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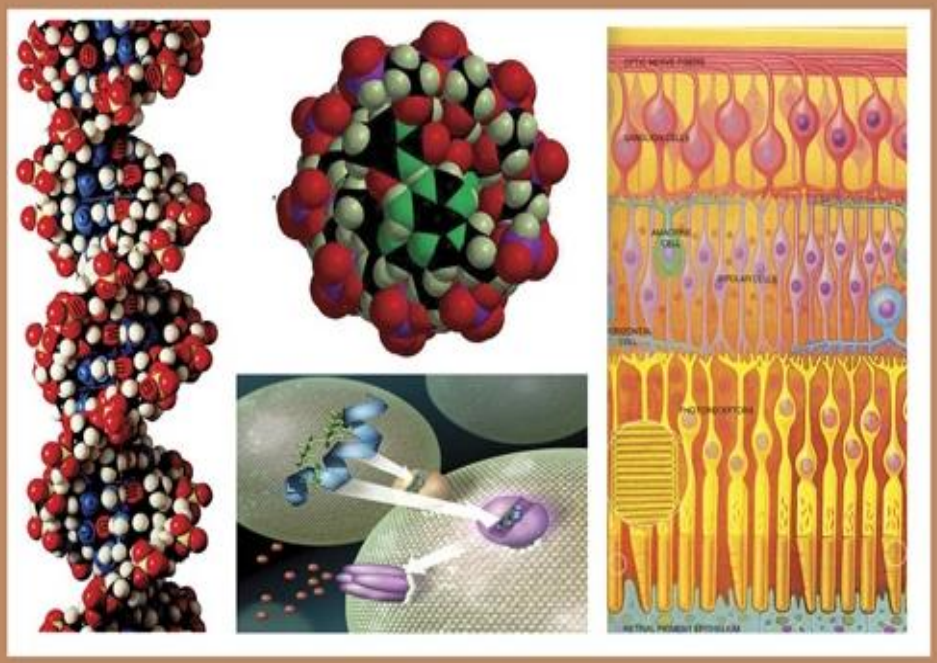

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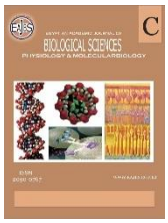

Evaluation of the Efficacy of *lawsonia inermis* (Lythraceae) Leaves Extracts on the Main Metabolites in Fat Bodies and Haemolymph of *Schistocerca gregari* (Forskal) (Orthoptera: Acrididae).

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ABSTRACT

The current work was conducted to check the biological effects of the henna tree, *Lawsonia inermis* ethanol extracts on the *Schistocerca gregaria* main body metabolites in fat bodies and hemolymph. LC_{50} of ethanol (8.5%) were applied on penultimate instar nymphs and the metabolite was determined for last instar nymphs and newly emerged adult females. Clearly decreased protein content was determined in fat bodies of late-aged nymphs, after treatment with ethanol extract, as well as of newly emerged adult females. With regard to protein content in hemolymph, strong inducing effects were exhibited on early, mid-aged nymphs and newly emerged adults while reducing effects were exhibited on early-aged nymphs. A reducing effect of *L. inermis* on the carbohydrate content was observed in fat bodies of late-aged nymphs and newly emerged adults whereas aninducing effect on the nymphs of early ages was displayed. Carbohydrate content in heamolymph has the same trend. A prevalent inhibitory action of *L. inermis* extracts on the lipid content in fat bodies of nymphs and adults was recorded with few exceptions to mid-aged nymphs. In hemolymph, a major reducing action was achieved on both nymphs and adults, except mid-aged nymphs.

