Global Advanced Research Journal of Environmental Science and Toxicology (ISSN: 2315-5140) Vol. 1(6) pp. 172-177, September, 2012
Available online http://garj.org/garjest/index.htm

Full Length Research Paper

Insecticidal potentiality of *Datura innoxia* leaf extracts against the cluster bug (*Agonoscelis pubescens* (Thunberg))

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Accepted 20 September, 2012

The cluster bug (*Agonoscelis pubescens*) is a major pest of *Sorghum bicolor* and many other crops in Sudan. Despite its costly chemical control, the pest retains more or less similar trend every year. This advocated the search for effective, cheap and safe alternatives. Hence, laboratory experiments were carried out to evaluate the insecticidal potentiality of *Datura innoxia* leaf extracts compared with a standard insecticide. Three extracts (water, ethanol and hexane) were studied concerning their application routes (spraying, dipping and filter paper methods) and bioassays against the adult *A. pubescens*. Mortality levels were recorded at different intervals (2, 3, 5 and 7days), post treatments. The results revealed that spraying of bugs on host plant gave better effects than the other application methods. The leaf hexane extract exerted significantly the highest mortality levels as compared with the other extracts, though was exceeded by the insecticide. In all extracts, the mortality rates generally increased in response to concentration and exposure time. Moreover, the hexane extract also manifested a hormonal effect on the pest emerged in deformed hind legs. In conclusion, the study proved variable biocidal activities of *D. innoxia*, and invites more research to isolate and characterize its active compounds particularly of hexane extract for proper assessment as natural biocides.

Keywords: *Agonoscelis pubescens*; *Datura innoxia*; bioassays; mortality effect; hormonal effect.

INTRODUCTION

The sorghum bug or cluster bug [*Agonoscelis pubescens* (Thunberg)] (Heteroptera: Pentatomidae), locally known as the "Dura Andat", is a major pest of *Sorghum bicolor* and many other cultivated and wild plants in Sudan. Despite the costly chemical control directed annually against this pest, its population that attacks the sorghum crop retains more or less similar trend every year. Such demerits of synthetic insecticides have advocated the search for effective, cheap and ecologically sound control alternatives. Although, the use of botanical insecticides in agriculture had suspended after the discovery and use of synthetic chemicals during the last century, the scientists resorted again to the natural products as a group of the important available pesticide alternatives. Accordingly, hundreds of secondary metabolites were isolated and showed variable biological activities against various pests. These bioactivities included mainly, stomach poison, feeding deterrent, repellent or growth regulatory effects. So far, only a handful of botanical insecticides were commercialized, in spite of rich plant resources that occur particularly in tropical countries.

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In the above context, the Solanaceae which represents one of the largest families in plant kingdom (>3000 spp.), is known to comprises several species with potent secondary metabolites of pharmaceutical and pesticidal importance. Such plants are recognized to posses numerous bioactive compounds including steroids, alkaloids and flavonoids (Silva *et al*., 2005; Singh and Kaushal, 2007). However, several plant derivatives detected in Solanaceae were proved to be effective as natural insecticides since earlier times, and the nicotine present in *Nicotiana* spp. Is one of the eminent insecticidal alkaloid worldwide. Hence, new records of active solanaceous plants are always declared in different regions (Silva *et al*., 2005; Satti and Abdelrahman, 2008).

Among widely distributed wild solanaceous plants in Sudan are two species of night shade or Devil's apple, *Datura innoxia* Mill. And *Datura stramonium* L., which are called "Sakran" (Elhadi, 2002). As this local name implies, these plants generally induce toxic or narcotic effects on grazing animals, and therefore they have been used by traditional herbalists for different purposes. Generally, *Datura* species are widely distributed in different parts of the world including Mexico, U.S., Caribbean Island, India, China and Africa (Howard, 1989; Schultes and Hofmann, 1992; Burkill, 2000). The phytochemical studies showed that *Datura* species are rich in alkaloids (e.g., hyoscyamine, hyoscine, atropine, scopolamine), saponins, flavonoids, phenols, essential oils and cardiacs glycosides (Gilman, 1990; Ayuba *et al*., 2011). Thus, the insecticidal activities of these plants were documented against some pests in different parts of the world (Khalequzzaman and Islam, 1992; Lohra *et al*., 2002).

