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OVICIDAL, NYMPHICIDAL AND JUVENOMIMETIC EFFECT OF *ADHATODA VASICA* EXTRACT ON THE RED COTTON STAINER, *DYSDERCUS CINGULATUS* (FAB.) (HETEROPTERA: PYRRHOCORIDAE)

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ABSTRACT

With an ever increasing population the need to produce more yield in limited area has become indispensable. Synthetic pesticides use to suppress pest population have caused the development of resistance in pests over the years and they have also been proved to cause health hazards. In such a scenario, botanical insecticides have helped control rampant damage to plants caused by pests. *D. cingulatus*, also called red cotton stainer is a major pest of cotton in many parts of India. The extract of *A. vasica* showed promising ovicidal, nymphicidal and juvenomimetic activity against this pest.

KEYWORDS: *Adhatoda vasica,* Cotton, *Dysdercus cingulatus,* Juvenomimetic Activity, Nymphicidal, Ovicidal

INTRODUCTION

Cotton is one of the major crops in India plagued with insect infestation. India is the second largest producer of cotton in the world after China accounting for about 18% of the world cotton production. It has the distinction of having the largest area under cotton cultivation in the world ranging between 12.2 million hectares and constituting about 25% of the world area under cotton cultivation. The yield per hectare is however, the lowest against the world average. The Cotton Association of India (Ramkumar, 2013) (CAI) has declared the 2013-14 seasons' cotton production to be 37.5 million bales (170 kg each). Cotton is damaged by over 160 insect species from the seeding stage throughout its growth period including the red cotton stainer, *Dysdercus cingulatus* (Fab.) (Heteroptera: Pyrrhocoridae), which causes serious damage by feeding on developing cotton bolls and ripe cotton seeds and by transmitting fungi that develop on the immature lint and seeds (Yasuda, 1992; Natarajan and Rajendran, 2005). Heavy infestations on the seeds affect the crop mass, oil content and the marketability of the crop (Sontakke *et al.,* 2013). In order to curb the pest population many synthetic pesticides have been employed.

Over the years, the use of conventional insecticides has raised concerns about the threat to the environment (Huang *et al.,* 1998). Many pesticides that are used in agro ecosystems target insect pests along with beneficial and natural enemies. This disrupts the ecological cycle which results in backlashes causing resistance, resurgence and replacement. Most pesticides which are currently being used also pose serious health hazards. Thus it is indispensable that we use natural predators or plant based compounds to control them.

Insect control using plant material is an ancient practice all over the world. As synthetic or petroleum based insecticides pose serious health hazards for mammalia, efforts have been devoted to search for new classes of insecticides derived from plants with lower mammalian toxicity and a lesser persistence in the environment, such as pyrethrinoids or

rotenone compounds. However, increased insecSt resistance to pesticides has led to the finding of new molecules from botanicals as alternative pest control agents, a well established approach in control strategies for pests (Roger *et al.,* 1993).

Botanical insecticides are one of the best alternatives for the harmful chemical pesticides. Phytochemicals are able to induce different types of abnormalities in insects which could safely be used in pest control (Sreelatha *et al.,* 2011). In research carried out around the world to assess the insecticidal properties in plants, it has been realized that disruption of growth and reproduction are the main aspects of pest control rather than antifeedants and repellents (Mordue and Nisbet, 2000).

Adhatoda vasica belonging to the family Acanthaceae, a popular Indian medicinal plant has long been used commonly in the Ayurvedic system of medicine. It is an evergreen, gregarious perennial shrub, 1-2.5 meter in height having opposite ascending branches (Baquar *et al.,* 1989). It is mostly recommended for a variety of ailments such as bronchitis, asthma, fever, jaundice, and leprosy (Seema *et al.,* 2010). It is also known for its antibacterial, anticholinesterase, wound healing, hypoglycaemic, abortifacient/Oxytocic, antitussive, digestive, cardioprotective, anti-inflammatory, hepatoprotective, antiulcer, antimutagenic, radiomodulation, antitubercular properties (Ahmad *et al.,* 2009). Adding to these properties, it also exhibits insect repellent activity which has been made use of over centuries (Kokate *et al.,* 1985). It is vernacularly known as Malabar nut, Adulsa, Arusha, Vasaka, Justicia adhatoda, Adhatodai, Bakash, Adhatoda, Adalodakam, Adusoge, Addasaramu, Lion's muzzle and Stallion's tooth. It is known as Adhatoda in tamil and is a small evergreen and sub-herbaceous bush which is commonly found in India, Sri Lanka, Myanmar and Malaysia (Sreelatha *et al.,* 2011).

