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Research Article

EVALUATION OF NYMPHICIDAL EFFECT OF TWO INDIGENOUS PLANT EXTACTS ON COTTON PEST, *DYSDERCUS CINGULATUS* (FAB.)

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ABSTRACT

Antifeeding effect varied concentrations of *Adathoda vasica* and *Vitex negundo* crude methanolic leaf extracts was evaluated against an economically important cotton pest, *Dysdercus cingulatus* (Fab.) (Hemiptera: Pyrrochoreidae). All the concentrations (0.2, 0.4, 0.6, 0.8 and 1.0%) tested effectively caused varied ranges of nymphal mortality in all instar groups. The results revealed that among two plant antifeedants, *Vitex negundo* caused more nymphal deaths. However, both plant extracts produced more than 50% nymphal mortality at two higher concentrations (0.8 and 1.0%). It was evident that earlier nymphal instars (II and III) were more susceptible than the late nymphal instars (IV and V) to both plant antifeedant concentrations. Presence of Alkaloids, Terpenoids and Phenolic compounds might be the reason for these activities. It is concluded that *V.negundo* possess both antifeedant and nymphicidal activities. It can be used for the management of sucking pests of agricultural crops.

Keywords: *Adathoda vasica, Vitex negundo*, antifeedants, Nymphal mortality, Cotton pest.

INTRODUCTION

In recent years, great emphasis is given on the use of plant extracts which is non-toxic, safe biodegradable alternatives to the conventional control of crop pests by chemical insecticides (Wink, 1993; Shah, *et al.*, 2005; Akhtar *et al.*, 2008; Alagumeena, 2010; Sayed *et al.*, 2011) Many of the reported indigenous plants came under scrutiny, leading to extraction and characterization of other active constitutents which accounted for various uses by man. The most important of these constituents are alkaloids, terpenoids. phenols, saponins, tannins (Sehmutterer, 1990; Koul and Dhaliwal 2001; Isman 2008; Kamaraj *et al.*, 2010; Sahayaraj and Kalidas 2011). The deleterious effects of plant extracts or pure compounds on insect pests can be manifested in several manners including toxicity, mortality, antifeedants growth inhibitors, fecundity and fertility. Opender Koul (2009) reviewed the plants used for pest control and explained that there is a strong connection between insect pest control and phyto insecticides. The environmental hazards posed by synthetic chemical insecticides have necessitated the search for alternatives of natural origin such as plant extracts for applying viable pest control strategies (Chadha 1986; Anil Kakker and Pandey 2001; Wheeler and Isman, 2001; Cartoni 2002; Bakkali *et al.*, 2008; Adeniyi *et al.*, 2010). Though considerable works are available on the control of insects by plant antifeedants, data on the nymphal mortality of fibre crops are meagre.

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Cotton (*Gossypium histurum*) is the most economically important natural fiber material yield crop in the world. One of the major obstacles hindering cotton cultivation is insect - pest menace. In particular red cotton bug *Dysdercus cingulatus* is a serious pest, sucking on developing cotton bolls and ripe cotton seeds. For the control of such sucking insect in cotton fields, it is advisable to use plant extracts of resistant varieties for their insecticidal properties. Therefore, the present study was undertaken to evaluate the antifeedant effect of methanolic extracts of *Adathoda rasica* leaves (Fam: Acanthaceae) and *Vitex negundo* leaves (Fam: Verbenaceae) against the cotton bug, *Dysdercus cingulatus*. In addition, preliminary phyto-chemical screening of leaves of chosen plants was also recorded.

MATERIALS AND METHODS

Rearing test insect

For the present study, a stock culture of red cotton bug, *D. cingulatus* was reared from the freshly laid eves collected from the severely infested areas of Sattur, Virudhunagar District, Tamil Nadu. Newly hatched nymphs were sorted out from the stock culture and were reared on cut cotton bolls and cotton seeds in the laboratory at 27 ± 1 °C Temp; 65.5% R.H.

