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Evaluation of Neem (*Azadirachta indica*) Extracts Against the Eggs and Adults of *Dysdercus cingulatus* (Fabricius)

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Abstract: *Azadirachta indica* (neem) belongs to the family Meliaceae and is an indigenous tree of India. In the present study, the toxic efficacy of neem leaves, neem green seed coat, neem yellow seed coat and neem seed kernel were tested against *Dysdercus cingulatus* (Fabricius) eggs and adults at different concentrations. The concentrations used were 0.005%, 0.01%, 0.025%, 0.05%, 0.1%, 0.25%, 0.5% and 1.0% (v/v) respectively. The adult insects were allowed to feed upon the cotton seeds previously soaked in the respective extracts of the neem. It was observed that *D. cingulatus* (Fab.) adults showed highest mortality (75.00%) at 1.0% concentration of the neem seed kernel, whereas, the least mortality was (5.00%) recorded with yellow neem seed coat at 0.005%. It was further observed that when the eggs of *D. cingulatus* (Fab.) was treated with same neem extracts an increased percent mortality (12.25%) was noticed at the concentration of 0.005% of neem green seed coat, indicating least efficacy of this natural product. The least survival of egg (5.00) was observed at 0.005% neem seed kernel after 72 hours.

Key words: *Azadirachta indica* (neem) · Cotton pest · *Dysdercus cingulatus* (Fabricius) · Neem Seed Kernel

INTRODUCTION

Dysdeercus cingulatus (Fab.), commonly known as red cotton bug is an important pest of cotton, lady's finger, samphal and hollyhock. Due to hazards associated with the increased use of synthetic pesticides the use of phyto-toxins (pesticides) has gained considerable attention on the eco-friendly approaches of insect management. Thus, the exploitation of pesticides of plant-origin in agriculture has reduced the risk factor appreciably not only for the public health and human wellbeing but also for the environmental pollution.

As a whole, Indian neem, *Azadirachta indica* is an environmentally safe biopesticide and act as insect repellent and feeding inhibitor. The main active ingredient of neem is azadirachin. In addition to this the antifeedant effects of azadirachtin and growth regulatory properties, have also been extensively studied [1]. In fact, the efficacy of neem based products is determined on the basis of amount of azadirachtin and the type of formulation [2]. The extracts from Indian neem or its most active compound, the limonoid azadirachtin (AZA), have been extensively investigated in recent years, with demostrated activity reported against more than 200 species of insects [3, 4].

In present investigation, in order to enhance the cotton and vegetable production, the toxic effects of alcoholic extracts of various parts of *Azadirachta indica* viz. leaves, green seed coat, yellow seed coat and seed kernel were evaluated against eggs and adult of *Dysdercus cingulatus.*

MATERIALS AND METHODS

Maintenance of the Stock Culture of *Dysdercus cingulatus* (Fabricius): Adults and nymphs of *Dysdercus cingulatus* (Fabricius) were collected from the Jawahar Park and Agricultural Farm of Aligarh Muslim University, Aligarh. The culture of this pest was maintained in the insectory under controlled conditions, at 28±2°C and 70-80 % relative humidity. They were maintained in glass rearing jars measuring approximately, 20 x 15 cm, containing a layer of moist and coarse sand (4 cm thick), which was previously sterilize in autoclave. The mouth of these jars was covered with a piece of muslin cloth fixed with rubber band. All the stages were fed on the fresh healthy soaked cotton seeds and over-crowding was avoided.

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Preparation of Plant Extracts: The neem leaves, neem seed kernel and neem seed coat were collected from the university premises during spring seasons. The identification of these collected plants was confirmed by Former Professor Wajahat Ali Khan, Plant Taxonomist of Department of Botany, Aligarh Muslim University, Aligarh.

Preparation of Alcoholic Extract: *Azadirachta indica* leaves, seed kernel and seed coat were washed thoroughly with distilled water, dried at room temperature necessary for the preparation of fine grinding. The crystals thus obtained were considered technically 100% pure. From this pure material 2% stock solution was prepared as per recommendation of Pearson's square method, using double distilled water with the help of magnetic stirrer in order to dissolve the material completely and stock solution was refrigerated until needed.

Test of Neem Extracts Toxicity Against the Test Insects: In view of the specificity of insecticidal action, the neem extracts were used against adults of *D. cingulatus*. The cotton seeds were soaked into solutions containing different concentration of neem extracts, separately. The concentrations were prepared in fresh and sterilized double distilled water. From the stock solution of neem extracts further dilutions *viz.*, 0.005, 0.01, 0.025, 0.05, 0.1, 0.25, 0.5 and 1.0 were prepared. After treatment, insects were removed carefully with the help of brush. The mortality was noted after 24 hours. All experiments were repeated thrice and there were 50 insects in each repeat.