OBJECTIVES OF THE STUDY

To study ovicidal, nymphicidal, juvenomimetic effect and repellent activity of *Adhatoda vasica* extract on the red cotton stainer, *Dysdercus cingulatus*

METHODS

Preparation of Extract

Fresh, mature leaves of *A. vasica* were collected from Vadaloor (11.7431° N, 79.7656° E) and Madurai (9.9197° N, 78.1194° E) in Tamil Nadu. The leaves were washed thoroughly twice with tap water and once with sterile distilled water to remove dust particles and were dried in the shade for two weeks. The dried leaves were finely powdered using a domestic blender to about 40-60 mesh size. The extract of *A. vasica* was prepared on per cent basis as per the method of Gahukar (1996) and Sharma *et al.* (1997) for which a stock solution of 20% concentration was prepared by dissolving 20 gram of each plant material in 80 ml of water and used at different concentrations. The powdered plant material was loaded into soxhlet apparatus for extraction with the solvents chloroform and methanol. The crude extract was concentrated in the vacuum rotary evaporator and stored in the refrigerator for later use.

Rearing of Insects

Nymphs and adults of *Dysdercus cingulatus* were collected from the Adyar Theosophical Society, Adyar, Chennai, Tamil Nadu. The collected insects were maintained in transparent, plastic containers with a layer of sterile coarse sand (3 cm) under laboratory conditions. They were maintained at a temperature regime of 28±2ºC and a light: dark cycle of 13L:11D was used. *D. cingulatus* was fed with water soaked cotton seeds and fresh cotton saplings. The pests were maintained for 2 generations and laboratory emerged 6-24 hour old third instar larvae were used for the experiments.

Ovicidal Effect

30 freshly laid eggs were treated 5 times with varying percentages of the extract (0.5, 1.0, 1.5, 2.0%). 30 control eggs were treated with the same amount of methanol. The egg mortality was calculated depending on the number of successful hatch outs.

Nymphicidal Effect

Bioassay studies were carried out using uniform sized 6-24 hrs old third stadium *D. Cingulatus* (Figure 4) which were selected randomly from the stock culture. Ten insects were placed in a transparent plastic container with a perforated lid. Four concentrations (0.5%, 0.1%, 1.5% and 2.0%) were prepared with 1 mL of the *A. vasica* extract, which was then diluted with 10 mL water for use in the experiment. 100 g of cotton seeds were taken separately in four conical flasks and 250 mL of plant extract was added. The flask was agitated at 65 rpm in a shaker for 12 hours at room temperature and the soaked seeds were fed to *D. cingulatus.* 24 hours starved third instar *D. cingulatus* were allowed to feed the treated cotton seeds. Ten replications were maintained for each concentration. , cotton seeds were replaced every day by new plant extracts of seeds soaked for 12 hours. Mortality was recorded every 24 hrs, till 96 hrs.

Juvenomimetic Effect

Newly emerged adult females were separated and 2 µl of the extract at four concentrations (0.5%, 1.0%, 1.5% and 2%) was applied on the abdominal tergites using a Hamilton micropipette and released into petri dishes (Figure 2). The females thus treated were allowed to mate with normal males. Untreated males of the same age in 1: 1 ratio were introduced into the same petri dishes for observing mating behavior. The oviposition rate of the egg laying (fecundity) and percentage of hatching (fertility) were observed.

Repellent Activity

10 adult insects were released into each petridish that contained a whatman's filter paper one half of which was treated with 0.5 µl extract of each concentration (0.5, 1.0, 1.5, 2.0 %). The behavior of the insects was observed.

RESULTS AND DISCUSSIONS

The present work revealed the effect of *A. vasica* extract against *D. cingulatus*. In general, survival of the treated eggs and third instars of the pest upon exposure to the plant extract was found to decrease drastically as compared to the corresponding controls i.e, dose dependant mortality was observed. When *D. cingulatus* eggs were treated with *A. vasica* extract, the survival rate gradually decreased from lower concentration to higher concentration (Table 1). This might be due to the degrees of morphogenetic malformation in the recipient pest embryo and is found to be dose dependant (Sahayaraj and Kalidas, 2011). With respect to the nymphicidal activity of the extract on the third instars of *D. cingulatus*, a higher concentration was seen to bring about a higher rate of mortality (Table 2).

When the extract was applied topically on the abdomen of the female and released into petridishes, no abnormality in the mating process was observed. But the number of eggs laid by the females was much less in comparison with the control insects. The eggs thus laid took longer to hatch by an average of 4.83 ± 2.26 days. Egg development proceeded at normal rates for four days after which some of them turned pale brown and eventually black (Figure 1). The eggs that showed a change in color failed to hatch. This clearly shows the presence of anti-juvenile hormonal compounds in the extract of *A. vasica* (Table 3). The effect was more pronounced when the concentration of the extract was increased (0.5 to 2 %). Bioactive elements present in the plant blocked the normal development either at germ bund stage or at blastokinesis stage. Similar effects were seen when *D. cingulatus* was treated with the root extracts of

Pedalium murex (Sahayaraj *et al.*, 2006). Repellent activity was also found to be dose dependant (Table 4). Studies by Saxena *et al.*, 1986 have shown that various alkaloids from *Adhatoda vasica* (Acanthaceae) were found to be responsible for the antifertility and antifeedant activity against insects.

