: → International unit (the amount of enzyme which under defined assay conditions will catalyze the conversion of one micromole of substrate per minute).

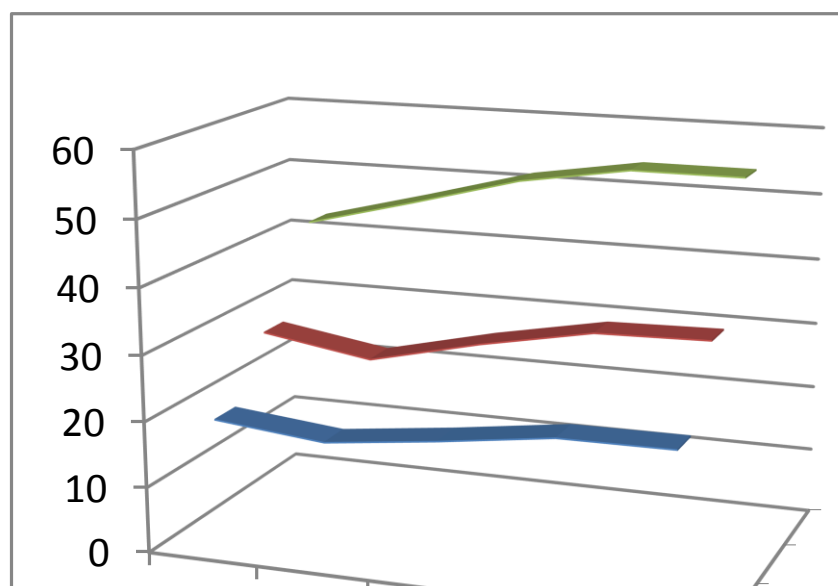


Fig. (1) Effect of *Artemisia herba alba* and *Allium sativum* on haemolymph Carbohydrase activity of the 3rd instar larvae of *Spodoptera littoralis* .

DISCUSSION

Bioassays of third- instar larvae of *S. littoralis* with *Artemisia* 3% , *Artemisia* 1% , *Allium* 3% and 5% *Allium* against the 3rd early instar larvae of *S. littoralis* were estimated as larval, Pupal durations and corrected in the control according to Abbot (1925). As shown in Table (2) the duration of the tested larvae was highly significant increased to reach (13.6 and 13.1 days) after treatment with *Artemisia* 3% and 1% and significantly increased to be (12.2 and 11.4 days) for *Allium sativum* 5% and 3% respectively. Concerning the pupal duration, the data in the same Table show the same trend, were the pupal period seemed to be significantly longer than check (10 ± 0.1), as they were 12.9, 11.9, 11.7, and 10.8 days for the tested plant extracts as the same arrangement, respectively.

The efficiency of *Artemisia* 3%, 1% and *Allium* 5% and 3% caused a significant prolongation in larval and pupal durations of 3rd instar larvae of *Spodoptera littoralis* (Abdel Galil *et al.*, 2005). There was an obvious effect on the inhibition percentage of pupation and adult of the tested larvae (Manal *et al.*, 2010). The results showed that not only the acute action was the indicator for plant extracts, but also the latent effectiveness must be taken as a parameter where the effect of tested extracts was extended to the pupae and adult ensuing from treated larvae (Kalarzyna, 2008). It could be concluded that the tested extracts resulted generally as reduction in carbohydrases enzymes with different ratios depending on the type of enzymes and extract used.

It worthy to say that carbohydrase enzymes are very important digestive enzymes which have received a great deal of attention, to be studied in order to know

insects can adapt themselves to a particular type of food (Meisner *et al.*, 1978). The rapid decrease of glucose concentration at the end of 4th larvae of *S. littoralis* was probably caused high metabolic of the epidermis (Florkin, 1964 and Gonzalez. Coloma *et al.*, 2002). So it is unable to utilize trehalose. However that carbohydrases (trehalase, invertase and amylase) attracted the researchers to know the digestive process in most insects (Ishaya and Swirski 1976).

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فحص فسيولوجي لكل من الثوم ونبات الشيح علي دودة ورق القطن

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اختبرت بعض المستخلصات النباتية مثل مستخلص نبات الشيح *Artemisia herba alba* الذي ينمو في شبة جزيرة سيناء ونبات الثوم *Allium sativum* وهو الثوم الذي ينمو في شمال الدلتا كمواد مكافحة للحشرات وأمنة علي البيئة وبعد المعاملة بهذه المستخلصات اتضح انه كان لهما التأثير الفعال علي العمليات الحيوية مما أدى إلي أحداث تغيرات لإنزيمات الهضم لتلك الآفه ، وأشارت النتائج إلي أن معاملة يرقات العمر الثالث لدودة ورق القطن لمدة ٤ ساعة لها تأثير واضح أدت إلي ٥٠% من نسبة الموت ليرقات العمر الثالث لدودة ورق القطن بالإضافة إلي أطالة فترات حياة كلا من اليرقات والعذارى وحدوث هذه الإطالة لكلا من انخفاض شديد في خروج العذراء والطور الكامل مما كان له تغيرات واضحة في إنزيمات الهضم الثلاثة Trehalase, Invertase and amylase.