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INSECTICIDAL ACTIVITIES OF STEM BARK EXTRACTS FROM *VITEX NEGUNDO* L. AGAINST *TRIBOLIUM CASTANEUM* (HERBST)

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Abstract

Context: Medicinal plants contains some pesticidal activities that may control stored product insects.

Objectives: To elucidate the insecticidal activity from the stem bark extracts of nishinda (*Vitex negundo* L.) and their toxicity against red flour beetle, *Tribolium castaneum* (Herbst) (Tenebrionidae).

Materials and Methods: The powdered stem bark (250 g) was extracted with methanol (MeOH), ethyl acetate (EtOAc), acetone and chloroform sequentially. Solvents were evaporated using a rotary evaporator to obtained dry extract, weighed and re-dissolved in the same solvent from which they were extracted for bioassay. Red flour beetles were reared for the experiment in 500 ml beakers containing food medium and kept in a control temperature room (30 ± 0.5^0C). Residual film bioassay was used to evaluate the toxicity. The adult mortality of *T. castaneum* (CTC-12, FSS-II and KANO strains) was recorded after 24, 48 and 72 h, but for larvae, the mortality was recorded on 12^{th} and 20^{th} larval day after treatment. The mortality percentages were subjected to probit analysis.

Results: The LD_{50} values for first through fourth instar larvae were more or less similar whereas, LD_{50} for fifth instar larva and adults were the lowest. Among the solvents, methanolic extracts elucidated more toxicity on both adult and larval insects. The degree of toxicity of the extracts according to solvents was MeOH>Pet spt.>acetone>EtOAc. The susceptibility order according to insect strains was FSS-II>CTC-12>KANO.

Conclusion: The results suggest the presence of active toxic substances of nishinda acting after consumption or topical application on *T. castaneum*. Methanolic extracts of the plant provided the most potent and reliable control of the flour beetle.

Key words: *Vitex negundo*, sovent extracts, toxicity, *Tribolium castaneum*

Introduction

Annual post-harvest losses resulting from insect damage, microbial deterioration and other factors such as humidity, temperature, aeration and cleanliness of the bulk storage, are estimated to be 10-25% of production worldwide (Mohan and Fields 2002). However, insects are the main problem in stored grain because they reduce the quantity and the quality of grains (Madrid *et al*. 1990). Insect pest control in stored food products relies heavily on the use of gaseous fumigants and residual chemical insecticides. The continual use of these synthetic insecticides has led to the development of pest strain resistance (Riebeiro *et al*. 2003).

Several plant species are known for their insecticidal activities (Klocke 1989). The insecticidal potentiality of many plant derivatives against stored-product insect pests has been demonstrated (Abubakar *et al*. 2000, Fields 2006, Pascual-Villalobos and Fernandez 1999, Tripathii *et al*. 2002). Azadirachtin extracts from the Indian neem tree (*Azadirachta indica*) (Jilani and Saxena 1990) and pyrethrum from *Chrysanthemum cinerareafolium* (Head 1966) have received the most attention.

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Vitex negundo L., a large aromatic shrub with bluish purple flowers is native to the tropical and semi-tropical countries, found in moist areas, often on banks of rivers throughout India, Bangladesh, Sri Lanka, Burma, China, Philippines, Malaysia, Tropical Africa, Afghanistan, Madagascar and Pakistan (Anonymous 1992, David *et al*. 1988). In Bangladesh it is very common in many parts and often occurs gregariously. Leaves of nishinda possess insecticidal properties (Ahmed and Koppel 1986, Dakshinamurthy 1988, Morallo-Rejesus *et al*. 1990, Singh *et al*. 1996, Mia *et al*. 1985). However, research on insecticidal activity of stem of nishinda is scanty. The objective of this work is to study the insecticidal activity of stem of *Vitex negundo* L.on the red flour beetle *Tribolium castaneum* (Herbst).

