EVALUATION OF INSECTICIDAL ACTIVITY OF HENNA *LAWSONIA INERMIS* LINN. EXTRACTS AGAINST THE COTTON LEAFWORM

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Abstract

Laboratory studies were carried out to evaluate the efficacy of *Lawsonia inermis* Linn. (Family: Lythraceae) against the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), Results of the five concentrations (1000,2000,3000,4000 and 5000ppm) of water extract against (1st and 2nd instars) and (3rd and over instars 4th, 5th and 6th) of *S. littoralis* after 24 and 48 hrs showed that, the *S. littoralis* mortality significantly increased when water extract concentration increased. Also, results indicated that treatment with water extract affects the biochemical activities of the cotton leafworm *S. littoralis*. There are an alterations in all tested enzymes (Phenoloxidase, chitinase, protease, alkaline and acid phosphatases) and disturbances in protein levels under investigations when the cotton leafworm *S. littoralis* treated with Henna extract at all concentrations.

Keywords: *Lawsonia inermis*, *Spodoptera littoralis*, Toxicity and biochemical activities.

INRODUCTION

The Egyptian cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) is one of the most destructive phytophagous insect pests in Egypt, not only to cotton but also to other field crops and vegetables (Kandil *et al*., 2003). This pest causes serious and considerable economic losses to many crops in both greenhouses and open fields. The noctuid *S. littoralis* is widely distributed all over the world; in Egypt, throughout Africa, Southern Europe, and several parts of Asia In addition to the cotton, the *S. littoralis* larvae infest more than 90 important plant species belonging to 40 families causing great losses in quantity and quality of the attacked crops (Kandil *et al*., 2003).

The prolonged application of chemical insecticides for controlling insect pests cause many problems such as pest resistance, environmental pollution, health hazards to farmers, food contamination and toxicity to non-target organism (parasitoids and predators). Most of these toxic chemicals enter into the food chain and cause pollution

of the environment. So it is demanded to develop selective and environmentally safe methods that will result in better control of insect pest control. Recently, great attempts have been done at screening plants in order to develop new botanical insecticides as an alternative to the existing insecticides**.** There is renewed interest in the application of botanical pesticides for crop protection. Botanical pesticides are biodegradable and their use in crop protection is a practical sustainable alternative and reduces environmental contamination and human health hazards (Tripathi *et al*., 2009). As a continuation of this type of searching, we have selected *Lawsonia inermis* L. which is a well known medicinal plant in many parts of the world and commonly known as *Henna*. There is no many studies on the insecticidal activity of *L. inermis* leaves, so, the objective of this study is to investigate the insecticidal activity of water extract of *L. inermis* leaves against (1st and 2nd instars) and (3rd and over instars4th , 5th and 6th) of cotton leafworm *S. littoralis* at different concentrations (1000,2000,3000,4000 and 5000ppm) and its effects on the following biochemical parameters: one defensive enzyme (Phenoloxidase), three hydrolytic enzymes (Chitinase, Acid and Alkaline phosphatase), one digestive enzyme (protease) and total proteins content at the same concentrations.

MATERIALS AND METHODS

1. Plant collection and preparation

Leaves of *L. inermis* were collected from El-Luxer, Governorate, Matthna Station, Plant Protection Researches Institute. The collected leaves of *L. inermis* were cleaned and dried. The dried plant materials were then pulverized into a coarse powder. The powdered leaves were successively extracted with water at room temperature. These extracts were evaporated under reduced pressure at 40°C using a rotary evaporator.

2. Bioassay of henna extract against *S. littoralis*

Dipping technique

Five concentrations of henna *L. inermis* extract were prepared in water ranged from 1000 to 5000ppm as required for the bioassay tests against (1st and 2nd larval instars) and (3rd and over larval instars) of cotton leafworm *S. littoralis.* The castor bean leaves were dipped in each concentration of the plant extracts for 20 second and left to dry then five replicates of 10 larvae of each concentration were fed on the treated leaves for 24 and 48 hrs. The surviving larvae were transferred to clean cups and supplied daily with untreated leaves until pupation. For control, plant leaves were dipped in fresh water. Mortality was recorded daily (24 and 48 hrs) after treatment and the LC_{50}s were determined.

