Insecticidal Potentialities of Leaf Extracts of *Azadirachta indica* (A.Juss.) and *Calotropis Procera* (Ait.) Against *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae)

BY

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INTRODUCTION

The increasing number of investigations on plant – insect chemical interactions in the last few decades unveiled the potential of utilizing secondary plant metabolites , or allelochemicals , as pest control agents. This interest in botanical pesticides resulted from the need to provide an alternative in IPM programmes to the synthetic insecticides , whose adverse effects on agroecological systems are well known (Whitte,1992).

Plants may provide potential alternatives to currently used insect – control agents because they constitute a rich source of bioactive chemicals (Wink,1993). Since those are often active against a limited number of species including specific target insects , are often biodegradable to non – toxic products and are potentially suitable for use in integrated pest management and they could lead to the development of new classes of safer insect – control agents. Much efforts have , therefore , been focused on plant – derived materials for potentially useful products as commercial insect – control agents (Ahmed *et al*., 2006).

Azadirachta indica (Meliaceae) , with the common name (Neem)and*Calotropis procera* (Asclepiadaceae) , with the commen name (Ushar), are growing widely throughout the tropic of Africa and Asia and they are grown abundantly in arid and semi-arid regions without irrigation, fertilization, pesticides or other agronomic practices. The contents of the green parts of these plants appear to be as defense strategies of the plant against virus, fungi and insects (Qari,2008)

Azadirachta indica, the neem tree, is the most promising species since chemicals extracted from it, besides showing antifeedant effects, can also cause growth disruption and mortality of the insects. Compound isolated from the neem tree can cause antifeedancy, growth reduction, moulting inhibition, anatomical abnormalities as well as mortality, in a vast range of insect species, many of them belonging to the order Lepidoptera (Martinez and Emden, 2001).

Calotropis procera , with the common name (Ushar) was reported having potential anti inflammatory, antidiarrhoeal, analgesic, antipyretic and schizonticidal activities (Dewan *et al*., 2000;Sharma and Sharma , 2000 and Kumar *et al.,* 2001). Bioactivities of the plants such as insectidal has been reported (Khan and Siddiqui, 1994 and Moursy,1997)) The genus *Spodoptera* includes many species which constitute pests of economic importance for different crops, in different countries. *Spodoptera Littoralis* is an important pest of cotton in Southern Europe, Africa and the Middle East (Hosny *et al*.,1986).

This study is aimed at developing insecticides using aqueous extract of leaves of plants that are safe and locally available with the cotton pest *Spodoptera Littoralis* as a target. It is also essential that appropriate system is developed to promote a direct preparation of traditional pesticides at the farm level for those resource – poor farmers who have no access to commercial pesticides or organic solvents to extract plant materials or can not afford them.

MATERIALS AND METHODS

Insects:

Spodoptera Littoralis eggs were obtained from Agriculture Research Centre – Giza – Egypt, and were reared in the laboratory of Biology Department in Taif University,(KSA) for two generations before experimental starting. Insects were reared under laboratory conditions and were fed on the leaves of caster oil plants *Ricinus Communis*.

Test plants:

Ushar, *Calotropis procera*, and Neem, *Azadirachta indica*, plants were collected during April to June 2010 from naturally growing plants in their natural habitats located in the area of Taif – university.

Extract Preparation:

Plant leaves were used in the present experiments. Leaves were washed and air – dried in a shady place. Dried materials were ground in a table mode grinder. The ground plant materials were dipped in distilled water (200 gm/liter) for 48 – 72 h. , filtered and the stock solutions were kept in tightly capped jars in the Frigidaire until used.

Bioassay:

Each of the two plant extracts was assayed for anti – insect biological activities using the following tests. All experiments were carried out at room temperature in triplicates of 10 individuals in each container fed on fresh caster oil leaves dipped in tested plant extract solutions. Control groups were fed on caster oil leaves dipped in distilled water.

Fecundity and longevity:

The emerged adults were sexed and paired; each pair was transferred to a separated chimney glass cage containing oleander leaves (*Nareium oleander*) for the egg deposition. Three groups of 10 pairs each of one-day old female and male insects were fed on a piece of cotton of the two tested solutions mixed with 10% sugar solution, which were replaced daily. The third group, control insects, was fed on a piece of cotton dipped in 10% sugar solution. The pre-oviposition period, the number of eggs daily laid by each female and longevity of male and female were recorded to determine the effect of either extracts on insect fecundity and longevity.