Materials and Methods

Plant collection: The barks of *V. negundo* were collected from the local areas of Rajshahi and were authenticated by the authority of Botany Department, Rajshahi University. The barks were sent to the laboratory where they were air-dried at room temperature for 5 days. The plant parts were chopped up into pieces and then finally powdered with the help of grinder. The powdered material 250 g each extracted respectively with methanol, ethyl acetate, acetone and chloroform. The extracts were made to stand in the dark for 3 days after which they were filtered. Solvents were evaporated using a rotary evaporator and obtained residues were redissolved in water for bioassay.

Test insect: Two hundred beetles were placed in a 500 ml beaker containing food medium covered with a piece of muslin cloth and kept in an incubator at 30 ± 0.5^0C. After 3-5 days the collected eggs were hatched. Newly hatched larvae were then with a fine pointed camel hairbrush and shifted to the fresh food medium for culture. The larval instars were determined using the methods of Mondal (1984). The second, third, fourth, fifth and six instar larvae were obtained from the larval culture in the 3rd, 6th, 9th, 12th and 16th day from hatching, respectively while the newly hatched larvae were considered as first instar.

Bioassay: Test the mortality rate of adult *T. castaneum* of CTC-12, FSS-II and KANO strains, residual film method (Busvine 1971) was applied. When the solvents were completely dried up, few adult of (3-5 days old), CTC-12, FSS-II and KANO strains of *T. castaneum* were released within petri dish separately. Five doses each five replications were taken. A control group was maintained in which only solvent was used. For larvae, from each dose 0.2 ml of liquid was dropped in glass vial (25 mm) and kept open for a few minutes to dry up the solvent leaving only the extracts. Then 2.0 gm of food was added and 10 larvae were released in each vial. Three replications were made for each dose. A control batch was maintained for each treatment where only the solvent was used. The adult mortality was recorded 24, 48 and 72 h after treatments. The doses were calculated by measuring the dry weight of the crude extracts applied in the petri dish divided by the surface area. For larvae, the mortality was recorded on 72 and 20th larval day after treatment.

The mortality percentage was corrected using Abbott's formula (Abbot 1925). The observed data was then subjected to Probit analysis according to Finney (1947) and Busvine (1971) using a software developed in the department of agriculture and Environmental Science, University of Newcastle Upon Tyne, UK.

Results

The effect of contact poisoning of four different extracts of the stem bark of nishinda on the mortality of three strains of adult *Tribolium castaneum* is presented in Table 1. The toxic effect of stem bark extracts on the adult insect was varied according to insect strains and solvent types. The α^2 values with 3 degrees of freedom for all the dose-mortality tests were non significant, which reveal any significant heterogeneity for all strains of adult insect.

Table 1. LD_{50} values with corresponding regression equation and χ^2 values for dose-mortality test of Pet. spt. extract of nishinda stem bark against *T. castaneum*

Strains	Life stages	Exposure time (h)	LD_{50} mgcm^2	Regression equation	χ^2
CTC-12	1st ins. larva		3.057	Y= 3.340955 + 1.11693X	0.228
	2nd ins. larva		3.462	Y= 3.122165 + 1.21986X	0.613
	3rd ins. larva	72	2.759	Y= 3.082996 + 1.33052X	0.820
	4th ins. larva		2.720	Y= 2.968917 + 1.415578X	0.334
	5th ins. larva		1.994	Y= 3.020737 + 1.52270X	1.880
		24	1.278	Y= 3.783597 + 1.09907X	0.281
	Adult	48	0.954	Y= 3.85092 + 1.17259X	0.631
		72	0.739	Y= 3.962189 + 1.19425X	0.858
FSS-II	1st ins. larva		3.040	Y= 2.622535 + 1.60322X	0.602
	2nd ins. larva		2.927	Y= 3.303497 + 1.15683X	0.404
	3rd ins. larva	72	2.174	Y= 3.410706 + 1.18835X	0.820
	4th ins. larva		2.046	Y= 2.953719 + 1.56095X	0.295
	5th ins. larva		1.766	Y= 3.062635 + 1.55342X	0.589
		24	1.077	Y= 3.66061 + 1.297526X	1.561
	Adult	48	0.675	Y= 3.716975 + 1.54642X	2.118
		72	0.498	Y= 4.034721 + 1.38287X	0.488
KANO	1st ins. larva		0.602	Y= 3.078165 + 1.21530X	0.759
	2nd ins. larva		4.787	Y= 3.174014 + 1.08680X	0.296
	3rd ins. larva	72	3.406	Y= 3.383681 + 1.05479X	0.238
	4th ins. larva		2.877	Y= 2.938260 + 1.41317X	0.294
	5th ins. larva		2.311	Y= 3.137315 + 1.36576X	0.427
		24	1.629	Y= 3.630682 + 1.12976X	2.153
	Adult	48	1.395	Y= 3.683876 + 1.14976X	2.827
		72	0.967	Y= 3.855891 + 1.16083X	3.672