Spraying technique):

The leaf spraying technique was used as toxicity test for henna *L. inermis* extracts against (1st and 2nd instars) and (3rd and over instars) of cotton leafworm insect *S. littoralis*. Uninfested plant leaves were selected from castor and placed in a petri dishes (9cm diameter, 1cm high by 3.5 cm diameter). Infested castor leaves were transported to the laboratory and *S. littoralis* individuals were separated from it and put in a container. After that each dish, leaf and *S. littoralis* individuals were sprayed with the different concentrations (1000,2000,3000,4000 and 5000ppm) of water henna extract. Dishes were leaved to dry at ambient temperature and covered. Five replicates were conducted for each treatment. A control leaf was spraying by water. After application, Petri dishes placed in laboratory conditions and examined for mortality and life after 24, 48 and 72 hrs.

3. Biochemical assays:

Determination of enzymatic activities.

Defensive enzyme assay (Phenoloxidase):

Phenoloxidase activity of *S. littoralis* 4th instar larvae was determined according to modifications of Ishaaya (I971), in a reaction mixture consisting of 0.5 ml phosphate buffer (0.1 M, PH 7), 200 ml enzyme solution and 200 ml catechol solution (2%). Prior to the initiation of the reaction, the substrate and other ingredients of the reaction mixture were separately incubated at the optimum temperature of the reaction (25°C). The enzyme reaction was initiated by adding catechol solution, then after exactly 1 min, the optical density was determined. Zero adjustment was against sample blank. The phenoloxidase activity was determined as O.D. units 10^3 at an absorbency of 405 nm.

Digestive enzyme assay (Protease):

The proteolytic activity of *S. littoralis* 4th instar larvae was determined according to Birk, *et al.*, (1962). The proteolytic activity was determined from bovine serum albumin standard curve; as *O.D.unitsx10^3/larvae body weight* and the absorbance at 280nm.

Hydrolytic enzymes activities.

Acid phosphatase (AC-P) and alkaline phosphatase (ALK-P) activities were determined according to the methods described by Powell and Smith (1954), in 100 mg tissue of whole homogenated 4th instar larvae.

Chitinase was assayed using a 3,5-dinitrosalicylic acid reagent to determine the free aldehyde groups of hexoaminase liberated on chitin digestion according to the method described by Ishaaya and Casida (1974).

Determination of energy reserve (Total proteins).

Protein content of *S. littoralis* 4th instar larvae samples was estimated spectrophotometrically by the method of Bradford (1976). Total proteins contents were calculated and expressed as mg/g of larvae body weight.

Statistics:

The results were analyzed by one way analysis of variance (ANOVA) using costat statistical software (cohort software,Berkeley). When the ANOVA statistics were significant ($P<0.05$), means were compared by the Duncan's multiple range test.

RESULTS AND DISCUSSION

Dipping technique

The results of toxicity assays by dipping technique of Henna *L. inermis* extract against (1st and 2nd instars) and (3rd and over instars) of cotton leafworm insect *S. littoralis* as represented in the table (1), showed that, water extract of henna achieved significant high mortality percentages against (1st and 2nd instars) and (3rd and over instars). The highest concentration 5000ppm was recorded 100% mortality against (1st and 2nd instars) and (3rd and over instars) after 24 and 48 hrs. The higher toxicity rate of water extract against (1stand 2nd instars) were 65.00 and 97.50 % at the concentration 4000ppm, after 24 and 48 hrs., respectively and were 60.00 and 77.50 %, with the concentration 3000ppm after the same two times respectively. 0n other hand, the toxicity rate against (3rd and over instars) after 24 hr. were 25.00 and 40.00 %, and after 48 hr. were 55.00 and 60.00 %, at the two concentrations (3000 and 4000ppm), respectively. Concentration 2000 ppm caused (45.00 and 15.00 %) and (45.00 and 15.00 %) mortalities against (1st and 2nd instars) and (3rd and over instars) after 24 and 48 hrs., respectively. The lowest mortality (32.50 and 37.5%) caused by concentration 1000 ppm against (1stand2nd instars) after 24 hrs and (5.00 and10.00%) against (3rdand over instars) after 48 hrs.

