Pak. J. Agri. Sci., Vol. 58(X), xxx-xxx; 2021
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI: 10.21162/PAKJAS/21.740
http://www.pakjas.com.pk

BIOACTIVITIES OF *Azadirachta indica, Murraya koenigii, liquorice* AND *Nicotiana tobacum* AGAINST TWO STRAINS OF *Callosobruchus chinensis* L.

Shireen Raza Haidri[1,*], Farooq Ahmad[1], Mansoor-ul-Hasan[1] and Nazir Javed[2]

[1]Department of Entomology, University of Agriculture, Faisalabad, Pakistan; [2]Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan
[*]Corresponding author's e-mail: shireenraza2309@gmail.com

The current research work was conducted for the evaluation of repellent and growth inhibitory potential of *Azadirachta indica*, *Murraya koenigii*, *Nicotiana tobacum* and *Liquorice* against *Callosobruchus chinensis* (L.). Each plant extract was tested with four concentrations viz; 5, 10, 15 & 20 % for bioassay experiments. Repellent potentials of acetone extracts of the plants were evaluated by using the area preference method against the pulse beetle. The four dilutions of the extracted plant materials (extract) were used at one half of the filter papers (of each) while remaining halves were solely treated with acetone (used as control experimental unit) for the comparison purposes. Data regarding repellence were taken after 12, 24 and 48 hours of the post treatment. In repellency bioassay, the highest (93.32%) repellence of *Callosobruchus chinensis* was observed for which against 20% concentration of *A. indica* extract, whereas lowest repellence (78.87%) was recorded in case of *Liquorice*. Repellency values of 90.07% and 84.76% were recorded in extracts of *M. koenigii* and *N. tobacum* in Faisalabad strain. In case of Lahore strain highest repellence of *C. chinensis* was observed for which 95.16% at 20% concentration of *A. indica* extract was whereas relatively lowest value (82.04%) was recorded in case of *Liquorice*. Repellency values of 87.27% and 91.06% were recorded in extracts of *M. koenigii* and *N. tobacum*. The results regarding growth inhibitory effect revealed that highest mean progeny inhibition (78.12%) was noted at 20% dose rate of *A. indica* extracted plant material after longest exposure time (60 days). Comparatively lowest inhibition (28.21%) was noted after 30 days exposure period at 5% dilution of *Liquorice* extract in Faisalabad strain. Mean inhibition of progeny was found directly dependent on both time as well as concentration, applied. Results revealed that highest mean progeny inhibition (85.03%) was recorded at 20% concentration of *A. indica* extract after longest exposure time (60 days). Comparatively, lowest inhibition (35.18%) was recorded at 5% after 30 days of application of *Nicotiana tobacum* extract in Lahore strain. Hence, use of plant based materials can be helpful for the eco-friendly management of the stored grains insect pests as a part of IPM program.
Keywords: *Callosobruchus chinensis*, chickpea, green pesticides, deterrence, growth inhibition, time of exposure.

INTRODUCTION

The Pulse beetles (*Callosobruchus chinensis)* one of the most damaging insect pest, is also known as Dhora beetle. It is responsible for infesting stored pulses *(*Righi-Assia *et al*., 2010). As a cosmopolitan insect it is found all over the world (Verma and Anandi, 2010). In storage and field conditions *C. chinensis* cause both qualitative and quantitative losses to stored chickpea (Das *et al.*, 2005 and Naveena *et al*., 2009). It causes more than 10% damages to chickpea during storage in Punjab (Aslam *et al*., 2004) It is reported that up to 60 percent losses occurs in pulses 50-67 percent losses in seed weight by the attack of the bruchids (Gujar and Yadav, 1978). All over the world the synthetic insecticides (Organophosphates and pyrethroids) and fumigants (methyl bromide and phosphine) have been used for the control of insect pests of stored commodities. In storage conditions for the protection of the food commodities these synthetic insecticides are used as protectants (EPA, 2001). But it is reported that these insecticides contribute as a biggest source of pollution in the environment. Similarly, removal of the beneficial organisms from the environment and residual effects in harvested crops may found by the use of the synthetic insecticides (Anand *et al.,* 2008; Chinnaiah *et al.,* 1998). Resistance has been observed in numbers of stored grain insect pests by the application of the synthetic insecticides (Subramanyam and Hagstrum, 1995; Srivastava and Singh, 2002).