Therefore, preliminary laboratory experiments were carried out to evaluate the insecticidal potentiality of *Datura innoxia* against the adult bugs of *A. pubescens*. Three application routes were tested against the pest coupled with a bioassay experiment of various leaf extracts.

MATERIALS AND METHODS

Two laboratory experiments were carried out in Khartoum aiming to study the insecticidal potentiality of Devil's apple (*Datura innoxia*) leaf extracts against the adult bugs of *A. pubescens*. The first experiment, conducted in July-August 2009, was intended to check the efficacy of three application methods (viz., spraying, dipping and filter paper techniques) of leaf water extract on mortality of the pest, while the second experiment in September-October 2009, compared the activities of different leaf extracts (water, methanol and hexane) applied through the best technique (i.e., spraying) chosen from the previous experiment. The test insects (*A. pubescens* adults) were collected from Sennar area, Blue Nile State, during the active period in autumn season (July and September). Sexed pairs of the bugs were reared in glass jars (9.5cm) to obtain the second generation adults for the bioassay. Sesame (*Sesamum indicum*) cultures were grown at different times in the field so as to secure the availability of fresh sesame pods for feeding the bugs both during rearing and the bioassay experiments.

Preparation of treatments

Leaves of *D. innoxia* were collected from Shambat area, Khartoum North, shortly prior to each experiment, washed and left for 7 days to dry under shade. The dry leaves were ground into fine powder using an electric blender (Moulinex model 276). Water extract was prepared following steps adopted by Siddig (1991) and Satti *et al*. (2003). Accordingly, 80 g of powder was mixed in one liter of distilled water in a conical flask, thoroughly shaken manually and left to stand for 24 h, then strained through a fine mesh to obtain the stock solution (8% w/v). Regarding organic solvents extraction, each 75 g of *D. innoxia* leaf powder was extracted separately by hexane and methanol in a soxhlet apparatus for 14 h, and dried in a rotary evaporator (Schmutterer, 1985; Sharma *et al.,* 2003; Alkhail, 2005). Four concentrations (1%, 2%, 4% and 8%) were prepared from all extracts to be screened in each experiment. However, to make the oil suitable for application, 0.1% liquid soap was added as an emulsifier.

Bioassay experiments

As mentioned above, only the four concentrations of water extract was used in the first experiment which designed to compare three methods of application (spraying, dipping and filter paper) on mortality of the adult bugs. The ways these methods applied were; spraying both insects and sesame pods together, dipping only the pods before given to insects, and spraying only a Whatman filter paper No.1 (9cm) before releasing of insects, respectively. On the other hand, the prepared concentrations of all water and organic solvent extracts were bioassayed in the second experiment against the pest. Each ten insects were introduced together with fresh sesame pods into the glass jars according to the different treatments, with three replications. Based on the results of the first experiment, the spray method was applied for the second bioassay experiment. Consequently, both sesame pods and insects

Table 1. Effects of three application methods of *Datura innoxia* leaf water extract on the adults of *Agonoscelis pubescens*, under laboratory conditions, during July-August 2009.

Treatments	Mortality mean (±S.E) percents at different intervals			
	2 days	3 days	5 days	7 days
Spray 1%	06.7±5.8 c	16.7±5.8 cd	20.0±10.0 cd	20.0±10.0 cd
Spray 2%	10.0±10.0 c	10.0±10.0 cd	23.3±20.8 cd	23.3±20.8 cd
Spray 4%	13.3±11.5 bc	23.3±20.8 cd	36.7±5.8 bc	36.7±5.8 bc
Spray 8%	36.7±32.2 b	50.0±26.5 b	56.7±35.1 b	60.0±36.1 b
Dipping 1%	06.7±5.8 c	06.7±5.8 cd	10.0±10.0 cd	10.0±10.0 cd
Dipping 2%	06.7±11.5 c	06.7±11.5 cd	13.3±11.5 cd	13.3±11.5 cd
Dipping 4%	16.7±20.8 bc	16.7±20.8 cd	23.3±20.8 cd	23.3±20.8 cd
Dipping 8%	20.0±10.0 bc	26.7±5.8 bc	33.3±5.8 bc	33.3±5.8 c
Filter paper 1%	00.0±0.0 c	00.0±0.0 d	00.0±0.0 d	00.0±0.0 d
Filter paper 2%	00.0±0.0 c	00.0±0.0 d	00.0±0.0 d	00.0±0.0 d
Filter paper 4%	00.0±0.0 c	00.0±0.0 d	00.0±0.0 d	00.0±0.0 d
Filter paper 8%	06.7±11.5 c	13.3±15.3 cd	13.3±15.3 cd	13.3±15.3 cd
Malathion 57%EC/sprayed	100.0±0.0 a	100.0±0.0 a	100.0±0.0 a	100.0±0.0 a
Control	00.0±0.0 c	00.0±0.0 d	00.0±0.0 d	00.0±0.0 d
C.V.%	77.4	64.0	59.3	59.4