Preparation of leaf extracts

Healthy leaves of *A vasica* and *V. negundo* were collected in Sendakottai village near Adirampattinam. They were separately air dried and powdered and soxhelt extracted with methanol. Each leaf extract was collected in a beaker and evaporated to dry in a vacuum evaporator. A stock solution was prepared by adding 10 ml of methanol to 100 mg of each leaf extract varied concentrations viz 0.2,0.4,0.6.0.8 and 1.0% of *A. vasica* and *V. negundo* were prepared using methanol (85%) as solvent.

Phytochemical analysis

Chemical tests were undertaken to identify the phytochemicals namely alkaloids. flavonoids, saponins, terpenoids, tannins, glycosides. steroids, as per methodology adopted by Harborne (1998) in the methanolic leaf extracts *A. vasica* and *V. negundo*. Phytochemicals identified were recorded and tabulated.

Antifeedant bioassay and treatments

Fresh cotton bolls and seeds were collected from the non-infested cotton fields cut into pieces and weighed in mg separately. Spraying of each leaf extract was done on the known weighed cut cotton bolls and seeds and significant care was taken to cover the cotton bolls and seeds adequately.

Nymphal instars of varied age groups (II and V) after 0-12 h moulting were sorted out from the stock culture and starved for 6 hrs Sprayed cotton bolls and seeds of each leaf extract concentration were offered as diet to these nymphs individually in circular petridishes (15 cm dia.) one placed over another, leaving a small interval for ventilation. For each experiment 15 nymphs were used and replicated 3 times. Nymphs (n=15) fed with cotton bolls and seeds sprayed with methanol only were kept as control. Both control and experimental nymphs were subsequently maintained at 27 ± 1° C Temp; and 65 ± 5% R.H.

Percent nymphal mortality

Both control and experimental nymphs of *D. cingulatus* were observed by one week to calculate percent mortality as per the equation descused by Thangam and Kathiresan (1992).

$$\text{Percent mortality} = \frac{\text{No. of dead insects}}{\text{No. of insects treated (n=15)}} \times 100$$

Data obtained on percent nymphal mortality were subjected to various statistical analyses (Palanichamy and Manoharan 1984).

RESULTS

Preliminary phytochemical investigation of methanolic leaf extracts of *A. vasica* and *V. negundo* revealed the presence of alkaloids, flavonoids, terpenoids, etc., These antifeedant chemicals vary in amounts (Table l) The phytochemical analysis of the leaf extracts of both plants *A. vasica* and *V. negundo* showed the presence of alkaloids in maximum amount in the methanolic extracts of *V. negundo* leaves which was higher than in other plant *A. vasica*. The

phenolic compound was also found in both leaf extracts of *A. vasica* and *V. negundo* but in lower quantity. In addition, flavonoids and terpenoids were also found to be present in *A.vasica* leaves but absent in *V. negundo* leaves. Two phytochemicals namely saponins and tannins were found to be missing from leaves of both two plants as determined by the chemical tests used in the present study. Steroids and glycosides could not be detected in the leaves but found in traces in the leaves of *V. negundo.*

Effect of antifeedants of *A. vasica* and *V. negundo* on percentage of *A. vasica* which they are mortality of varied nymphal groups of *D. cingulatus* are detailed in Table 2 and 3. The results obtained on the percentage mortality of II instar nymphs of *D. cingulatus* at 0.2, 0.4, 0.6. 0.8, 1.0% leaf antifeedant concentration of *A. vasica* and *V. negundo* were somewhat similar, the values ranging from 53.4% to 93.4%. A similar trend of results was also evident in the treatment of both leaf antifeedant concentrations against III instars nymphs and the values ranged in between 46.7% and 80% Percentage mortality recorded for IV instar nymphs varied from 26.7% to 46.7% and from 53.7% to 73.8% at *A. vasica* and *V. negundo* concentrations respectively.

V. negundo antifeedant concentrations showed superiority over *A. vasica* in effecting percentage nymphal mortality, the values ranging from 40 to 67 in *V.negundo* treatment while the % value recorded for *A. vasica* treatment were 13% and 33%.

Table1. Phytochemicals identified in the methanolic leaf extracts of *A. vasica* and *V. negundo*.