The least lethal concentration (LC_{50}) values obtained from probit analysis for mortality values after 24 and 48 hours of each *L. inermis* extract applied are given in the Table (1). According to the results, the Henna water extract has the lowest LC_{50} value 1933.67 and 1497.56 ppm after 24 and 48 hours against (1st and 2nd instars), respectively. While, LC_{50} values of Henna water extract against (3rd and over instars) were 3498.56 and 2390.08 ppm after 24 and 48 hours respectively. In general LC_{50} values of Henna water extract after 48hr were lower than 24hr against (1st and 2nd instars) and (3rd and over instars). Among all, Henna water extract after 48 hr. of exposure was found to be more effective against *S. littoralis* than after 24hr. Overall results indicated that, water extract of Henna *L. inermis* exhibited toxicity rate with

concentration dependent and the efficiency of those extracts against cotton leafworm *S. littoralis* increased when their concentrations and time exposure increased.

The insecticidal effect of *L. inermis* leaves against insects was reported in many researches and confirmed with our findings. The study of Kamal *et al*. (2016) indicated that, methanolic extract of leaf of *L inermis* was highly effective against red flour beetle, *Tribolium castaneum* and the mortality percentage was directly proportional to the level of concentration of plant extract. Also, The toxic effect of plant extracts against *S. littoralis* was confirmed by Mesbah *et al*. (2006) who reported that, most of the evaluated plant oils were found to have an insecticidal effect on the4th instar larvae of the cotton leaf-worm, *S. littoralis*. The development of the treated 4thlarval instar was blocked due to treatment with the tested plant oils. In addition, results of Abdel-Aziz *et al*. (2013) showed that all the tested oils (thyme, bitter and neem) caused deformations with various degrees for larvae, pupae and adults resulted from the treated 2nd and 4th instar larvae of *S. littoralis* .

The feeding on henna extract in the diet resulted in irreversible damage to physiological processes essential to the development of *S. littoralis*. Several anomalies, possibly related to defective moulting, were observed with the most concentrations, but particularly at 4000 ppm and 5000 ppm. Most of the tested extract concentrations significantly inhibited larval growth compared to the control. From our results we noticed that the development of the treated larval instars were blocked, the cuticle colour turned to a uniform dark grey, dorsally swollen and the larva to pupa moult stopped due to larvae instars fed on castor leaves treated with *L. inermis* extract. The results showed no clear evidence of any direct antifeedant effect at the concentrations tested which indicates that the *L. inermis* extract acted principally as a larval growth inhibitor and also an inhibitor of chitin synthesis (anti moulting agent) rather than antifeedants causing disruption of the insect development, abnormal larvae that were lead finally to death. However, additional detailed experiments are needed to determine the mechanism of the effects of our extract on insects, and shed light on these mechanisms.

Our results are in agreement with Martinez and Emden (2001) who showed that, a two-day feeding period promoted prolongation of the larval instars, reduction in the Mean Relative Growth Rate (MRGR), moulting disruption, morphological anomalies and mortality of *S. littoralis* in a dose-dependent manner. In an analogous study, the tested essential and/or volatile oils acted principally as Insect Growth Inhibitors (IGIs) rather than antifeedants causing disruption of the larvae of the cotton leaf-worm *S. littoralis* development, abnormal larvae, pupae and adults that were lead finally to death (Mesbah *et al* 2006).

The results obtained here are promising for the control of *S. littoralis*. A two-day feeding period was enough to promote prolongation of the larval instars, moulting disruption, morphological anomalies and mortality of the species. In conclusion, the present results indicate that *Henna* extract possessed toxic effects on *S. littoralis* and inhibited growth through various metabolic processes. Furthermore, the results suggest an interesting opportunity to develop bio-insecticides based on extracts from *Henna* extract for use in integrated pest management of insect pests that may affect crop production.

Spraying technique

Results in Table (2) indicated that after 24 hrs. of spraying water extract of *L. inermis* leaves, all different concentrations (1000 to 5000ppm) not caused any significant mortality percentages against cotton leafworm *S. littoralis*. The highest percent mortality (30.77%) was observed in water extract at concentration 5000 ppm compared to other treatments after 72hr. However, in general there is no significant increase in toxicity of water extract was observed with increasing of concentrations and time against *S. littoralis* and the percent mortality ranged between 2.77% to 13.62% after 24hr. from 6.67% to 23.33% after 48hr. and from 10.75% to 30.77% after 72hr.

Dipping technique caused higher toxicity rate against cotton leafworm insect *S. littoralis* than spray technique at all concentrations of the study. Generally, our results showed that the dipping technique was more efficient than the spraying technique in control of cotton leafworm insect *S. littoralis*.