Means followed by the same letter(s) in each column are not significantly different at (P = 0.05) according to Duncan's Multiple Range Test.

Table (2). Mortality levels of *Agonoscelis pubescens* adults treated with "Sakran" *Datura innoxia* leaf water and organic (hexane and methanol) extracts, during September- October 2009.

Treatments	Mortality mean (±S.E) percents at different intervals			
	2 days	3 days	5 days	7 days
Sakran leaf water extract, 1%	13.3±15.3cde	13.3±15.3 def	13.3±15.3 e	13.3±15.3 ef
Sakran leaf water extract, 2%	03.3±5.8 ef	16.67±20.8 cde	23.3±23.1de	23.3±23.1def
Sakran leaf water extract, 4%	16.7±5.8 cd	26.7±20.8 cde	26.7±20.8 de	26.7±20.8 def
Sakran leaf water extract, 8%	10.0±10.0 def	30.0±20.0 cd	40.0±36.1cd	46.7±37.9 cd
Sakran leaf hexane extract, 1%	06.7±5.8d ef	20.0±0.0c def	26.7±5.8 de	30.0±10.0 de
Sakran leaf hexane extract, 2%	10.0±0.0d ef	26.7±11.5 cde	43.3±11. 5 cd	43.3±11.5 cd
Sakran leaf hexane extract, 4%	16.7±11.5 cd	36.7±20.8 bc	56.7±15.3 bc	60.0±10.0 bc
Sakran leaf hexane extract, 8%	23.3±5.8 b	53.3±5.8 b	73.3±11.5 ab	73.3±11.5 ab
Sakran leaf methanol extract,1%	00.0±0.0 f	03.3±5.8 f	10.0±10.0 e	10.0±10.0 ef
Sakran leaf methanol extract,2%	03.3±5.8 ef	10.0±10.0 def	16.7±11.5 e	16.7±11.5 ef
Sakran leaf methanol extract,4%	06.7±5.8d ef	06.7±5.8 ef	23.3±15.3 de	23.3±15.3 def
Sakran leaf methanol extract,8%	03.3±5.8 ef	20.0±10.0 cde	26.7±15.3 de	33.3±5.8d e
Malathion 57%EC	100.0±0.0 a	100.0±0.0 a	100.0±0.0 a	100.0±0.0 a
Control	00.0±0.0 f	00.0±0.0 f	00.0±0.0 e	00.0±0.0 f
C.V.%	46.4	50.1	47.6	44.9

Means followed by the same letter(s) in each column are not significantly different at (P = 0.05) according to Duncan's Multiple Range Test.

were sprayed together with 2 ml of the different concentrations of water and organic extracts. A water treated control and a standard insecticide, Malathion 57% EC (malathion), were included in both experiments for comparison. Such experiments were assigned in Completely Randomized Design (CRD). The average room temperatures and relative humidity were recorded during the experimental periods. The number of dead insects and other observations were recorded at the 2nd, 3rd, 5th and 7th days from treatments. After the analysis of