INTRODUCTION

Episodes of the desert *Schistocerca gregaria* have compromised farming generation in Africa, the Center East, and Asia (Ceccato *et al*., 2007). Destruction is caused as a consequence of its polyphagous be behaviour, high density of the population, and the nature to aggregate and swarm. Each individual gregarious locust can consume roughly its own weight in foliage daily (Lindsey, 2002). The extensive use of synthetic pesticides has considerable drawbacks, such as the development of insect resistance to insecticides, increased costs, handling hazards, concerns about insecticide residues, and great threats to both human and environmental health (Garriga & Caballero, 2011). Hence, many institutions have intensified their efforts in the search for integrated locust control measures. Much attention has been devoted to the use of plant extracts or plant constituents that have insecticidal effects (Schmutterer, 1990). Furthermore, the prolonged application of chemical insecticides for controlling insect pests causes many problems such as pest resistance, environmental pollution, health hazards to farmers, food contamination, and toxicity to a non-target organism (parasitoids and predators). Utmost of these toxic chemicals enter into the food chain and cause pollution of the environment (Pimentel *et al*., 2009).

The natural products, such as plant extracts, form promising non-conventional pesticides against the damaging pests for crops and health (Weinzierl, 1998). The botanical control agents are generally pest-specific and relatively harmless to non-target organisms and they are biodegradable and consequently harmless to the environment (Rembold, 1994). Though, hundreds of plant natural products have established deleterious effects on insects only a handful of botanical insecticides are presently approved for use in industrialized countries (Isman, 2006). Therefore, we need to search for new resources and innovative approaches for effective and biodegradable pesticides for insect pest control. There is improved 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). By way of a continuation of this type of searching, we have selected *Lawsonia inermis* which is a well-known medicinal plant in many parts of the world and commonly known as Henna. There are numerous reports of the use of lawsone (from henna extracts) in agriculture for control of plant diseases caused by fungi, bacteria, or nematodes. Its distribution in Native range Algeria, Egypt, Eritrea, Ethiopia, Indonesia, Iran, Iraq, Jordan, Kenya, Kuwait, Lebanon, Malaysia, Morocco, Oman, Philippines, Qatar, Saudi Arabia, Syria. *L. inermis* leaves have been used traditionally as a remedy against diarrhea, dysentery, and other related diseases, (Aliyu, 2006). Additionally, the main constituents reported on this plant are naphthoquinone derivatives, aliphatic components, triterpenes, sterols, phenolic derivatives, coumarins, xanthones, and flavonoids (Jiny*et al*., 2010). The available literature reported morphology and mortality activity of the *L. inermis* extracts against *Locusta migratoria* (Outtar, *et al*., 2014). These extracts had been reported, also, the influence of *L. inermis* on *Strongyloides* species using scanning electron microscopy (Ismail *et al*., 2016) and rice weevil *Sitophilus oryzae* (Ahmed & Al-Moajel, 2005). Moreover, (Biswas *et al.,* 2016) examined the activity of *L. inermis* versus the red flour beetle, *Tribolium castaneum*. While, Abd ell Razzik, *et al*., (2018) examined *L. inermis* extracts against *Solenopsis tinsley*.

The present study was conducted aiming to explore the metabolic effects of ethanol extract from the leaves of *L. inermis* on the main body metabolites (proteins, carbohydrates, and lipids) in the fat body and haemolymph of nymphs and adults of the desert locust *S. gregaria*.

MATERAILS AND METHODS

Experimental Insect:

A weight of one Kg. The henna leaves, *Lawsonia inermis,* (Lythraceae) which was acquired from Plant Protection Researches Institute in Giza, The gathered leaves of *L. inermis* were cleaned and dried by air. Collected plants were free from any insecticide contamination. The dried plant materials were then pulverized into a coarse powder. Then the pulverized powdered leaves were successively extracted with ethanol at room temperature. This extract was evaporated under reduced pressure at 40^{o}C using a rotary evaporator to have ethanol extract (Ncube *et al*., 2008).