Larval development and survival:

The effect of Neem and Ushar leaf extracts on larval development and survival were investigated. The tests were carried as described by (Tewari and Moorthy 1985) with some modifications. Simply the larvae were taken just after egg hatching (1^{st} instar) and placed as three groups of three replicates of 10 larvae each in Petri-dishes

lined with moist filter papers. Two groups were fed on caster oil leaves dipped in the two tested plant leaf extracts separately. The third group was fed on caster oil leaves dipped in distilled water and was considered as control group.

Larval development and instar durations, moulting process and mortality rates were recorded. Morphological anomalies and abnormalities were observed and recorded.

Data analysis:

The levels of significances of the differences between means were determined using student's " t " test, significant

($P< 0.05 – 0.01$), highly significant ($P < 0.001$).

RESULTS AND DISCUSSION

Present study indicated variations in insecticidal efficacy of *A. indica* and *C. procera* tested plant extracts on fecundity and longevity of adults, larval development and mortality and also on larval morphological anomalies.

The fecundity and longevity of the adults emerged from the larvae fed on the *A. indica*_(neem) and *C. procera* (Ushar) leaf extracts were significantly reduced (Table 1,2) when compared with control adults. Ushar extract caused a reduction in adult longevity more than neem – extract. But, on the other hand neem – extract was more insecticidal effective in reducing the number of laid – eggs and the percentage of egg – hatching compared with control insects. Neem – extract also succeeded in increasing the pre-oviposition period more than usher – extract.

The longevity of *Spodoptera Littoralis* adults fed on neem – leaf extract was 7.1 days. They lived for 2.3 days as pre-oviposition period and 5 days as egg – laid period and all the insects were dead at the 6^{th} day. The longevity of the control insects for this experiment was 11.3 days .They lived for one day as pre-oviposition period and stayed 10 days in laying eggs. The numbers of laid – eggs were highly significantly reduced throughout the days of the experiment. The number started with 240.0 eggs during the 1^{st} day of the experiment in treated insects compared with 916.3 eggs in control insects.

The ratio of reduction maintained through the following days of experiment. Neem extract treated insects laid 240.0, 300.3, 226.6, 160.0 and 66.6 eggs/female during 1^{st} , 2^{nd} , 3^{rd} , 4^{th} and 5^{th} day compared with 916.3,890.0,876.6,700.2 and 150.0 eggs/female in control insects. Treated insects died after that time but control insects continued laying eggs up to the 10^{th} day. The sum of laid eggs in treated insects was 993.5 eggs/female, but in control insects it was 3566.57 eggs/female .

The percentages of egg – hatching were also highly significantly decreased during the different days of the experiment.Egg – hatching rate was 100% in control insects compared with 35.0%, 66.3%, 53.7%, 37.5% and 20.0% in treated insects from the 1^{st} to the 5^{th} day of the experiment respectively (Table, 1).

Ushar – leaf extract showed a highly insecticidal activity on fecundity and longevity of *Spodoptera Littoralis* insects. Pre-oviposition period of treated insects was 2.0 days compared with 1.2 days in control insects, and also the longevity of treated insects was 6.0 days compared with 11.6 days in control adults.

Egg laying period of ushar – leaf extract fed insects was 3 days and all the insects died at the 4^{th} day, while that of the control insects was 10 days.

The numbers of daily laid eggs were very highly significantly reduced in treated insects compared with control insects. During the three days, the number of eggs laid by treated adults was 300.0, 436.6 and 150.0 eggs during the 1^{st} ,2^{nd} and 3^{rd} day compared with 861.0, 830.0 and 798.3 eggs of control insects, respectively and the sum of eggs laid by treated insects was 886.6 eggs compared with 3861.2 eggs in control insects. The percentages of egg hatching were significantly reduced but the rate of reduction was less than that of neem – leaf extract treatment. The rates of egg - hatching were 100% in control insects compared with 91.6%, 92.3% and 80.8% during the three days of the experiment from the 1^{st} to the 3^{rd} day, respectively (Table, 2).