Plant extract	Name of the phytochemical							
	ALKA.	FLAV.	TERP.	SAPO.	TANI	STER	PHEN	GLYC
Adathoda vasica	++	+	+	-	-	+	+	+
Vitex negundo	+++	-	-	++	-	-	+	-

Key: + = Present, +++ = Abundance, ++ = Few, = Absent

Abbreviations: ALKA – Alkaloids, FLAV – Flavonoids, TERP – Terpenoids, SAPO – Saponins, TANI – Tannins, STER – Steroids, PHEN – Phenolics, GLYC – Glycosides.

Table 2. Nymphal mortality of *D. cingulatus* at varied methanolic antifeedant concentrations of *A. vasica* leaves within a period of 7 days.

Age of Instar	Treatments	Percent Nymphal mortality at concentrations				
		0.2%	0.4%	0.6%	0.8%	1.0%
II	Control	N.D.	N.D.	N.D.	N.D.	N.D.
	Experimental	53.34±5.42 (08)	53.34±5.42 (08)	60.00±3.15 (9)	73.34±5.42 (11)	80.00±5.47 (12)
III	Control	N.D.	N.D.	N.D.	N.D.	N.D.
	Experimental	46.67±5.47	53.34±5.42	62.67±5.42	73.34±3.15	73.34±3.15
IV	Control	N.D.	N.D.	N.D.	N.D.	N.D.
	Experimental	26.67±3.29	33.34±3.15	33.34±3.15	40.00±5.42	46.67±5.42
V	Control	N.D.	N.D	N.D	N.D	N.D
	Experimental	N.D.	N.D	13.34±3.24	26.67±3.29	33.34±5.29

N.D. = Not Detected.

Values are the Mean of three observations and S.D.

Values in parentheses indicate the number of nymphs dead.

Table 3. Nymphal mortality of *D. cingulatus* at varied methanolic antifeedant concentrations of *V. negundo* leaves within a period of 7 days.

Age of Instar	Treatments	Percent Nymphal mortality at concentrations				
		0.2%	**0.4%**	**0.6%**	**0.8%**	**1.0%**
II	Control	N.D.	N.D.	N.D.	N.D.	N.D.
	Experimental	60.00±3.42 (09)	66.67±4.31 (10)	73.34±5.29 (11)	80.00±4.45 (12)	93.34±2.49 (14)
III	Control	N.D.	N.D.	N.D.	N.D.	N.D.
	Experimental	53.34±4.45 (08)	60.00±3.45 (09)	66.67±4.33 (10)	66.67±4.31 (13)	80.00±4.45 (12)
IV	Control	N.D.	N.D.	N.D.	N.D.	N.D.
	Experimental	53.34±4.45 (08)	53.34±4.45 (08)	60.00±3.45 (09)	66.67±4.33 (10)	73.74±5.31 (11)
V	Control	N.D.	N.D.	N.D.	N.D.	N.D.
	Experimental	40.00±3.29 (06)	46.67±4.40 (07)	53.34±5.29 (08)	53.34±4.45 (08)	6.67±4.31 (10)

N.D. = Not Detected.

Values are the Mean of three observations and S.D.

Values in parentheses indicate the number of nymphs dead.

DISCUSSION

The search for plant - derived chemicals that have potential use as crop protectants (insecticides, antifeedants, growth inhibitors) often begin with the screening of plant extracts (Peta Devanand and Pathipati Usharani Usharani 2008). Accordingly, the methanolic leaf extracts of *A. vasica* and *V. negundo* showed the presence of maximum alkaloids which according to the concept of Schoonhoven (1982) and Nighat Begum *et al.* (2012) acted as potential antifeedants against the nymphs of *D. cingulatus*. The antifeedant effects of leaf alkaloids and other phytochemicals was reflected on the varied ranges percent nymphal mortalities. The active principles such as alkaloids contained in the leaves inhibited nymphal feeding behaviour of the nymphs, consequently the reduced feeding may cause the rejection of the plant food (mixed with non host plant extract) may affect the development and longevity of the insect or may lead to death. This idea has been favoured by several authors who reported antifeedant activity against phytophagous insects such as *Stomopteryx subsecivella* (Shah, 1996), *Spodoptera litura* (Ignacimuthu *et al.,* 2006), *S. litura* (Ulrichs *et al.,* 2008; Jayasankar *et al*.) (2010) and *H. armigera* (Sundararajan, 2011).