Biochemical analysis

In the present study, effect of water extract of *L. inermis* on biochemistry of the cotton leaf-worm, *S. littoralis* was evaluated and the estimations were done for analyzing the activity of one defensive enzyme (Phenoloxidase), three hydrolytic enzymes (Chitinase, Acid and Alkaline phosphatase), one digestive enzyme (protease) and total proteins content at the five different concentrations.

Effects of water *L. inermis* extract on enzymatic activities of *S. littoralis*.

***Effect on phenoloxidase and chitinase enzyme activities.**

Results in Table (3) showed that, there is significantly increasing in the phenoloxidase enzyme activity (10.30, 9.90, 13.77, 14.30 and 20.23 *O.D. units/min/gm*) compared to control (9.08*µg D,L alanine/min/g.b.wt*) with the increasing of the five assessed concentrations, respectively. Also, there is increasing in the chitinase enzyme activity (1076.60, 814.30, 1396.60, 1523.33 and 2011.33 *µg*NAGA/min/ *g.b.wt*) compared to control (969.33*µg* NAGA/min/g.b.wt) with the same concentrations, respectively except concentration 2000 ppm. Our results are in

agreement with these finding by Abd El-Mageed and Shalaby (2011) who noticed that, the most effective insecticide Kingbo, caused a significant increase in phenoloxidase activity of *S. littoralis* (2858.33% higher than in the control) followed by engeo (1191.67%) and chlorosan (291.67%). Phenoloxidase activity elevation because it's an oxidative enzyme in insects and playing an important role in the development and immunity of insects (Ashida and Brey 1995).

The changes in the chitinase activity in *S. littoralis* larvae was observed by many authors. Abdel-Aziz *et al*. (2013) demonstrated that, the tested oils (thyme, bitter and neem) caused highly significant stimulation in chitinase activity against 4th and 6th instars of *S. littoralis* for 48 hrs and the most effective ones was thyme followed by bitter and neem compared with control. Also, Abd El-Mageed and Shalaby (2011) found that chitinase activity was increased more than control after treatment of cotton leafworm larvae *S. littoralis* by mixtures of bioinsecticids. Chitinase activity increasing interpreted by Yu and Terriere, (1977) who stated that, the increase in chitinase activity could be attributed to the secondary effect of chitin synthesis inhibitor, or may be a secondary effect for the reduced activity of β-ecdysone metabolizing enzymes, followed by β-ecdysone accumulation which result in hyperchitinase activity**.**

Effect on hydrolytic enzyme activity (Acid and Alkaline phosphatase)

Results in Table (4) showed that, alkaline phosphatase caused continuous gradual decrease in enzyme activity with increasing concentrations compared to control except at concentration 2000ppm. Also, acid phosphatase activity of *S. littoralis* significantly decreased at five different tested concentrations compared to control. In general acid and alkaline phosphatase enzyme activities were decreased with the increasing of the assessed concentrations. From these findings, acid and alkaline phosphatases activities are depending on the plant extract concentrations.

The inhibition of acid and alkaline phosphatases activities with increasing plant extract concentrations seem to be in agreement with the results reported by Mead *et al*. (2016) who stated that, both mahlab and KZ mineral oil decreased the activities of alkaline and acid phosphatases of *S. littoralis* as compared to the control, with the exception of mahlab oil on acid phosphatase. Also, our results were confirmed by Younes *et al.,* (2011) who reported that, the acid and alkaline phosphatases activities in treated larvae of khapra beetle *Trogoderma granarium* were less than in control one when using garlic, onion, sunflower, and rosemary oils in the treatments. Also, they stated that, the inhibition of acid phosphatase activity that could be due to the presence of anti-insect protein in the tested oils that its defense function is correlated

with acid phosphatase activity and cause loss of acid phosphatase activity and significantly delayed their development of insects and increases their mortality.

Effects on energy reserve proteins and its enzyme (protease).

Effect on total proteins:

Protein content of the treated larvae of *S. littoralis* show significant increase with increasing of the five tested concentrations as compared with the control (Table 5). Protein content significantly increased in all treatments, generally, changes in protein content probably reflect the balance between synthesis, storage, transport and degradation of structural and functional nutrients during ontogeny as well as response to particular physiological conditions. The increase in protein content with different treatments may be attributed to the increased activity of protein biosynthesis. Many workers obtained similar findings on other species of insects. Shakoori and Saleem (1991) attributing the greater protein synthesis with insecticidal treatment to synthesis of the proteinases needed for insecticide detoxification. This finding may be due to the conversion of carbohydrates and lipids to proteins as stated by Kinnear *et al*. (1968) who suggested that increased protein levels was due to increased synthesis of new proteins by the fat body, haemolymph and other tissues of the larvae.