Plant extracts are organic substances derived from the different plant parts. In soil they are easily degradable and there is no deposition of the plant extracts in the tissues of the living organisms. Tropical farmers are well known by the application of extracted plant materials and applicate their crops against the insect pests (Araya and Emana, 2009). Botanicals are environmentally pollution free and had no harmful effects for human beings and other living things in comparison with the other pesticides (Isman, 2002, Debashri and Tamal, 2012). Degradation of plant based insecticides takes place within a few hours or days. Extracted plant materials are pest specific and are harmless for non-target organisms (Guleria and Tiku, 2009).

Among the natural plants, powders of the plants were used as protectants for the safety of the crops (Isman, 2002). Plant extracts caused repellency when the insects contact with these botanicals (Boeke *et al.,* 2004). A number of plants recognized for the insecticidal properties (Kamakshi *et al.,* 2000, Maheshwari and Dwivedi, 1996). Sharma (2013) conveyed the effectiveness of many extracted plant materials against *C. chinensis*. Most of the extracted plant materials had a number of characteristics like fast knock down, antifeedant, repellency, biodegradability and cause reduction in resistance of insects also (Reddy *et al.,* 2012).

Keeping in view, following were the objectives of the proposed study;

- To check the repellent effects of dissimilar concentrations of *A. indica*, *M. koenigii*, *N. tobacum* and Liquorice against two geographical strains of *Callosobruchus chinensis*.
- To evaluate the progeny inhibition potential of the dissimilar concentrations of the plant extracts against the two strains of *Callosobruchus chinensis*.

MATERIALS AND METHODS

Experiments were performed in Stored Grain Research, Training and Storage Management Cell, Department of Agricultural Entomology, University of Agriculture, Faisalabad in year 2018-19. Materials consists of *Azadirachta indica*, *Murraya koenigii*, *Liquorice* and *Nicotiana tobacum* and insects (*Callosobruchus chinensis*).

***Rearing of insects*:** From the grain market, mix aged adults of *C. chinensis* were collected from Lahore (LHR), and Faisalabad (FSD) and were reared in the laboratory at optimum conditions to get the homogenous population of *C. chinensis* up to six generations. To get the homogenous population of *C. chinensis* in the laboratory at optimum conditions, *C. chinensis* population was reared in sterilized glass jars. For the coupling and egg lying of test insect 100 adults of *C. chinensis* were released into the jars after adding 500 g grains of chickpea. The jars were tightened with rubber bands after covering with muslin cloth to avoid the escape of test insects. Optimum conditions 25± 2˚C and 60% R.H. were maintained for the rearing of the test insects. Adults were sieved after five days and the jars filled with grains along with eggs were kept under optimum conditions to get homogenous population.

***Preparation of plant extracts*:** Leaves of *Nicotiana tobacum* (tobacco), stem of *Liquorice* (mulathi), *Murraya koenigii* (curry patta) and *Azadirachta indica* (neem) were collected. After drying of plant materials to get the homogenous powder, the plant materials were grinded in electric grinder. About 50 gm of powder were mixed with 100 ml of acetone and then rotary shaker was used to shake the mixture for 24 hours. After that the extracts were filtered. For the removal of excess acetone in primary extract the rotary evaporator was used and after this the concentration served as mother liquor. Acetone was used to prepare altered concentrations i.e. 5, 10, 15 and 20%. For the preparation of 5% concentration of tested plant extracts; 5 ml of the extracted plant material was added in 95 ml concentrated acetone. In the same way 10, 15 and 20% concentrations were prepared by using acetone.

***Repellency bioassay*:** Filter papers were used to check the repellent actions of the tested plants against *C. chinensis* adults. In repellency bioassay the Area preference method was used to evaluate the potential of repellency. The filter papers were cut into two halves and the diameter of each filter paper was 9cm. One half of each filter paper was coated with four concentrations of each plant extract whilst the other half of the filter paper was coated with acetone serving as non-experimental unit (control) for the comparison purposes. Completely Randomized Design (CRD) was used to perform this experiment. Filter paper will be dried for few minutes. Then treated and untreated both the halves of filter papers were clipped together and counted beetles (fifty) were placed in clipped area (center) of each Petri dish. After this number of tested insects on filter paper (on both halves) were calculated after 24 hours intervals.