The efficiency of both neem and ushar leaf extracts as larvicidal was proven against *S. Litttoralis* larvae. Larval – extracts feeding promoted prolongation of the larval instars (especially the 2^{nd} instar), moulting disruption and blockage, morphological anomalies and mortality of the larvae before reaching to the next

instars (Tables 3 & 4). Leaf extracts of both plants showed non – significant toxic effect on the 1st larval instar. The 2nd instar larvae had the length of that instar significantly prolonged .

Neem leaf extract prolonged the length of 2nd larval instar from 3 days in control to 7 days in treated larvae and ushar leaf extract prolonged it to 12 days compared with the same control.

The number of dead larvae during the 1st seven days were higher in case of neem – leaf extract and the larvae were dead after that time. Larvae treated with ushar – extract continued their 2nd larval instar and the number of dead larvae increased gradually until they all died after the 12th day. The larvae in both cases failed to moult and to complete the process and the defects intensified with the sequence of moults into the later instars.

The mortality rates were significantly high and gradually increased during the duration of the 2nd instar larvae fed with neem – extract (7 days) until 100% mortality at the 8th day(Table, 3). But in case of ushar leaf – extract fed larvae mortality rate started highly significant from the 3rd day and increased gradually until 100% mortality at the 13th day (Table,4).

Reduction in oviposition and decreased longevity of the adults were observed in this study (Tables, 1 and 2). Other studies also showed that extracts from different plant parts of *C. procera* and *A. indica* have insecticidal effect Abbassi *et al.* (2003) Reported that alkaloid extracted from the leaves of *C. procera* was able was able to cause a considerable mortality of *Schistocera gregaria* .(Jahan *et al* 1991) showed the toxicity of leaf powder of *C. procera* against larvae of *Tribolium confusum*.

The suppression of reproductive activity and adults longevity showed by plant extracts may be due to malnutrition effect because the larvae exposed to the treated leaves consumed considerable fewer amount than the control (Ahmed *et al.*, 2006) . Boekea *et al.* (2004) reported that botanical insecticide reduced the oviposition and longevity under laboratory conditions. *Azadirachta* can be shown to cause profound effects on both male and female insects. Rembold and Sieber, (1981) mentioned that

Azadirachta inhibits both oogenesis and ovarian ecdysteroid synthesis in *locusta migratoria* so preventing oviposition. When female aphids are fed on diets containing *Azadirachta*, their fecundity decreases dramatically within 48 h of feeding (Mordue (Luntz) *et al*. 1996). Male reproduction is also affected by *Azadirachta*. Injection of male *Oncopeltus fasciatus* with 0.125 mg per insect severely reduces male potency by 9.80 % reduction in the fecundity of normal females when mated with treated males (Dorn 1986). Singhi *et al*.,(2004) mentioned that *C. procera* latex solution showed a remarkable effect a larvicide against *Aedes aegypti* and brought forward very important observations on the ovipositing behavior.

Das *et al*., (2007) mentioned that phytochemicals derived from plant sources can act as larvicide, insect growth regulators, repellent and ovipositor attractant. Mordue (Luntz) and Nisbet (2000) and Martinez and Emden (2001), reported that the neurosecretory system of the brain affected by *C. procera* and *A. indica* extracts which causes a blockage of the release of morphogenetic peptide hormones and allatostatins. These control the function of the prothoracic glands and corpora allata respectively . Moulting hormone from the prothoracic glands in turn controls new cuticle formation and ecdyses whereas juvenile hormone from the corpora allata controls the formation of juvenile stages at each moult. In the adults both hormones can be involved in the control of yolk deposition in the eggs. Any disruption in these cascade events by plant extracts results in the many various but well defined effects as seen as moult disruption, moulting defects and sterility effects.

Morphological abnormalities as observed in this study focused on the larval cuticle which was usually tanned on the abdomen, and on the dorsal region of the head and thorax. The remaining parts became darker and finally black, shrinkage of the larval body and paralyzed – like appearance when the insect was close to death. These observations agreed with those of Martinez and Emden (2001). Therefore, leaves which gave a highest extractive amount could be used as insecticidal agent in an integrated vegetable pests control programs. The use of the plant materials in the pest

control could become important supplements to imported synthetic pesticides, especially in developing countries.

(Table, 1): Effect of *Azadirachta indica* leaf extract on fecundity and longevity of *Spodoptera littoralis* adults.