Test of Neem Extract Toxicity Against Eggs of the Test Insect: Bioassay studies were carried out on egg survival under laboratory conditions at 28±2°C temperature and 70-80% relative humidity using glass Petri-dishes (150 x 15 mm). Clean, healthy, 0-6 hour old eggs of *D. cingulatus* were collected from the culture. The neem extracts were applied at 0.005% concentration on 25 eggs in each set. In each experiment, 25 eggs with different concentrations of neem extracts were arranged equidistantly in the glass Petri-dish, the base of which was covered with a Whatman No.1 filter paper. We applied neem extracts on eggs and seen its effects for 72 hours. Parallel control set was maintained where the eggs were heated with solvent only (DDW).

Statistical Analyses: The following statistical procedures were used during the course of present investigation:

Arithmetic Mean (AM) and Standard Deviation (SD): The Arithmetic Mean and Standard Deviation were calculated for each neem extracts, concentration as well as experimental pest used.

$$A.M.=\frac{\Sigma fx}{f}$$

Where:
'x' number of insect and 'f' corresponding frequency and Σ f is sum of all frequencies.

$$S.D.=\sqrt{\frac{\sum x^2-(\sum x)^2}{N-1}}$$

Where:
'x' is percent mortality and 'N' is number of observations.

Chi-sequire (χ^2) Analysis: χ^2 for homogeneity / heterogeneity was applied to verify the significant difference b/w samples.

$$\chi^2=\sum\left[\frac{(fo-fe)^2}{fe}\right]$$

Where:
(f_o) and (f_e) are the observed and expected frequencies respectively.

Coefficient of Correlation (r): Coefficient of correlation was used and calculated as

$$r=\frac{\sum xY-\frac{(\sum x)(\sum Y)}{N}}{\sqrt{\left(\sum x^2\frac{(\sum x)^2}{N}\right)\left(\sum Y^2\frac{(\sum Y)^2}{N}\right)}}$$

Where:
'x' is concentration applied and 'y' is the percent mortality noted after the respective treatment.

Linear Regression Equation: The coefficient of linear regression, □ (slope) of Y on x is calculated as follows-

$$\frac{\Delta y}{\Delta x}=\frac{Change\ in\ the\ value\ of\ x}{Change\ in\ the\ value\ of\ y}$$

Y intercept is calculated as,

$$\bar{y}=a+bx$$

This equation has been obtained to study the effect in terms of transformed mortality as a linear regression of different neem extracts at different concentrations, respectively.

Lethal Concentrations: LC_{50} values were calculated from the transformed mortality concentration graph.

Relative Ratio (RR)/ Toxicity Ratio: Relative ratio for neem extracts for the LC_{50} values was calculated by taking LC_{50} as unity and dividing it by the respective LC_{50} of the same vertical column.

RESULTS AND DISCUSSION

Toxicity of plant extracts was evaluated against the *Dysdercus cingulatus* (Fab.) to suggest a safe method for their control. The adults of the pest were reared and subjected to the toxicity of four neem extracts i.e neem leaves, green neem seed coat, yellow neem seed coat and neem seed kernel. The concentrations used were 0.005%, 0.01%, 0.025%, 0.5%, 0.1% 0.25%, o.5% and 1.0% of each neem extract (Table 1).

Relative Toxicity of Neem Extracts

Toxicity: The observations were made on the comparative response of the highest mortality (75.0%) was observed when the insects were allowed to feed on cotton seeds treated with 1.0% concentration of neem seed kernel whereas the least mortality (5.0%) was observed by 0.005% concentration of yellow neem seed coat (Table 1). The toxicity response of leaves, green neem seed coat, yellow neem seed coat and neem seed kernel was found to be significant at 5% level as obtained from chi-square (χ^2) test (Table 2). The slope (β) values were highest (95.704) for leaves and lowest (44.657) for yellow neem seed coat (Table 2).

Lethal Concentrations: The LC_{50} values were found to be highest (0.475) for green neem seed coat and yellow neem seed coat, however, a lowest value (0.462) was observed for neem leaves and neem seed kernel (Table 2).

Coefficient of Correlation: A positive correlation (r = 0.94143) was noted for yellow neem seed coat and least value (r = 0.8097) was obtained for neem leaves in case of *D. cingulatus* adults (Table 2).

Relative Ratio: The statistical analysis shows that the leaves and neem seed kernel are most effective (relative ratio = 1.0541) (Table 2) and green neem seed coat and yellow neem seed coat are least effective (Relative ratio = 1.0252) at LC_{50} (Table 2). The order of toxicity was noted as leaves and neem seed kernel (1.0541) > yellow neem seed coat (1.0252) = green Neem seed coat (1.0252) (Table 2).

Application of Neem Extracts on the Eggs of *Dysdercus Cingulatus* (Fabricius): The observations were made on the comparative response of the neem extracts on the eggs of *Dysdercus cingulatus* (Fabricius). The application of neem extracts on the eggs showed that highest survival (12.25%) at 0.005 concentration of green neem seed coat. The least survival of egg (5.00) was observed at 0.005% neem seed kernel extract after one week (Table 3).