***Progeny inhibition studies*:** Counted sex pairs (Twenty five) of the test insect were placed in small plastic jars having plant extract treated diet (50 gm grains). Jars having grains treated with only acetone were used for comparison (as a control). Released target insects were discarded from the rearing jars after 7 days and insect population buildup findings were computed after the exposure period of 30 and 60 days. Then percent inhibition rates were computed using by Abbott's formula (1925):

$$(\text{Corrected growth inhibition }\%) = \frac{\mathrm{T} - \mathrm{C}}{100 - \mathrm{C}} \times 100$$

Here, C = presents the progeny in control units; T = denotes the numbers (progeny) in treated jars

***Data analysis*:** The collected data were analyzed Analysis of Variance using Statistical software (Stat Soft 8.0) and the comparison of treatment means was done by using Tukey's HSD test at α= 5 percent.

In case of FSD strain of the *Callosobruchus chinensis A. indica* and *M. koenigii* caused highest repellence (93.32 and 90.07 %) at (20%) dilution after longest time of exposure (48 hrs). In case of *N. tobacum* extract, maximum repellency (84.76%) was observed at the peak dilution (20%) after longest time of exposure (48 hrs). In case of *Liquorice*, maximum repellency (78.87%) was noticed at 20% dilution of the extracted plant material used after 48 hrs (longest exposure time) of the post treatment whilst comparatively lowest mean repellency (48.89%) was noticed at lowest concentration (5%) the treatment after post treatment period of 12 hrs (shortest exposure period of the treatment, applied). In repellency, 95.16% value against Lahore strain of *C.*

chinensis was observed in case of *Azadirachta indica* followed by *N. tobacum* 91.06 %. *M. koenigii* produced 87.27 % repellency of the *C. chinensis* whereas lowest repellency 82.04% was exhibited by *Liquorice*.

Table 4 and 5 shows the progeny inhibition % of Faisalabad and Lahore straThe bioassay (progeny inhibition) revealed that in case of *A. indica* highest mean ins of *Callosobruchus chinensis* L. caused by various dilutions of *Murraya koenigii*, *Azadirachta indica*, *Nicotiana tobacum* and *Liquorice* after 30 and 60 days respectively. Results shows that in case of *A.indica* highest mean inhibition (78.12%) were observed at the highest dose rate (20%) of plant extract used after longest post treatment period (60 days). Relatively lowest inhibition (34.57%) was noticed at 5% after 30 hrs of time period. Similarly, within the exposure period of 30 and 60 days at 20 percent concentration inhibition values were 60.89 and 78.12 respectively. In case of *Liquorice* highest mean inhibition (61.26%) was observed at the peak dose rate (20%) of the treatment (extract) used after longest post treatment period (60 days). While comparatively minimum inhibition (28.21%) was observed at 5% after 30 hrs of time period in *Liquorice*. Similarly, within the 30 and 60 days of the exposure time at 20 percent concentration inhibition values were 50.35 and 61.26 % respectively. In repellency, 95.16% value against Lahore strain of *C. chinensis* was observed at 20 % concentration in case of *A. indica* whereas lowest 82.04% was by *Liquorice*. In case of progeny inhibition of Lahore strain, highest 85.03% was recorded at 20 % concentration of the *A. indica* while relatively lowest 67.41% was recorded at 20 % concentration of the *N. tobacum*.

DISCUSSION

Findings of the repellency bioassay revealed that repellency ranged from 60-93% during the bioassay with the application of *A. indica* extract. Mean repellency was found increased with rise in the extract concentration and exposure period and vice versa. Highest mean repellency (93.32%) was observed at (20%) peak dilution of the applied treatment (extracted plant material) used after longest time of exposure (48 hrs). However, relatively low repellency (60.87%) was examined at lowest concentration 5% after 12 hrs of the treatment (extract), applied. Repellency ranged from 55-90% during the bioassay with extract of *M. koenigii*. Results showed that maximum repellency (90.07%) was observed at 20% dilution of the extracted plant material (*M. koenigii*) used after longest time of exposure (48 hrs). While relatively low value (55.53%) of repellency was noticed at lowest concentration (5%) of the extract. Maximum repellency (84.76%) was noted after the longest exposure time (48 hrs) at the peak dilution (20%) of the extracted plant material (*N. tobacum*). Comparatively lowest repellency (52.21%) was noted after 12 hrs of exposure period at 5%. In case of *Liquorice*, maximum repellency (78.87%) was noticed at 20% dilution of extracted

Table 1. Repellence (%) of Faisalabad and Lahore Strains of *C. chinensis* L. caused by various dilutions of *Azadirachta indica, Liquorice, Murraya koenigii* and *Nicotiana tobacum* after 12 hours exposure period.