The LD_{50} values with Pet. spt. extracts for strain CTC-12 were 1.279, 0.955 and 0.740 mgcm^{-2}; for FSS-II 1.077, 0.676 and 0.499 mgcm^{-2} and for KANO 1.630, 1.395, 0.968 mgcm^{-2}, respectively after 24, 48 and 72 h of treatment.

The LD_{50} values of steam bark EtOAc extract were higher than the stem bark extracts with other solvents. The LD_{50} values were 1.796, 1.429, 2.660 mgcm^{-2} after 24 h; 1.314, 1.068, 2.093 mgcm^{-2} after 48 h and 1.006, 0.754, 1.913 mgcm^{-2} after 72 h of treatment for the strains CTC-12, FSS-II and KANO, respectively (Table 2). On the other hand, LD_{50} values for stem bark acetone extract after 24 h of treatment are 1.280, 1.125 and 2.227 mgcm^{-2} for the strains CTC-12, FSS-II and KANO respectively. After 48 h of treatment the values were 1.147, 1.003, 1.734 mgcm^{-2} after 72 h of treatment the values are 0.949, 0.734, 1.426 mgcm^{-2} for the strains CTC-12, FSS-II and KANO, respectively. The methanolic stem bark extract of nishinda exhibited more toxicity to adult *T. castaneum*. The LD_{50} values for the methanolic stem bark extracts were 1.864, 1.281, 2.247 mgcm^{-2} after 24 h of treatment; 1.496, 0.939 and 2.018 mgcm^{-2} after 48 h of treatment; 1.142, 0.742 and 1.555 mgcm^{-2} after 72 h of treatment for the strains CTC-12, FSS-II and KANO, respectively.

The dose-mortality data of nishinda steam bark extracts reveal that FSS-II showed the highest adult mortality and KANO showed the lowest mortality. Solvent wise dose-mortality order of all three strains of *T. casataneum* were MeOH>Pet.spt.>acetone>EtOAc.

Table 2. LD_{50} values with corresponding regression equation and χ^2 values for dose-mortality test of EtOAc extract of nishinda stem bark against *T. castaneum*