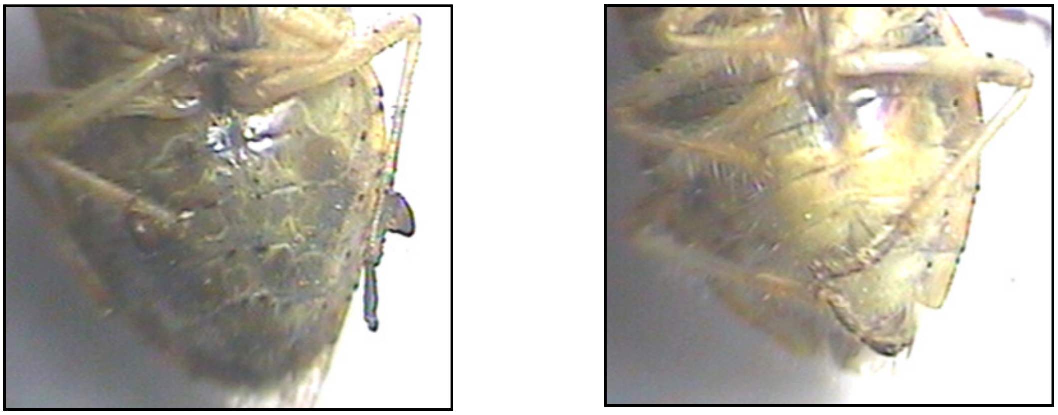

Plate 1. A growth regulatory effect of *Datura innoxia* leaf hexane extract on adult *Agonoscelis pubescens*: left) affected adult with malformed hind legs, right) normal adult.

variances, means were separated according to Duncan's Multiple Range test.

RESULTS AND DISCUSSION

In the course of screening botanical extracts as promising natural alternatives to hazardous synthetic chemicals for combating agricultural pests, the current preliminary research was attempted to evaluate the potentiality of *Datura innoxia* against the adults of sorghum bug (*A. pubescens*), a deleterious economic pest of sorghum and other crops in Sudan. Accordingly, two laboratory experiments were executed to find out the suitable application method of these botanical treatments, and to evaluate the potential activities of *D. innoxia* leaf extracts in combating the pest. The average room temperatures and relative humidity during the experimental periods of the two experiments were 34±2°C and 40–48%, respectively.

Application methods

The results of mortality percents of *A. pubescens* as affected by the three different application methods of *D. innoxia* leaf water extract are shown in Table (1). The overall scene of the table reflected that there were significant variations among the three methods of application, with spray treatments generally revealed better mortality percents than the other application methods. However, the sprayed Malathion was the most effective treatment exerting 100% mortality since the first count. However, during the second day only the 8% sprayed leaf extract showed significant mortality result (36.7±32.2%) as compared with the untreated control (0.0±0.0%). This followed by the high doses of dipping (8% and 4%) and spray (4%) methods, but without significant differences from the control. The highest sprayed dose (8%) also kept significantly the superior effect among the other routes of application during the subsequent counting intervals up to the 7^{th} day. The 4% spray and 8% dipping rates lied next in order with significant differences from the control, during the 5^{th} and 7^{th} days. Most treatments in both spray and dipping methods showed increased mortality in relation to concentration and exposure time. However, it is clear that the filter paper technique revealed no toxic effect on the pest, except at the highest concentration which manifested low non significant mortality levels.

The current results may explained that the leaf water extract of *D. innoxia* acts mainly through stomach action, but also seemed to induces slight contract effect which became more apparent with the uppermost concentration of filter paper application. Therefore, the highest effect resulted from spraying method compared to the other tested techniques could be an advantage of all possible modes of actions, though studies on mode of actions of such botanical extracts are still wanted. Hence, it can be concluded from this experiment that spraying of extract treatment on infested plants based on insect count, such as the economic threshold level, was expected to give better effect than prophylactic application prior to infestation. Bearing in mind the quick degradation of botanical extracts, such conclusion seemed to be rational. These aspects need to be confirmed both at laboratory and field levels.

Bioassay of different extracts

The results of the second experiment regarding screening of different leaf extracts of *D. innoxia* as insecticides against *A. pubescens* adults were presented in Table (2). After 48h (2days) post treatments, Malathion caused 100% mortality, whereas few *Datura* treatments showed significant results with the highest dose (8%) of hexane extract being the best (23.3±5.8% mortality). On the third day nearly all extracts treatments reflected prominent increases in mortality percents. The high doses (8% and 4%) of leaf hexane extract were significantly superior (53.3±5.8% and 36.7±20.8% mortalities, respectively). Afterward, the former treatment (8% hexane extract) attained a comparable significant effect (73.3±11.5%) with that of the Malation insecticide at the fifth day of the experiment commencement. The next lower dose (4%) of hexane extract was also the second in order without statistical difference from the highest dose. These two concentrations of leaf hexane extract kept the same top ranking at the seventh day. In addition to such superior mortality results, the second low rate (2%) of leaf hexane extract was noticed to manifest a hormonal effect on the pest appeared in deformed hind legs (Pl. 1).