Nymphal Treatments:

In an initial experiment, different concentration levels of ethanol extract of *L. inermis* leaves were applied on the newly moulted penultimate (4th) instar nymphs of *S. gregaria* over the fresh food leaves of *Trifolium alexandrinum* dun ked once in the extract for 4 minutes. A day after treatment, all nymphs (treated and control) were provided, individually, with untreated fresh food leaves. Each separate nymph was lonely in a glass vial provided with a thin layer of pasteurized sand as a floor. All vials were located in a large cage having a

suitable electric bulb. LC_{50} values were estimated for ethanol extract by 8.5%. After the treatment of penultimate instar nymphs with LC_{50}, the successfully moulted last instar nymphs and newly emerged female adults were undergone to determine the influenced main body metabolites in two tissues: haemolymph and fat body. Three ages of last instar nymphs were only used: early- (1 –day old), mid- (4-day old), and late-aged (7-day old) nymphs.

Quantitative Determination of the Main Metabolites:

Determination body metabolites in haemolymph, was gathered from early-, mid-and late-aged last instar nymphs, as well as newly emerged adult females. Haemolymph was drawn into Eppendorff Pipetman containing few milligrams of phenoloxidase inhibitor (phenylthiourea) to prevent tanning or darkening and then thinned 5 with a saline solution 0.7%. For the whole blood examination, the diluted haemolymph was solidified for the20s to crack the haemocytes. The haemolymph tests were then centrifuged at 2000 r.p.m. for 5 min, and lone the supernatant fractions for evaluation directly or frozen until use. Three duplicates were used and the haemolymph of two individuals was never mixed. For the determination of the main body metabolites in the fat body, samples were collected from last instar nymphs (early-, mid-and late-aged last instar nymphs) and newly emerged adults. The fat body samples were weighed and then homogenized in a saline solution (the fat body of one insect / 1 ml saline solution 0.7 %) using a fine electric homogenizer, tissue mortar for 2 min. Homogenizes were centrifuged at 4000 r.p.m. for 15 min. The supernatant was utilized straightforwardly or solidified until the utilization of the metabolite assurance. Three replicates were used and the fat bodies of two individuals were evaded to be mixed. The total protein content of the haemolymph or fat body was conducted according to Doumas (1975) by a kit of Bioadwic company. at 550 nm using a spectrophotometer. Total carbohydrate (as glycogen) content of haemolymph or fat body was quantitatively determined by using the anthrone reagent according to Singh and Sinha (1977) at 620 nm. Quantitative determination of the total lipid content of haemolymph or fat body was conducted according to the technique of Folch *et al*. (1957) using the Spectrophotometer at 520 nm.

Statistical Analysis:

Data obtained were analyzed by the Student's t distribution, for the test significance of the difference between means ± SD. (Moroney, 1956).

RESULTS

Protein Content:

It is effectively observed that ethanol extract of *lawsonia inermis* insignificantly affected the protein content in haemolymph or fat body. However, decreased proteins was determined in the fat body of late-aged nymphs and newly emerged adult (121.7 ± 2.4 vs. 125.4 ± 1.9 and 134.6 ± 1.5 vs 140.3 ± 1.8 mg/ml respectively). Also, a slight enhancing effect of ethanol extract on early- and mid-aged nymphs. However, reduced proteins were determined in the haemolymph of newly emerged adult and all last instar nymphs except for late-aged nymphs (Table 1).

Table 1: Total protein content (mg/ml ± SD) in fat bodies and hemolymph of the desert locust *Schistocerca gregaria* after-treatment of the newly moulted penultimate instar nymphs with LC_{50} of ethanolic extract of *lawsonia inermis*.