Strains	Life stages	Exposure time (h)	LD_{50} values mgcm^2	Regression equations	χ^2 values
CTC-12	1st ins. larva	72	7.119	Y= 3.580886 + 1.6648X	0.363
	2nd ins. larva		5.685	Y= 4.042476 + 1.26869X	1.602
	3rd ins. larva		5.190	Y= 4.07651 + 1.291191X	0.167
	4th ins. larva		4.737	Y= 3.923497 + 1.59360X	0.304
	5th ins. larva		4.390	Y= 4.085362 + 1.42359X	3.998
	Adult	24	1.796	Y= 3.531269 + 1.17080X	1.178
		48	1.313	Y= 3.67354 + 1.85943X	2.411
		72	1.005	Y= 3.853613 + 1.14361X	0.898
FSS-II	1st ins. larva	72	5.681	Y= 3.794742 + 1.59758X	0.585
	2nd ins. larva		4.624	Y= 4.203504 + 1.19756X	0.766
	3rd ins. larva		4.676	Y= 4.155291 + 1.26094X	0.457
	4th ins. larva		3.589	Y= 4.141949 + 1.54584X	0.361
	5th ins. larva		4.180	Y= 3.871801 + 1.81337X	2.415
	Adult	24	1.429	Y= 3.268531 + 1.49903X	0.222
		48	1.067	Y= 3.410939 + 1.54501X	0.592
		72	0.753	Y= 3.662206 + 1.52494X	0.908
KANO	1st ins. larva	72	7.467	Y= 3.813797 + 1.35852X	0.798
	2nd ins. larva		9.495	Y= 3.796758 + 1.23093X	0.441
	3rd ins. larva		7.630	Y= 3.768182 + 1.39571X	0.591
	4th ins. larva		6.667	Y= 3.739429 + 1.52989X	0.538
	5th ins. larva		6.226	Y= 3.76759 + 1.55187X	0.839
	Adult	24	2.660	Y= 3.513622 + 1.12543X	0.287
		48	2.092	Y= 3.352308 + 1.12543X	0.111
		72	1.913	Y= 3.499631 + 1.156327X	0.131

*Effect of stem bark extracts on larval mortality:*The mortality percentage in terms of LD_{50} with corresponding regression equations and α^2 values of different larval instars of the three strains *T. castaneum* in different doses of nishinda steam bark extracts are presented in Table 1. The results reveal that the does-mortality of larvae were different among different strains, different extracts and even among different instars of the same strain of the test insect. The mortality order of the larvae for all instars were FSS-II>CTC-12>KANO. It was observed that MeOH stem bark extract of nishinda exhibited the highest degree toxicity as observed for leaf extract to larval instars than Pet. spt., acetone and EtOAc extracts. The non-significant α^2 values with three degree of freedom did not show any significant heterogeneity for first, second, third, fourth and fifth instar larvae of all the strains in all stem bark extracts.

In the first instar larvae, nishinda stem bark MeOH extract demonstrated more toxicity than the extracts with other solvent showing LD_{50} values 2.962, 2.408, 3.833 mgcm^{-2} for CTC-12, FSS-II and KANO, respectively. This is followed by Pet. spt. (LD_{50} values are 3.057, 3.040 and 0.603 mgcm^{-2}); EtOAc (LD_{50} values are 7.119, 5.641 and 7.467 mgcm^{-2}) and acetone (LD_{50} values are 8.106, 6.609 and 10.365 mgcm^{-2}) for the FSS-II, CTC-12 and KANO, respectively.

MeOH extract was found to be more toxic to second instar larvae too showing LD_{50} values 3.362, 2.712 and 3.560 mgcm^{-2} for CTC-12, FSS-II and KANO, respectively. Pet. spt. and acetone extracts showed more or less same level of toxicity to second instar larvae.

In the third instars, the toxicity showed similar trends as observed in first and second instar larvae. MeOH extract showed the highest order toxicity that is followed by Pet. spt., acetone and EtOAc extracts (Table 3). The LD_{50} values reveal that the fifth instar larvae were found to be more susceptible to the toxicity of all extracts from nishinda bark. The LD_{50} were comparatively lower for most of the extract types than those observed for the other instars (Table 4).