Treatment	Pre-oviposition period (days)	Day	No. of eggs/ female mean ± S.E	% Hatching mean ± S.E	Longevity (days)
Control	1.0 ± 0.032	1st 2nd 3rd 4th 5th 6th 7th 8th 9th 10th 11th	916.3 ± 0.077 89 0.0 ± 0.50 876.6 ± 0.395 700.2 ± 0.126 150.0 ± 0.180 150.0 ± 0.09 153.0 ± 0.93 191.0 ± 1.91 163.0± 0.67 76.6 ± 0.850 0.0 ± 0.0	100 ± 0 100 ± 0 100 ± 0 100 ± 0 100 ± 0 100 ± 0 100 ± 0 100 ± 0 100 ± 0 100 ± 0 0.0 ± 0.0	11.3 ± 0.10
Neem – leaf extract	2.3* ± 0.103	1st 2nd 3rd 4th 5th 6th	240.0 ± 1.60** 300.3 ± 1.012** 226.6 ± 0.950** 160.0 ± 0.321** 66.6 ± 0.861** D	35.0 ± 0.670** 66.3 ± 0.25** 53.7 ± 0.94** 37.5 ± 1.29** 20.0 ± 1.41** D	7.1* ± o.983

(Table, 2): Effect of *Calotropis procera* leaf extract on fecundity and longevity of *Spodoptera littoralis* adults.

Treatment	Pre-oviposition period (days)	Day	No. of eggs/ female mean ± S.E	% Hatching mean ± S.E	Longevity (days)
Control	1.2 ± 0.369	1st 2nd 3rd 4th 5th 6th 7th 8th 9th 10th 11th	861.0 ± 1.29 830.0 ± 1.19 798.3 ± 0.94 702.6 ± 1.85 175.2 ± 1.06 162.0 ± 0.85 178.0 ± 0.79 133.6 ± 0.86 58.3 ± 1.20 7.2 ± 1.385 0.0 ± 0.0	100 ± 0 100 ± 0 100 ± 0 100 ± 0 100 ± 0 100 ± 0 100 ± 0 100 ± 0 100 ± 0 100 ± 0 0.0 ± 0.0	11.6 ± 1.09
Ushar – leaf extract	2.0* ± 0.091	1st 2nd 3rd 4th	300 ± 1.610** 436.6 ± 0.723* 150.0 ± 0.95*** D	91.6 *± 1.950 92.3 *± 1.826 80.8 *± 0.990 D	6.0** ± 0.985

(Table,3): Effect of *Azadirachta indica* leaf extract on *Spodoptera littoralis* larval development and survival.

Treatment	Larval Stage	Day	No. of live larvae	No. of dead larvae	% Mortality
Control	1st	1st	30.0 ± 0	0.0 ± 0.0	0.0 ± 0.0
		2nd	30.0 ± 0	0.0 ± 0.0	0.0 ± 0.0
		3rd	30.0 ± 0	0.0 ± 0.0	0.0 ± 0.0
	2nd	1st	30.0 ± 0	0.0 ± 0.0	0.0 ± 0.0
		2nd	30.0 ± 0	0.0 ± 0.0	0.0 ± 0.0
		3rd	30.0 ± 0	0.0 ± 0.0	0.0 ± 0.0
	3rd	1st	30.0 ± 0	0.0 ± 0.0	0.0 ± 0.0
		2nd	30.0 ± 0	0.0 ± 0.0	0.0 ± 0.0
	4th	1st	30.0 ± 0	0.0 ± 0.0	0.0 ± 0.0
		2nd	30.0 ± 0	0.0 ± 0.0	0.0 ± 0.0
	5th	1st	30.0 ± 0	0.0 ± 0.0	0.0 ± 0.0
		2nd	30.0 ± 0	0.0 ± 0.0	0.0 ± 0.0
	6th	1st	30.0 ± 0	0.0 ± 0.0	0.0 ± 0.0
		2nd	30.0 ± 0	0.0 ± 0.0	0.0 ± 0.0
Neem – leaf extract	1st	1st	30.0 ± 0	0.0 ± 0.0	0.0 ± 0.0
		2nd	30.0 ± 0	0.0 ± 0.0	0.0 ± 0.0
		3rd	30.0 ± 0	0.0 ± 0.0	0.0 ± 0.0
		4th	30.0 ± 0	0.0 ± 0.0	0.0 ± 0.0
	2nd	1st	23.0 ± 1.950*	7.0 ± 0.02	23.3 ± 1.29*
		2nd	19.0 ± 0.950*	11.0 ± 1.19*	36.7 ± 0.951*
		3rd	11.0 ± 0.791*	19.0 ± 1.09*	63.3 ± 1.09**
		4th	10.0 ± 0.623*	20.0 ± 0.98*	66.7 ± 0.595*
		5th	10.0 ± 0.723	20.0 ± 0.91**	66.7 ± 1.031**
		6th	7.0 ± 1.090**	23.0 ± 0.62**	76.7 ± 1.070*
		7th	2.0 ± 0.630**	28.0 ± 0.591**	93.3 ± 0.959***
		8th	0	30.0 ± 0	100