Time (hours)	Concentra-tions (%)	Faisalabad Strain				Lahore Strain			
		A. indica	*M. koenigii*	*N. tobacum*	*Liquorice*	*A. Indica*	*M. koenigii*	*N. tobacum*	*Liquorice*
12	5	60.87±2.54g	55.53±1.11i	52.21±1.11i	48.89±1.11g	62.12±1.54i	57.04±1.31g	60.32±1.43i	50.67±1.33g
12	10	70.01±1.92f	65.56±1.11h	60.03±1.92gh	55.54±1.92f	65.43±1.75h	66.17±1.33fg	68.16±1.63h	56.00±1.33fg
12	15	75.56±1.11de	73.51±2.44e	62.24±2.23gh	58.86±1.92e	76.31±1.33efg	71.00±1.33ef	74.7±1.33gh	57.67±1.33ef
12	20	85.54±1.92b	77.79±1.32d	67.75±2.54ef	63.31±1.11d	82.17±1.87def	74.24±1.33bc	76.02±1.53ef	68.27±1.33b

Table 2. Repellence (%) of Faisalabad and Lahore Strains of *C. chinensis* L. caused by various dilutions of *Azadirachta indica, Liquorice, Murraya koenigii* and *Nicotiana tobacum* after 24 hours exposure period.

Time (hours)	Concentra-tion (%)	Faisalabad Strain				Lahore Strain			
		A. indica	*M. koenigii*	*N. tobacum*	*Liquorice*	*A. indica*	*M. koenigii*	*N. tobacum*	*Liquorice*
24	5	74.43±1.11def	68.89±1.11fg	65.53±2.87g	54.43±1.11f	76.60±1.28efg	67.33±1.33ef	69.29±1.43fgh	58.26±1.18ef
24	10	76.67±1.92de	70.02±1.92f	69.54±1.92ef	63.32±1.92d	83.67±1.33cde	71.16±1.63de	74.41±2.62efg	61.20±1.33de
24	15	82.23±1.11cd	74.42±1.11e	71.12±1.11e	67.76±1.34cd	87.64±1.33cd	73.00±1.33cd	77.04±1.33de	64.14±1.33cd
24	20	86.56±1.24bc	84.45±1.92c	76.67±1.11d	68.87±2.56cd	91.07±1.13ab	80.10±2.27ab	83.37±2.87cd	69.30±1.87b

Table 3. Repellence (%) of Faisalabad and Lahore Strains of *C. chinensis* L. caused by various dilutions of *Azadirachta indica, Liquorice, Murraya koenigii* and *Nicotiana tobacum* after 48 hours exposure period.

Time (hours)	Concentra-tions (%)	Faisalabad Strain				Lahore Strain			
		A. indica	*M. koenigii*	*N. tobacum*	*Liquorice*	*A. Indica*	*M. koenigii*	*N. tobacum*	*Liquorice*
48	5	80.02±1.52cde	78.89±1.57d	77.78±1.11d	58.88±1.12e	84.00±1.33cde	76.03±2.08cd	84.17±1.58cd	63.14±1.23cd
48	10	84.43±1.11cd	82.23±1.11c	81.12±2.93bc	70.03±2.11c	86.04±1.33cd	78.43±1.83ab	89.32±1.33bc	65.21±1.13bc
48	15	88.87±1.11b	87.76±1.27ab	83.32±1.92ab	75.56±1.92ab	92.19±1.43ab	82.18±1.33ab	90.89±2.16ab	70.08±1.33b
48	20	93.32±1.92a	90.07±1.11a	84.76±1.92a	78.87±1.11a	95.16±1.54a	87.27±2.33a	91.06±1.53a	82.04±2.03a

Table 4. Progeny inhibition % of Faisalabad and Lahore Strains of *C. chinensis* L. caused by various dilutions of *Murraya koenigii*, *Azadirachta indica*, *Liquorice* and *Nicotiana tobacum* after 30 days post treatment development.