Tissues		Last instar nymphs			Newly emerged adults
		Early-aged	Mid-aged	Late-aged	
Fat body	Mean±SD	114.5 ± 1.3 [n]	116.6 ± 1.2 [n]	121.7 ± 2.4 [n]	134.6 ± 1.5 [n]
	Control	112.3 ± 1.6	110.3 ± 1.7	125.4 ± 1.9	140.3 ± 1.8
Hemolymph	Mean±SD	55.4 ± 2.5 [n]	51.2 ± 2.4 [n]	52.9 ± 2.3 [n]	41.8 ± 2.2 [n]
	Control	60.1 ± 2.2	54.1 ± 2.0	44.2 ± 2.1	51.1 ± 2.1

Mean ± SD followed with the same letter ([n]): is not significantly different (P>0.05), Early-aged: 1- day old nymphs, Mid-aged: 4- day old nymphs, Late-aged: 7- day old nymphs.

Carbohydrate Content:

Table (2) shows the insignificant decreasing effect of *L. inermison* on the carbohydrate content of the fat body and haemolymph of late aged nymphs and newly emerged adults whereas a significant increase was exhibited in early-aged nymphs and mid-aged nymphs except for the haemolymph of early-aged nymphs.

Table 2: Total carbohydrate content (mg/ml ± SD) in fat bodies and hemolymph of the desert locust *Schistocerca gregaria* after-treatment of the newly moulted penultimate instar nymphs with LC_{50} of ethanolic extract of *lawsonia inermis*.

Tissues		Last instar nymphs			Newly emerged adults
		Early-aged	Mid-aged	Late-aged	
Fat body	Mean±SD	46.2 ± 1.0 **	40.3 ± 2.7 ***	33.7 ± 2.1 [n]	30.8 ± 2.0 [n]
	Control	30.1 ± 1.8	38.9± 1.6	40.6 ± 1.8	41.3 ± 1.7
Hemolymph	Mean±SD	66.6 ± 1.1 [n]	62.2 ± 1.6 *	58.3 ± 1.4 [n]	52.1 ± 2.1 [n]
	Control	67.1 ± 1.0	60.1 ± 1.8	61.9 ± 2.2	62.2 ± 2.0

Early-aged, Mid-aged, Late-aged, [n]: See footnote of Table (1), (*): Significantly different (P<0.05), (**): Highly significantly different (P<0.01), (***): Very highly significantly different (P<0.001).

Lipid Content:

A prevalent inhibitory effect of *L. inermis* extract on the lipid content in fat body and haemolymph of nymphs and adults were determined with exception to mid-aged nymphs in haemolymph (Table 3). In late-aged nymphs, ethanol extract displayed the strongest inhibiting effect for lipid content in the fat body (65.4 ± 3.0 vs 71.7 ± 1.8 mg/ml). Additionally, an insignificant reduction of total lipid content was recorded in the newly emerged adults irrespective of the tissues**.**

Table 3: Total lipid content (mg/ml ± SD) in fat bodies and hemolymph of the desert locust*,Schistocerca gregaria* after-treatment of the newly moulted penultimate instar nymphs with LC_{50} of ethanolic extract of *lawsonia inermis*.

Tissues		Last instar nymphs			Newly emerged adults
		Early-aged	Mid-aged	Late-aged	
Fat body	Mean±SD	85.3 ± 2.0 [n]	83.9 ± 2.3 [n]	65.4 ± 3.0 **	58.4 ± 3.0 [n]
	Control	88.7 ± 1.9	85.7 ± 2.1	71.7 ± 1.8	73.1 ± 2.8
Hemolymph	Mean±SD	26.1 ± 2.1 [n]	22.3 ± 2.1 [n]	16.9 ± 2.0 [n]	16.0 ± 2.1 [n]
	Control	27.3 ± 1.6	19.4± 2.0	18.3 ± 1.8	19.3 ± 1.9

Early-aged, Mid-aged, Late-aged, [n]: See footnote of Table (1), **(**):** See footnote of Table (2).

DISCUSSION

Disturbed Protein Content in *S. gregaria* by *L. inermis* Extracts:

Changes in protein content probably reflect the balance between synthesis, storage, transport and degradation of structural and functional nutrients during metamorphosis as well as a response to particular physiological conditions.