Effect on digestive activity (Protease)

The results obtained in Table (5) indicated that protease activity increased significantly with increasing of concentrations as compared to control**.** These results are in agreement with those reported by Abdel-Aal *et al*., (2012) recorded significant increase in protease of *S. littoralis* larvae with treated with Diple 2x alone and its mixture with two insecticides. In the same context, Abdel-Aziz *et al*. (2013) revealed that, the protease activity fluctuated between increase and decrease. Bitter and thyme treatments exhibited remarkable inhibition in protease activity. While high significant stimulation was recorded neem treatment compare with control.

The secondary metabolites of plants are compounds with wide range of insecticidal activity. Phytochemical investigations on leaf and fruit of *L. inermis* showed the presence of many secondary metabolites such as tannins, cardiac glycosides, saponins, flavonoid, steroids, phenolic derivatives, coumarins, xanthones, naphthoquinone derivatives, alkaloid and terpenoids and these compounds of *L. inermis* had great impact on insecticidal activities Kamal *et al*. (2016). The results of Adedeji *et al*. (2017) showed that the extractives compounds from *L. inermis* leaves such as alkaloids, phenols, tannins and saponins had significant biocide actions against wood termites. On the other hand Karamanoli *et al*. (2011) reported that tannin combine with protein inhibit the enzyme activity and reduce the availability of protein in haemolymph insect. Naphthoquinones and Coumarins are wide-spread

phenolic compounds in nature, Juglone, lawsone, plumbagin (Naphthoquinone) and Esculetin, fraxetin (Coumarin) extracted from *L. inermis* leaves have significant antibacterial, antifungal, antiviral, insecticidal, anti-inflammatory, and antipyretic properties (Abulyazid, *et al.* 2013). The exact mechanism action of *L. inermis* is not yet known, the insecticidal activity of *L. inermis* may be attributed to not only a single active compound but to a variety of bioactive compounds. So, the pesticidal activity of *L. inermis* against *S. littoralis* may be due to the presence of these different bioactive compounds especially naphthoquinone derivatives. The overall results of this study reported the toxic effect of leaf of *L. inermis* on *S. littoralis* and our next approach will be targeted to isolate possible active compounds.

CONCLUSION

It was perceived from the present study, Henna leaves extracts showed alterations in all the enzymes and protein levels under investigations when larvae of *S. littoralis* treated with Henna extract at all concentrations. In conclusion these findings of the present study suggest that water extract can be used to control *S. littoralis* but further study needs to be conducted to know the compatibility of these extracts in insect control to help in integrated pest management strategy.

Table 1. Effect of five different concentrations of water Henna extract on larvae mortality of *Spodoptera littoralis* by dipping technique at 24 hours and 48hr.

Conc./ppm	Mortality ±SD (%)			
	1st and 2nd Instars		3rd and over Instars	
	24hr.	48hr.	24hr.	48hr.
Control	0.00	0.00	0.00	0.00
1000	32.50±17.08^{c}	37.50±5.00^{c}	5.00±5.00^{c}	10.00±5.77^{c}
2000	45.00±28.87^{bc}	55.00±25.09^{bc}	15.00±9.57^{bc}	50.00±10.00^{b}
3000	60.00±11.55^{bc}	77.50±5.70^{ab}	25.00±15.00^{bc}	55.00±9.57^{b}
4000	65.00±5.00^{b}	97.5±5.00^{abc}	40.00±8.16^{b}	60.00±8.16^{b}
5000	100.00±0.00	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
LC_{50}	1933.67	1497.56	3498.56	2390.08
F	10.39***	5.877**	17.235***	14.83***
LSD	24.15	22.533	27.24	25.15

Table 2. Toxicity effect of five different concentrations of water Henna extract on larvae mortality of *Spodoptera littoralis* by spraying technique at 24, 48and 72hrs.