Days	Conentra-tions (%)	Faisalabad Strain				Lahore Strain			
		A. Indica	*M. koenigii*	*N. tobacum*	*Liquorice*	*A. Indica*	*M. oenigii*	*N. tobacum*	*Liquorice*
30	5	34.57±2.54f	30.13±1.11f	29.41±1.11f	28.21±1.11g	46.10±0.77f	41.28±0.57f	35.18±0.77g	36.29±0.47g
30	10	51.26±1.11d	42.10±1.92e	40.36±1.11e	37.03±1.92f	53.21±0.67e	46.15±0.77e	37.41±0.91f	39.12±0.57f
30	15	54.46±1.92e	49.62±2.44d	46.36±1.92d	44.14±2.23e	64.35±0.76d	59.05±0.47c	43.78±0.87d	48.08±0.77e
30	20	60.89±1.92c	58.39±1.32c	55.21±2.54b	50.35±1.11c	72.17±2.13b	64.49±0.87b	54.13±0.94c	60.42±1.07c

Table 5. Progeny inhibition % of Faisalabad and Lahore Strains of *C. chinensis* L. caused by various dilutions of *Murraya koenigii*, *Azadirachta indica*, *Liquorice* and *Nicotiana tobacum* after 60 days post treatment development.

Faisalabad Strain						Lahore Strain			
Days	Concentra-tions (%)	*A. Indica*	*M. koenigii*	*N. tobacum*	*Liquorice*	*A. Indica*	*M. koenigii*	*N. tobacum*	*Liquorice*
60	5	56.17±1.57d	51.08±1.52d	47.28±1.11d	41.48±1.12e	65.14±0.27d	55.04±0.47d	40.26±0.37e	45.19±0.55d
60	10	62.79±1.11c	58.43±1.11c	52.12±1.92c	48.13±2.11d	68.01±0.31c	63.77±0.37c	46.13±0.47d	61.01±0.55c
60	15	72.51±1.11b	67.16±1.27b	64.32±2.93a	58.87±1.11b	74.54±0.98b	69.24±1.07b	58.02±1.03b	64.30±0.34b
60	20	78.12±1.92a	71.17±1.11a	66.56±1.92a	61.26±1.92a	85.03±0.97a	78.31±1.37a	67.41±1.37a	71.26±1.45a

plant material used after 48 hrs (longest exposure time) of the post treatment. Comparatively lowest repellency (48.89%) was noted after the time of exposure of 12hrs at 5% of the concentration of the extract. Our repellency findings are also close to Muntaha *et al*., 2017 who used extract of *A. indica* and recorded up to 90 %. Findings of our study are close with Rehman *et al*. (2018). Results are in accordance with Sharma *et al*. (2003) who evaluated repellency results.

Studies on post treatment progeny inhibitory effect of the plant extracts revealed that in case of *A. indica* highest mean inhibition (78.12%) was observed at the peak dose rate (20%) of the treatment (extract) used after longest post treatment period (60 days). Relatively lowest inhibition (34.57%) was noticed at 5% after 30 hrs of time period. Similarly, within the exposure time of 30 and 60 days at 20 percent concentration inhibition values were 60.89 and 78.12 respectively. In case of *Liquorice* highest mean inhibition (61.26%) was observed at the peak dose rate (20%) of the treatment (extract) used after longest post treatment period (60 days).While comparatively minimum inhibition (28.21%) was observed at 5% after 30 hrs of time period in *Liquorice*. Similarly, at 20 percent concentration inhibition values were 50.35 and 61.26 % within the exposure time of 30 and 60 days, respectively. Our findings are close to Sultana *et al*. (2012) who evaluated the growth inhibition effects of three edible oils against *Callosobruchus chinensis*. Results of our study are also close to Sagheer *et al*. (2011) who evaluated the inhibitory effects of some plant extracts against *Tribolium castaneum*. Slight difference may be due to difference in two insect species. In repellency, 95.16% value against Lahore strain of *C. chinensis* was observed at 20 % concentration in case of *A. indica* whereas lowest 82.04% was by *Liquorice*. In case of progeny inhibition of Lahore strain, highest 85.03% was recorded at 20 % concentration of the *A. indica* while relatively lowest 67.41% was recorded at 20 % concentration of the *N. tobacum*. Our outcomes progeny inhibitions are close to Muntaha *et al*., 2017 who used *A. indica* against *C. chinensis*.

***Conclusion*:** Keeping in view the results of current work, it is concluded that all the concentrations of plant extracts have repellent effects against *C. chinensis. A. indica* and exhibited highest mean repellency at high concentration (20%) of the applied treatment used after (48 hrs) in both strains. *N. tobacum*, *M. koenigii* also showed highest repellency whereas lowest repellency was exhibited by *Liquorice*. Therefore, plant based materials can be useful for the eco-friendly controlling of the stored insect pests.

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