(Table, 4): Effect of *Calotropis procera* leaf extract on *Spodoptera littoralis* larval development and survival.

Treatment	Larval Stage	Day	No. of live larvae	No. of dead larvae	% Mortality
Control	1st	1st	30.0 ± 0	0.0 ± 0.0	0.0 ± 0.0
		2nd	30.0 ± 0	0.0 ± 0.0	0.0 ± 0.0
		3rd	30.0 ± 0	0.0 ± 0.0	0.0 ± 0.0
	2nd	1st	30.0 ± 0	0.0 ± 0.0	0.0 ± 0.0
		2nd	30.0 ± 0	0.0 ± 0.0	0.0 ± 0.0
		3rd	30.0 ± 0	0.0 ± 0.0	0.0 ± 0.0
	3rd	1st	30.0 ± 0	0.0 ± 0.0	0.0 ± 0.0
		2nd	30.0 ± 0	0.0 ± 0.0	0.0 ± 0.0
	4th	1st	30.0 ± 0	0.0 ± 0.0	0.0 ± 0.0
		2nd	30.0 ± 0	0.0 ± 0.0	0.0 ± 0.0
	5th	1st	30.0 ± 0	0.0 ± 0.0	0.0 ± 0.0
		2nd	30.0 ± 0	0.0 ± 0.0	0.0 ± 0.0
	6th	1st	30.0 ± 0	0.0 ± 0.0	0.0 ± 0.0
		2nd	30.0 ± 0	0.0 ± 0.0	0.0 ± 0.0
Ushar – leaf extract	1st	1st	30.0 ± 0	0	0
		2nd	30.0 ± 0	0	0
		3rd	28.0 ± 1.90	2.0 ± 0.930	6.7* ± 0.21
	2nd	1st	26.0 ± 0.890	4.0 ± 0.321	13.3* ± 0.35
		2nd	25.0 ± 1.086**	5.0 ± 1.320*	16.7 *± 0.93
		3rd	24.0 ± 1.362*	6.0 ± 1.601*	20.0 **± 1.01
		4th	21.0 ± 0.98*	9.0 ± 1.008*	30.0 *± 0.65
		5th	19.0 ± 0.632*	11.0 ± 0.321*	36.7 **± 0.09
		6th	17.0 ± 0.751*	13.0 ± 0.621	43.3 **± 0.17
		7th	16.0 ± 1.09	14.0 ± 1.860	46.7 ± 1.87
		8th	11.0 ± 0.431**	19.0 ± 0.865**	63.3 **± 0.90
		9th	11.0 ± 0.392	19.0 ± 0.631*	63.3 *± 1.03
		10th	10.0 ± 1.03**	20.0 ± 1.851**	66.7 **± 0.85
		11th	10.0 ± 0.670*	20.0 ± 0.732*	66.7 *± 1.07
		12th	9.0 ± 0.236**	21.0 ± 1.981***	70.0 ***± 0.09
		13th	0	30.0 ±	100

Summary

In an attempt to find natural and cheaper methods for the control of vegetable pest, locally available plants *A. indica* (neem) and *C.procera* (ushar) were used. Leaf – extracts of both plants were effective in controlling *Spodoptera littoralis* fecundity and longevity of adults and also larval development and survival , ushar – leaf extract caused a reduction in adult longevity more than neem extract. But, on the other hand neem extract was more insecticidal effective in reducing the numbers of laid – eggs and the percentage of eggs hatching. Neem extract also succeeded in

elongating the preoviposition period more than ushar – extract. The efficiency of both neem and ushar extracts as larvicidal was proven. They promoted prolongation of larval instars (2nd instar) , moulting disruption and blockage, morphological anomalies and mortality of the larvae before reaching to the next instars.

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