Table 3. LD_{50} values with corresponding regression equation and χ^2 values for dose-mortality test of acetone extract of nishinda stem bark against *T. castaneum*

Strains	Life stages	Exposure time (h)	LD_{50} values mgcm^2	Regression equations	χ^2 values
CTC-12	1st ins. larva	72	8.106	Y= 3.775131 + 1.34775X	1.269
	2nd ins. larva		8.879	Y= 3.563552 + 1.51461X	1.363
	3rd ins. larva		5.274	Y= 3.896551 + 1.52787X	0.332
	4th ins. larva		4.088	Y= 4.254185 + 1.21949X	0.758
	5th ins. larva		5.186	Y= 3.910592 + 1.52397X	0.977
	Adult	24	1.280	Y= 3.413458 + 1.43287X	0.233
		48	1.147	Y= 3.41808 + 1.492671X	0.228
		72	0.948	Y= 3.267919 + 1.77277X	0.675
FSS-II	1st ins. larva	72	6.609	Y= 3.96188 + 1.26578X	0.233
	2nd ins. larva		5.486	Y= 4.156582 + 1.14085X	0.123
	3rd ins. larva		4.357	Y= 3.96478 + 1.619994X	2.791
	4th ins. larva		3.126	Y= 4.194665 + 1.62684X	0.799
	5th ins. larva		3.219	Y= 4.155687 + 1.662504X	1.665
	Adult	24	1.125	Y= 3.458115 + 1.46674X	0.327
		48	1.003	Y= 3.440469 + 1.1055718X	0.818
		72	0.734	Y= 3.569756 + 1.65168X	2.058
KANO	1st ins. larva	72	10.36	Y= 3.746607 + 1.23417X	0.263
	2nd ins. larva		9.379	Y= 3.829338 + 1.203874X	1.971
	3rd ins. larva		5.225	Y= 4.239998 + 1.05836X	0.370
	4th ins. larva		6.045	Y= 4.155424 + 1.08079X	0.225
	5th ins. larva		5.573	Y= 4.118284 + 1.18177X	0.335
	Adult	24	2.227	Y= 3.169347 + 1.35832X	0.154
		48	1.734	Y= 3.117142 + 1.519532X	0.877
		72	1.425	Y= 3.150523 + 1.60256X	0.603

Table 4. LD_{50} values with corresponding regression equation and χ^2 values for dose-mortality test of MeOH extract of nishinda stem bark against *T. castaneum*

Strains	Life stages	Exposure time (h)	LD_{50} values mgcm^2	Regression equations	χ^2 values
CTC-12	1st ins. larva	72	2.961	Y= 2.650067 + 1.59687X	1.121
	2nd ins. larva		3.362	Y= 3.153999 + 1.20919X	0.469
	3rd ins. larva		2.342	Y= 2.859756 + 1.56265X	0.821
	4th ins. larva		1.810	Y= 3.146697 + 1.47345X	0.663
	5th ins. larva		1.835	Y= 3.06901 + 1.577038X	0.757
	Adult	24	1.863	Y= 3.082685 + 1.50919X	0.799
		48	1.496	Y= 3.546865 + 1.23673X	0.772
		72	1.142	Y= 3.69614 + 1.23233X	0.280
FSS-II	1st ins. larva	72	2.407	Y= 2.960954 + 1.475847X	0.754
	2nd ins. larva		2.712	Y= 2.783165 + 1.54660X	1.881
	3rd ins. larva		1.575	Y= 2.896582 + 1.75667X	0.380
	4th ins. larva		1.615	Y= 2.752932 + 1.859673X	1.532
	5th ins. larva		1.241	Y= 3.318744 + 1.536874X	3.085
	Adult	24	1.281	Y= 3.259037 + 1.57177X	2.097
		48	0.939	Y= 3.458348 + 1.58457X	2.374
		72	0.741	Y= 3.614344 + 1.59243X	1.687
KANO	1st ins. larva	72	3.832	Y= 3.394054 + 1.01418X	0.420
	2nd ins. larva		3.560	Y= 3.298226 + 1.09688X	0.841
	3rd ins. larva		3.736	Y= 2.812289 + 1.39128X	0.447
	4th ins. larva		2.416	Y= 3.216206 + 1.289269X	0.612
	5th ins. larva		1.980	Y= 3.20657 + 1.382982X	0.962
	Adult	24	2.247	Y= 3.779655 + 0.90289X	0.490
		48	2.017	Y= 3.631951 + 1.048388X	0.236
		72	1.554	Y= 3.646901 + 1.13549X	0.557