It has been suggested that the penetration of active chemical constituents of leaves such as alkaloids into the soft cuticle and then affects on growth are said to be major reason for the death of the nymphs. Yet, there is another concept for the mortality and it may be due to the failure of proper moulting.

It was also observed in the present study that the percentage mortality of each of nymphal instar group was directly proportional to the dose of the leaf antifeedant used. In general, mortality decreased with the dilution of both leaf antifeedants tested against the varied nymphal stages. The same trend of results was reported by previous workers. Islam Ahamed and Mirdula Gupta (1981) found that the Hydroprene was found effective is resulting in 100% mortality of 1.0% and 2.0% concentrations in *Culex fatigans* while 0.5% produced 48% larval mortality. Shah (1992) reported that leaf and root alkaloids extract of *Catharanthus rosens* were effective in producing 11.0% to 100.0% nymphal mortality in horse crickets. *Gryllodes sigillatus*. Amanulla Hameed *et al,* (2003) studied the larvicidal activity of *Spheranthus indicus* in II-VI instar larval age groups of *S. litura* and repaired 20 to 60% larval mortality and 50 to 80% larval mortality at two higher concentration viz 750 and

1000 ppm respectively. Sundararajan (2011) studied the antifeedant effect of 9 plant extracts against the larvae of gram pod borer, *Helicoverpa armigera*. Percentage mortality rate reported by this author varied from 10.8% to 72.8%. Among the leaf extracts tested, three plants namely *Andrographis paniculata*, *Catharanthus roseus* and *Datura metel* exhibited high rate of mortality i.e. more than 70%. Shah and Maheswari (2002) reported the potentiality of leaf alkaloids extract of *Vitex negundo* in the mosquito *Culex fatigans* and their studies exhibited that two higher concentrations viz. 0.75% and 1.0% produced 100% mortality. Nalla Mohamed *et al.*, (2003) also reported 100% mortality in fourth instar larvae of *S. litura* when treated with two higher leaf antifeedant concentrations of *Tridax procumbers* (0.75% and 1.0%).

It is significant in the present study that the early nymphal instars (II and III) were more susceptible than the successive instars (IV and V) to the plant antifeedants. The manner in which the plant antifeedants do have their impact on the mortality appears to be also significant. The two higher concentrations 0.8% and 1.0% were more potential than the other two lower and middle concentrations 0.2%, 0.4%, and 0.6% respectively, since they affected invariably more than 50% deaths in all pre adult groups. Some of the treated nymphs in each group died within 24h of treatment either due to toxicity or moulting failure (Kubo *et al.*, 1981; Omer Erturk, 2006; Sundararajan, 2010).

It is quite evident from the results that in all concentrations at various intervals, effect of *V.negundo* was better than *A.vasica* as far as mortality values are concerned. The results as mentioned in the present study concide with the earlies works reported on other indigenous plant extracts on the antifeeding and growth inhibitory activities against agricultural pests (Nakajima and Kawazu, 1980; Adhiya Choudhary *et al.*, 1985; Sunita Gupta and Sushma Gupta, 1993).

It appears that the selected plants *A. vasica* and *V. negundo* contain different antifeedant chemicals that act upon target cells effectively. The activity of these plant antifeedant chemicals also suggests into potential insect management chemicals with a minimum environment impact. It is advantageous as the extracts at higher doses effect mortality while the lower dose of the same plant is oral toxicant.

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REFERENCES

Adeniyi, S.A., Orjiekwe, C.L., Ehlagbonare, J.E., and Arimah, B.D., 2010. Preliminary phytochemical analysis and insecticidal activity of ethanolic extracts of four tropical plants (*Venonia anygdalina, Sida acirta, Ocimum gratissimums* and *Telfaia occidentalis*) against beans Weeveil (*Acanth scelides obtectus*) *Int. J. Physic. Sci.*, 5(6): 753-762.

Adiya Choudhery, N., Bhattacharya, A., Choudhary, A., and Shri Kumar, P., 1985. Chemical constitutents and of plant exhibiting insecticidal antifeeding and insect growth regulating activities. *J. Sci Ind. Res.*, 44: 85-101.