As clearly shown in the available literature, some authors reported increased protein content in various insect species as responses to actions of different botanicals, in the present study, ethanol extracts from *L. inermis* leaves induced the protein content in haemolymph of only late-aged nymphs of *S. gregaria*. Also, pronounced increased proteins were determined in fat bodies of early- and mid-aged nymphs. The current results agree with some results of induced protein content in several insect species after treatment with different botanicals. For example, rhodojaponin III, extracted from *camellia sinensis* induced proteins in diamondback moth, *Plutella xyllostella* (Xiaolin *et al*., 2013). Also, treatments with extracts from *Ricinus communis* resulted in a significant increment of protein content in haemolymph of *Spodoptera littoralis* larvae (Khatter&Abuldahb, 2010). However, the induced protein content in certain developmental stages of *S. gregaria* by *L. inermis* extract, in the present study, may be attributed to the increased activity of protein biosynthesis by its tool (amino acids). Shakoori& Salem (1991) reported that the increased protein content in haemolymph or fat body of some insect species may be caused after insecticidal treatments in order to the synthesis of proteins needed for insecticide detoxification. In other words, the increasing protein content may be a method of detoxification mechanism since Wilkinson (1976) explicated that protein helps to synthesize microsomal detoxifying enzymes that assist in detoxification.

On the other hand, remarkably depleted protein content was determined in haemolymph of the early, mid-aged nymphs and newly emerged adult of *S. gregaria*, in the present work.

The late-aged nymphs, as well as the newly emerged adult females, were subjected to a slight prohibitor effect to attain normal protein content in the fat bodies. However, the decreased protein content in haemolymph and fat body, at some ages of last instar nymphs and the newly emerged adults of *S. gregaria*, after nymphal treatment with *L. inermis* extract, in the present study, may be explained in the light of some acceptable suggestions. With regard to foreign compounds, proteins help insects to synthesize the microsomal detoxifying enzymes (Wilkinson, 1976), therefore the decrease in proteins may reflect the decrease in the activity of these enzymes (Kyung & Kim, 1990). It may be appreciated to suggest that different stresses on the insect can inhibit the total protein in the haemolymph. This could be due to the breakdown of protein into amino acids, so with the entrance of these amino acids to TCA cycle as a kito acid, they will help to supply energy for the insect (Etebari &Matindoost, 2004 a, b). Another point of interest is the protein depletion in haemolymph and fat body in adults of *S. gregaria*, in the current work, which may be attributed to the major mobilization of this metabolite as well as a reduction of its synthesis.

Disturbed Carbohydrate Content in *S. gregaria* by *L. inermis* Extracts:

Some of the reported works in the literature obviously indicate inconsistent effects of botanicals, on the carbohydrate content, depending on the insect species and its developmental stage, the efficiency of the plant extracts and their concentration levels.

In the present study, the mid-aged nymphs of *S. gregaria*were slightly stimulated by ethanol extract from *L. inermis* leaves to gain more carbohydrates

in their haemolymph and fat bodies. Moreover, it increases in the fat body in the early aged nymphs. These results, to a certain extent, in accordance with the increasing carbohydrates in haemolymph and fat bodies of *Spodoptera littoralis* larvae after treatment with oils extracted from *R. communis*, and *Brassica nigra* (Khatter&Abuldahb, 2010). In contrast, a general reducing effect of *L. inermis* extract on the carbohydrate content in haemolymph of early and late-aged nymphs, as well as the newly emerged adults of *S. gregaria*, in the present study, was estimated. Also, carbohydrate content was declined in the fat body of the late-aged nymphs and newly emerged adults. This carbohydrate depletion is in consistent with several reported findings for some insects by different botanicals. A considerable reduction in the carbohydrate content in haemolymph of *Agrotis ipsilon* (Abo El-Ghar*et al*., 1995) and *S. littoralis*(Chitra& Reddy, 2000) was caused by extracts from *Ammi majus,Apium graveolens,Melia azedarach* and *Vinca rosea*. A similar reducing effect was achieved on the greater wax moth larvae of *Galleria mellonella* by volatile oils from *Lantana camara* and *Vitexagnuscastus*(Shoukry& Hussein, 1998). A great reduction in carbohydrate content was determined after the treatment of *S. littoralis* larvae with different plant extracts (Bakr *et al*., 2002).