Table 1: Toxic efficacy of neem (*Azadirachta indica*) extract on *Dysdercus cingulatus* (Fabricius) adults

	Percent Mortality of Various Concentration							
Name of neem extracts	0.005%	0.01%	0.025%	0.05%	0.1%	0.25%	0.05%	1.0%
Azadirachta indica (Leaves)	11.00±0.829	20.00±2.915	29.00±2.772	43.00±2.947	50.00±4.500	57.00±4.493	63.00±4.918	73.00±4.205
Azadirachta indica (Green Neem seed coat)	6.00±0.500	10.00±0.500	15.00±0.829	16.00±0.707	29.00±3.961	31.00±2.165	49.00±4.815	53.00±2.947
Azadirachta indica (Yellow Neem Seed coat)	5.00±0.433	8.00±0.707	12.00±0.707	17.00±0.829	25.00±2.165	28.00±2.236	46.00±6.184	51.00±6.796
Azadirachta indica (Neem Seed Kernel)	12.00±0.707	18.00±0.500	40.00±0.707	44.00±0.707	49.00±0.829	53.00±1.299	60.00±1.414	75.00±3.766

Table 2: Toxic Efficacy of neem (*Azadirachta indica*) extracts against *Dysdercus cingulatus* (Fabricius) adults

Name of Neem Extracts	No. of Insects (N)	Chi-square (□2)	Coefficient of correlation (r)	Regression equation	Slope (□)	Intercept	LC_{50}	Relative Ratio (RR)
Azadirachta indica (Leaves)	100	3.52*	0.8097	y= 50.24x+31.06	95.704	35.717	0.462	1.0541
Azadirachta indica (Green Neem seed coat)	100	4.68*	0.9002	y= 45.33x+15.13	67.475	22.455	0.475	1.0252
Azadirachta indica (Yellow Neem Seed coat)	100	5.23*	0.9143	y= 44.65x+13.17	44.657	13.171	0.475	1.0252
Azadirachta indica (Neem Seed Kernel)	100	9.51*	0.8134	y= 48.48x+32.11	48.489	32.116	0.462	1.0541

□^{2} significant values: df = 7

* Significant at 5% level

Table 3: Toxic efficacy of neem (*Azadirachta indica*) extracts on eggs of *Dysdercus cingulatus* (Fabricius)

Name of Neem extracts	Survival of Eggs (25 individuals in each Replicate) After 1 Week old (egg)
Azadirachta indica (Leaves)	7.50 (±1.50)
Azadirachta indica (Green Neem seed coat)	12.25 (±0.829)
Azadirachta indica (Yellow Neem Seed coat)	7.50 (±2.692)
Azadirachta indica (Neem Seed Kernel)	5.00 (±2.915)

In another experiment, effect of RD-9repelin on growth and development of *D. Koenigii* showed that neem based products have been found effective against a wide range of pests of import ant crops [5-9]. Azadirachtin is found to be the most potent neem fraction that adversely effects the growth and development of different insects in specific manner by its multifarious actions like repellent, morphogenetic variation, oviposititon inhibition and sterilent [10, 11]. The data presented in (Table 2), shows that the extracts of leaves, green neem seed coat, yellow neem seed coat and neem seed Kernel (NSK) showed significant lethal effects on the *D. cingulatus* (Fab.). The 1.0% concentration of neem seed kernel extract resulted in 75% mortality of *D. cingulatus* (Fab.). Azadirachtin also acts as a powerful inset antifeedant and repellent [12, 13]. However, ether and ethanol extracts of various parts of neem against red cotton bug, *Dysdercus Koenigii* under the laboratory conditions [7]. Ethanol was found as better solvent for extracting more amount of toxic component from leaves and epicarp, while ether was effective for extraction from fruits and epicarp. It was maximum at 1.5 percent of ether extract of seed kernel followed by same dosage of epicarp and leaves,extract after 72 hrs of treatment. However, in this study when the eggs of *D. cingulatus* (Fab.) were treated with various neem extracts, the highest sunrvival (12.25) was observed at 0.005% concentration of green neem seed coat (Table 3).

Some parts of neem plant showed relatively more toxicity than other parts. The present data shows that green neem seed coat found to be the least toxic against *D. cingulatus* (R.R = 1.0252) as compared to other neem products namely neem leaves (R.R. = 1.0541) and neem seed kernel (R.R. = 1.0541) (Table 2). On the other hands, Azadirachtin and other neem pesticides (Azadirachtin-iodine, neem seed kernel extract (NSKE and oil) also most effective on the survival and growth red cotton bug, *D. Koenigii* (Fab.) [8]. However, complete mortality of larval instars of *spodoptera littoralis* was recorded at 0.2-0.5% of neem exteract [14].

Undoubtedly neem extracts, as toxicants are invaluable sources of potential insecticides and enhance the protection against *Dysdercus cingulatus* (Fabricius). Plant products are therefore, being considered in agro-ecosystems and are implemented as pest management tool.

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