Akhtar, Y., Yeoung, Y.R., and Isman, M.B., 2008. Comparative bioactivity of selected extracts from Meliaceae and some commercial botanical insecticides against two noctuid caterpillars, *Trichoplusia ni* and *Pseudaletia unipuncta*. *Phytochem. Rev.,* 9: 77-88.

Alagumeena, E., 2010. Efficacy of plant extracts on biology of a Coleopteran pest, *Mylabris pustulata J*. *Ecobiol.*, 27(3): 269-272.

Amanulla Hameed, S.V.S., Nazir Ahamed, T.A., and Shah D.S.M., 2003. Larvicidal activity of crude leaf extract of *Spheranthus indices* in cutworm, *Spodoptera litura* (Lepidoptera; Noctuidae). *Bionotes*., 5(2): 46.

Anil Kakker and Pandey, M.C. 2001. Role of Biopesticides in plant protection system. Biopest. Conf. BET Feb 7-9, 2001. Chandigarh, India, P.113.

Bakkali, F., Averbeck, S., Averbeck, D. Idaomar, M. 2008. Biological effects of essential oils: *Rev. Food Chem. Toxicol.,* 46: 446-475.

Cartini, C.R., Grossi, de Sa, M.F., 2002. Plant toxic properties. A review on the potentialities as bioinsecticides. *Toxicon.*, 40: 1515-1539.

Chadha, M.S., 1986. Response of *Sitophilus oryzae* Linn. to extracts of *Adathoda vasica* Neer. *Sci. Cul.*, 53(7): 213-215.

Harborne, J.B. 1998. Phytochemical methods: A guide to modern techniques of plant analysis. Chapman and Hall, New York, p. 1-286.

Ignacimuthu, S., Maria Packiam, S., Pavunraj, M., and Selvarani, N., 2006. Antifeedant activity of *Spheranthus indicus* L. against *Spodoptera litura* Fab. *Entomon*, 31: 41-44.

Islam Ahamed and Mridula Gupta, 1981. Effectiveness of JH mimic Hydroprene for the control of *Culex fatigens* Weid. *Curr. Sci.*, 50(1): 38-39

Isman, M.B., 2008. Perspective botanical insecticides : for richer, for poor. *Pest Manag. Sci.*, 64:8-11.

Jayasankar, A. Raja, N. and Ignacimuthu, S. 2010. Antifeedant and growth inhibitory activities of *Syzygium lineare* (Myrtaceae) against *Spodoptera litura* Fab. (Lepidoptera: Noctuidae) *Curr. Res. J. Biol. Sci.*, 2(3): 173-177.

Kamaraj, C., Rahiman, A.A., Mahapatra, A. Bagavan, A. and Elango, G. 2010. Insecticidal and larvicidal activities of medicinal plant extracts against mosquitoes, Parasitol. Res., 107(6): 1337-1349.

Koul, O. and Dhaliwal, G.S., 2001. Phytochemical Biopesticides. Harwood Academic Publishers, Amsterdam, The Netherlands, p. 1-12.

Kubo, 1., Klocke, J.A., and Asano, S., 1981. Insect ecdysis inhibitors from the east African medicinal plant *Ajuga remota* (Labiatae). *Agri. Biol. Chem.*, 45: 1925-1927.

Nalla Mohamed, K.E.N., Shah, D.S.M., Amanulla Hameed, S.V.S., Raveendran, S., and Amsath. A., 2003. Antifeedant effect of *Tridax procumbens* extract on *Spodoptera litura Proc. Nat Sem. Cont. Insect Plant* Insecticides, May 28-29, 2003, p. 4-6.

Nakajima, S. and Kawazu, 1980. Insect development inhibitors from *Coleops lanceolata* L. *Agri. Biol. Chem.* 40: 2283-2287.

Nighat Begum, Bechan Sharma and Pandy, R.S., 2011. Evaluation of insecticidal efficacy of *Calotropis procera* and *Annona squamosa* ethanol extract against *Musca domestica. J. Biofertilizers Biopestic.*, 1(1): 101-106.