Effects of insecticial efficacy of plants against several pests have been reported and confirmed over the years. The effect of *Citrus sinensis* against housefly, *Musca domestica* (Kumar *et al.*, 2012); plant oils against coho salmon and rainbow trout (Stroh *et al.*, 1998); orange oil against Haemonchus contortus (Squires e*t al.*, 2010); *Azadirachta indica* against *Epilachna varivestis* (Rembold *et al.*, 1980); *Adhatoda vasica* against *Spodoptera littoralis* (Sadek, 2003); orange peel against mosquito (Anaso *et al.*, 1990); Eucalyptus against many persistent pests (Batish *et al.*, 2008). With respect to the cotton stainer, the efficacy of polyphenolic rich fractions from the stem bark of *Streblus asper* (Hashim and Devi, 2003), kernel extracts of neem (Abraham and Ambika, 1979), inhibition of ovarian growth by tepa (Sugumar and Naidu, 1973), Juvenile-hormone-like activity of *Catharanthus roseus*, *Parthenium hysterophorus* and *Nephrolepis exaltata* (Rajendran and Gopalan, 1980) have reported potent activity. Studies by Sharma *et al.*, 2010, Prabhu and John, 2011, Satyanarayana and Sukumar, 1988, Sahayaraj and Kalidas, 2011, also mention the effect of various plant extracts against the various stages of *D. cingulatus*.

Table 1: Ovicidal Effect

Conc. (%)	No. of Treated Eggs	No. of Dead Eggs	Egg Mortality (%)	Adult Emergency
0.5	30	8	26.66	22
1.0	30	11	36.66	17
1.5	30	19	63.33	10
2.0	30	27	90.00	2
Control	**30**	**0**	**0**	**28**

Table 2: Nymphicidal Effect

Conc. %	No. of 3rd Instars Taken	Dead 3rd Instars (24 Hrs)	Dead 3rd Instars (48 Hrs)	Dead 3rd Instars (78 Hrs)	Dead 3rd Instars (96 Hrs)
0.5	10	0	3	5	9
1.0	10	2	5	8	10
1.5	10	6	9	10	-
2.0	10	8	10	-	-
Control	**10**	**-**	**1**	**-**	**-**

Table 3: Juvenomimetic Effect

Conc. (%)	Average No. of Eggs Laid	% of Hatching
0.5	26	72.53
1.0	20	68.66
1.5	9	59.33
2.0	-	-
Control	**48**	**98**

Table 4: Repellent Activity

Conc. %	Average Number of Insects Repelled (%)		
	Chloroform Crude Extract	Methanol Crude Extract	Purified Extract
1.0	46	54	68
1.5	52	62	77
2.0	63	67	81
2.5	69	71	86
Control	**0**	**0**	**0**

Figure 1: Treated Eggs Turning Brown or Black

Figure 2: Application on Extract on the Abdominal Tergites of the Female Insect

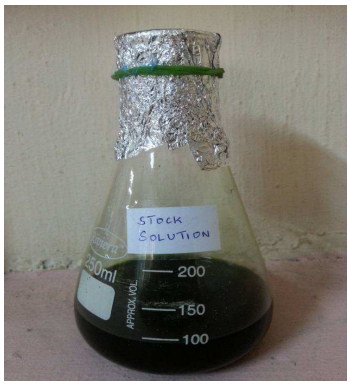

Figure 3: Stock Solution

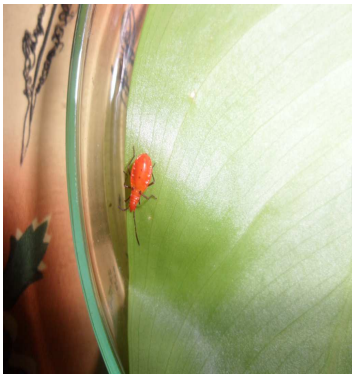

Figure 4: Third Instar of *D. Cingulatus*

CONCLUSIONS

Apart from the above findings that show a marked decrease in the survival rate of the pest, the leaf extract at much higher concentrations could potentially induce developmental deformities, metabolic abnormalities and reproductive aberrations which might be competent enough to distort normal growth and reproduction. The results of this study indicate that *A. vasica* extract is promising enough to be incorporated into integrated pest management techniques.

Thus, a diversified use of indigenous plants by the development of their use in the pest management sector could be of both economic and ecological benefit.

We can thus conclude that chloroform and methanol extracts of *A. vasica* possesses toxic principles with significant insecticidal effect and could be a potential cotton protectant against *D. cingulatus* and could be useful for developing new types of insecticides or biorational management agents for controlling the cotton pest.

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