Lipid Perturbation in *S. gregaria* by *L. inermis* extracts:

In the present study, a prevalent inhibitory action of *L. inermis* extract on the lipid content in haemolymph of nymphs and adults was exerted with the exception of mid-aged nymphs. To a great extent, a similar result of inhibited lipid content was reported in the 3 rd instar larvae of *G. mellonella* after treatment with extracts from L. *camara* and *V. agnus-castus* (Shoukry&Hussein,1998). The depleted lipid level was recorded pupae of *Rhynchophorus ferrugineus* by Azadirachtin and Jojoba oil (Bream, 2002). The prevalent inhibitory effects of *L. inermis* on the lipid content in the haemolymph and fat bodies of nymphs and adults of *S. gregaria*, with few exceptions, as recorded in the present study, demonstrate the pronounced interference of these extracts with not only the synthesis of lipids but also in their mobilization as promoted to convert into other metabolites of fatty acids. Also, it is of interest to suggest that these *L. inermis* extract can be useful as biocontrol agents for *S. gregaria* because this locust, like nearly all insects flying long distances, mobilizes lipids stored in its storage tissues to meet the energy requirements for sustained flight.

Conclusions:

The disruptive effects of *L. inermis* extract on the main body metabolites in *S. gregaria,* in the current work, may be explained by the intervention of certain chemical constituents in the hormonal regulation of the metabolite mobilization. The production or utilization of these metabolites has been under the control of juvenile hormone (JH), as reported by Gäde (2004) and Sugumaran (2010). Therefore, the occurrence of certain active ingredient (s) in *L. inermis* extracts exhibiting JH activity can be accepted. Also, recorded disturbance in the main metabolites of *S. gregaria* by this extract can be understood in the light of their ability to modify the synthesis of some metabolites and disrupt the functionality of the organism.

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ARABIC SUMMARY

تقييم فعالية مستخلص أوراق نبات الحنة البلدي على المستقلبات الرئيسية في الأجسام الدهنية والدم لحشرة الجراد الصحراوي) شيستوسيركا جريجاريا(الجراديات : مستقيمات الأجنحة.

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يهدف البحث الحالي لدراسة تأثيرات مستخلص الإيثانول لأوراق نبات الحنة البلدي علي محتويات الأيض الرئيسية في الأجسام الدهنية ودم الجراد الصحراوي شيستوسيركا جريجاريا (الجراديات : مستقيمات الأجنحة). تم تطبيق التركيز المميت 50٪ من مستخلص الإيثانول (8,5٪) على الحوريات قبل الأخيرة. تم تحديد محتوى البروتين المنخفض بشكل واضح في الأجسام الدهنية للحوريات ذات العمر المتأخر ، وكذلك الإناث اليافعات حديثي الظهور. فيما يتعلق بمحتوى البروتين في الدم ، وجد آثار مثبطة قوية على الحوريات والحشرات حديثة البزوغ. تم تسجل تأثير اختزال للمستخلص على محتوى الكربوهيدرات في الأجسام الدهنية في الحوريات ذات العمر المتأخر والحشرات اليافعة حديثة البزوغ. في الدم، سجل تأثير محفز فقط في العمر الأوسط لليرقات من قبل المستخلص. أظهر المستخلص بوجه عام تأثير مثبط لمحتوى الدهون في الأجسام الدهنية في الحوريات والحشرات اليافعة حديثة البزوغ. أما في الدم ، تم تسجيل نفس الإنخفاض باستثناء الحوريات في منتصف العمر.