The overall mortality results revealed that almost all concentrations of *D. innoxia* extracts had low knockdown effects 48h post treatments, but showed progressive increases in mortality thereafter. They seem to achieve their maximum activities at the fifth day, when comparable effect with the insecticide was attained as previously stated, after which they performed slight or no further effects. Moreover, the increases in performance of the different treatments were found to be relevant to concentrations. Comparing the three studied extracts, it is clear that water extract lied next to hexane extract treatments, while methanol extract was the least effective.

As with respect to water extract, it was observed that the mortality levels achieved by the different concentrations in this experiment were relatively lower than those reported for the spray method in the first experiment. For instance, the 8% extract concentration recorded (60.0±36.1%) and (46.7±37.9%) mortalities after 7days in the first and second experiments, respectively. Two reasons, mostly connected with the experimental timings, were suggested to contribute to such differences in results. It is a fact that the first experiment was conducted in July-August and the second one in September-October, coinciding with the onset and pre-termination periods of the insect active phase, respectively. Therefore, early generation insects after the resting phase were expected to be hungrier and ingest more food than those of the later generations, hence acquired higher quantity of the active compounds treated. The other justification could be attributed to the fact that the weak insects after resting may be more susceptible to the toxicant compounds than those of later generations which accomplished their full strength and viabilities, through storage of fats and other metabolic compounds, ready for the resting stage (Khalid, 2002; Mariod *et al*., 2007). The verification of these assumptions in coming research might be of great values to design strategies targeting the control of earlier generations with high competence.

However, the insecticidal activities of *D. innoxia* extracts could be attributed to certain active secondary metabolites in the leaves. *Datura innoxia* was found to be rich in saponins, flavonoids, alkaloids, phenols, sterols, triterpenes and tannins (Gilman, 1990; Ayuba *et al*., 2011; Sakthi *et al*., 2011). Although, the exact mechanisms underlying the actions of most secondary metabolites on insects are not fully understood, the deleterious insecticidal effects of different compounds were proved in literature. For instances, saponins were thought to have multiple insecticidal activities against various insects, such as feeding deterrence, moulting disturbance and increasing mortality levels (Chopra *et al*., 1986; Oliver-Bever, 1986; De Geyter *et al*., 2007; Chaieb, 2010), and phenolic compounds have negative effects on insects and can decrease fertility and shortening their life span (Zivanov-Curils *et al*., 2004; Golawska *et al*., 2008) whereas tannins in particular may cause oxidative stress and nutrients problems (Summers and Felton, 1994). Also, the growth regulatory effects of certain plant secondary metabolites including some triterpenoids were reported in different works (Schmutterer, 1985; Mordue (Luntz) and Nisbet 2000). Nevertheless, no literature was found considering the efficacy of *D. innoxia* on sorghum bug, but extracts of this plant were found to cause significant mortality and affecting oviposition and development of some other insect pests (Behera and Satapathy 1996; Maheshwari and Dwivedi, 1996; Gang *et al*., 2000; Lohra *et al*., 2002). Therefore, further studies are needed to ascertain the real biological activities of *D. innoxia*, particularly of hexane extract, in order to determine the LC_{50} for the intended active compounds after being isolated and characterized through advanced techniques. This may help to improve the extract solution through proper formulation to enhance its spraying and biocidal efficiencies.

CONCLUSION

The results obtained from the current preliminary studies showed manifold biological activities of *Datura innoxia* leaf extracts as toxicant and growth regulatory agents against the adult *A. pubescens*, when both host and pest were sprayed together under laboratory conditions. The specific active principles responsible for these effects are waiting for further investigations. However, hexane extract was the best portion that should be focused for the control of this pest. The present findings also suggested that early generations of sorghum bugs produced immediately after resting period might be more vulnerable to botanical treatments than later generations which built robust fattening bodies prepared for resting.

The answering of this question may open new door in the management strategy of *A. pubescens* in the future.

ACKNOWLEDGMENTS

The Sudan Academy of Science (SAS) is greatly acknowledged for the scholarship and the finance devoted to this study. Sincere thanks are also due to the staff members of the Environment and Natural Resources Program, Council of Biosciences, Advanced Technologies and Environment, SAS, for their kind assistance.

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