Conc./ppm	**Mortality±SD (%)**		
	24hr.	**48hr.**	**72hr.**
Water control	0.00	0.00	0.00
1000	2.77±4.80^{a}	6.67±1.56^{b}	10.75 ±5.00^{a}
2000	6.67±1.52^{a}	9.05 ±7.94^{ab}	21.50±9.49^{a}
3000	11.43±3.50^{a}	10.67±2.89^{ab}	26.67±10.00^{a}
4000	10.67±8.66^{a}	16.11±6.63^{ab}	28.50±15.10^{a}
5000	13.62±5.00^{a}	23.33±5.77^{a}	30.77±9.44^{a}
LC_{50}	62382.75	46665.04	14014.75
F	**0.637^{ns}**	**2.33^{ns}**	**0.729^{ns}**
LSD	**16.09**	**14.75**	**30.44**

Table 3. Effects of five different concentrations of Henna extract on defensive enzyme phenoloxidase and chitinase activities of treated 4th instar *S. littoralis* larvae.

Conc./ ppm	**Enzyme activity ±SD**	
	Phenoloxidase (*O.D. units/min/gm*)	**Chitinase (*µg* NAGA/min/g.b.wt)**
Control	9.08±0.530^{d}	969.33±17.04^{e}
1000	10.30±0.264^{c}	1076.67±20.82^{d}
2000	9.90±0.435^{c}	814.33±22.28^{f}
3000	13.77±0.321^{b}	1396.67±25.17^{c}
4000	14.30±0.100^{b}	1523.33±31.21^{b}
5000	20.23± 0.680^{a}	2011.33±72.28^{a}
F value	**279.65*** **	**442.52*** **
LSD	**0.767**	**65.15**

Table 4. Effect of five different concentrations of Henna extract on two hydrolytic enzymes activities of treated 4th instar *Spodoptera littoralis* larvae.

Conc./ppm	**Enzyme activity ±SD**	
	Acid phosphatase (*µg anaphthol/min/gm*)	**Alkaline phosphatase (*U x 10^{3}/g.b.wt.*)**
Control	235.33±7.57^{a}	1088.67±34.42^{a}
1000	204.67±9.07^{b}	978.33±100.75^{b}
2000	228.67±13.31^{a}	792.00±23.06^{c}
3000	205.00±5.291^{b}	743.00±15.72^{c}
4000	194.67±5.507^{b}	725.00±18.73^{c}
5000	125.67±5.5071^{c}	635.00±5.51^{d}
F-Value	**73.68*** **	**42.25***
LSD	**14.03**	**81.19**

Table 5. Effect five different concentrations of Henna extract on total proteins content and digestive enzyme (protease) of 4th instar *Spodoptera littoralis* larvae.

Conc./ppm	Energy reserves ±SD	Enzyme activity ±SD
	Total Proteins (mg/g.b.wt.)	**Proteases (*µg D,L alanine/min/g.b.wt*)**
Control	**10.93±0.71**^{c}	1.99±0.085^{d}
1000	**12.70±0.70**^{b}	2.15±0.145^{cd}
2000	**11.60±0.76**^{c}	3.00±0.152^{a}
3000	**11.33±0.98**^{c}	2.51±0.090^{b}
4000	**12.47±2.98**^{b}	2.22± 0.105^{c}
5000	**14.20±2.85**^{a}	2.43±0.087^{b}
F-Value	**18.63*** **	**28.64*** **
LSD	**0.85**	**0.203**

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تقييم النشاط الابادى لمستخلص الحنة ضد دودة ورق القطن

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تمت الدراسات المعملية لتقييم كفاءة مستخلص نبات الحنة ضد دودة ورق القطن وإشارت نتائج التركيزات الخمسة (1000,2000,3000,4000و5000 ppm) من المستخلص المائى ضد العمر الاول و الثانى وايضا الاعمار التى تلى العمر الثالث من دودة ورق القطن بوجود زيادة معنوية فى نسبة الموت مع زيادة تركيز المستخلص المائى بعد 24 ، 48 ساعة . ايضا اشارت نتائج تلك المعاملة بتأثير المستخلص المائى على الأنشطة البيوكيميائية لدودة ورق القطن . وهناك تغيرات فى كل الأنزيمات المختبرة (فينولواكسيديز والكيتينيز،البروتينيز،الالكالين واسيد فوسفاتيزوايضا اضطراب فى مستويات البروتين تحت الاختبارات عند معاملة دودة ورق القطن بكل تركيزات مستخلص نبات الحنة.