Omer Erturk, 2006. Antifeedant and toxic effects of some plant extracts on *Thaumetopoae solitaria* Frey. (Lepidoptera: Thaumetopoeidae). *Turk J. Biol.*, 30: 51-57.

Palanichamy, S., and Manoharan, M., 1994. Statistical methods for biologists. Palani Paramount Publication, Palani, pp. 285.

Peta Devanand and Pashipati Usha Rani 2008. Biological potency of certain plant extracts in management of two Lepidopteran pests of *Ricinus communis* L. *J. Biopestic.*, 1(2): 170-176.

Sahayaraj, K., and Kalidas, S., 2011. Evaluation of nymphicidal and ovicidal effect of a seaweed, *Padina pavonica* (Linn.) (Phacophyceae) on cotton pest, *Dysdercus cingulatus* (Fab.) *Ind. J. Geo-Marvine Sci.*, 40(1): 125-129.

Sayed, M.R., Bakry, F.A. and mansour, A.A., 2011. Biochemical and histopathological effect of crude extracts on *Spodoptera littoralis* larvae. *J. Evol. Biol. Res.*, 3(5): 67-68.

Schmutterer, H., 1990. Properties and potentials of natural pesticides from the neem tree. *Azadirachta indica. Ann. Rev. Entomol.*, 35: 271-297.

Schoonhoven, L.M., 1982. Biological aspects of antifeedants. *Ent. Exp. et Appl.*, 31: 57-69.

Shah, D.S.M. and Maheswari, V. 2002. Effect of alkaloids extract of leaves of. *Vitex negundo* on the development of mosquito, *Culex fatigans. Bionote*s, 4(4): 102.

Shah, D.S.M., 1992. Effect of plant extracts on the mortality of *Gryllodes sigillatus* (Orthoptera: Gryllidae) *J. Ecotoxicol. Envt. Monit.*, 2(1): 27-30

Shah, D.S.M., Raveendran. S. and Amanulla Hameed, S.V.S., 2005. Bioefficacy of plant extract profile on the ovipositional behaviour of groundnut cutworm, *Agrotis ipsilon* (Lepidoptera: Noctuidae) *J. Ecobiol.*, 17(2): 131-136.

Sundararajan, G., 2010. Bioassay of botanical extracts as antifeedant activity in *Helicoverpa armigera. J. Ecotoxicol. Envt. Monit.*, 20(6): 535-539.

Sundararajan, G., 2011. Antifeedant activity of aqueous extracts of selected plants on the larva of *Helicoverpa armigera. J. Ecotoxicol. Envt. monit.,* 21(2): 189-193.

Shah, D.S.M., 1996. Antifeedant effect of two indigenous plant extracts against larval of *Stomoptelyx subsecivella* (Lepedoptera: Noctuidae) on groundnut. *J. Ecobiol.*, 8(4): 293-298.

Sunita Gupta and Sushma Gupta, 1993. Growth disrupting activity of *Adathoda vasica* and *Annona squamosa* on *Poikilocercus pictus. Bioved,* 4(2): 237-242.

Thangam, T.S. and Kathiresan, K. 1992. Smoke repellency and killing effect of mangrove plants against the mosquito, *Aedes aegypti* L. *Trop. Biomed.*, 10: 125-128.

Ulrichs, C.H., Mews, S., Adikary, A., Bhattacharya and Goswami, A., 2008. Antifeedant activity and toxicity of leaf extracts from *Protesia coarctata* Takeoka and their effects on the physiology of *Spodoptera litura* (F). *J. Pest. Sci*., 18:79-84.

Wheeler, D.A., Isman, M.B., Sanchez-vindas, P.E., Arnason, J.T., 2001. Screening of costa Rican, *Trichilla* species for biological activity against the larvae of *Spodoptera litura* (Lepidoptera: Noctuidae). *Biochem. Syst. Ecol.,* 29: 347-358.

Wink, M., 1993. Production and application of phytochemicals from an agricultural perspective. In: Phytochemistry and Agriculture (Eds. Van Beek., T.A., Breteler, H.) Clarendon Press, Oxford, U.K. p. 171-213.