Discussion

Most of the authors trails to assess contact toxicity of different adult pests through nishinda leaves (Tiwari 1994, Morallo-Rejesus *et al*. 1990, Dakshinamurthy 1988, David *et al*. 1988, Mannan *et al*. 1993). In the present experiment, among the four solvents, MeOH yielded the highest. Plant extract yield is dependent on the chemical nature and the solubility of the components present in a defined organ of a plant. Moreover, the distribution and accumulation of particular plant product is also varied in different plant organs. Pet. spt. generally extracts oils, fats and fatty acids; EtAc extracts tarpin, alkaloids, and steroids; acetone separates dyes and some alkaloids non-soluble in ethyl acetate and MeOH extracts remaining alkaloids and acidic compounds. MeOH is polar but Pet. spt., EtOAc and acetone are non-polar solvents. Therefore, extract yielded from nishinda stem bark with MeOH may be related with the presence of higher amount polar solvent soluble alkaloids and acidic compounds (Ho *et al*. 1997).

The extracts in different doses were directly applied to the adult and larval stages of *T. castaneum*, they became lethargic, ceased moving and feeding, and finally died. The mortality of the test insect was found directly proportional to the concentration of the extracts in the doses and exposure times. Different workers elucidated the contact toxicity of nishinda, mostly in the form of crude leaf powder on different pests. Mannan *et al*. 1993, Abubakar *et al*. 2000, Khanam and Khalequzzaman 2000).Toxic effect of both crude leaf and bark extract of *V. negundo* against cockroach was reported (Morallo-Rejsus and Carino 1984).

Present study reveals that larval growth of *T. castaneum* is affected by the toxic effect of the nishinda extract. However, a variation was observed in the susceptibility as indicated from LD_{50} values. It is observed from the present study that younger in developmental instars of the all strains of test insect were less sensitive to the nishinda extract and the susceptibility was increased with the developmental stages. It is interesting to observe that in all extracts LD_{50} values are more or less similar from first to fourth instar but in the fifth instar LD_{50} values are certainly dropped indicating more toxic to this larval stage. This may be due to the maximum feeding stage at fifth instar larvae were apparently repelled by nishinda extracts and they do not settle and feed well. As a result, the mortality of the fifth instar larvae and adult was higher than other developmental stages. These observations on *T. castaneum* with nishinda extracts are concomitant to other studies on contact insecticides (Bushvine 1971), carbon dioxide (Leong and Ho 1993), clove flower extract (Ho *et al*. 1996).

Present study also elucidates that the phytotoxic effect of nishinda extract varied with the strains of *T. castaneum*. Among the three strains of test insect, FSS-II showed the highest degree of susceptibility to all dose-mortality tests. The susceptibility order among the test insect genotypes was FSS-II>CTC-12>KANO. Different susceptibility of these strains of *T. castaneum* may be due to their different genetic background. FSS-II is a malathion susceptible strain whereas, both CTC-12 and KANO are resistant to malathion (Sokoloff 1972, Lloyd and Ruczkowski 1980). Khanom and Khalequzzaman (2000) used neem seed kernel extract and observed different mortality rate of adults among different strains of *T. castaneum*. The greater mortality response of FSS-II than other strains of *T. castaneum* to different plant extracts has also been reported (Islam 1996).

In adult as well as larvae, the mode of action of nishinda stem bark extract is not properly known but it seems to act as chitin synthesis inhibitor. Due to its effect on the integument of larvae and adult, becomes much less extensive which might have the effect of increasing internal pressure in the body, restricting movement and hampering feeding. This may change the elasticity of the cuticle as well as the body protective

mechanism and may be responsible for adult and larval death. In the contact treatment, the adult mortality is due to the physiological factor. It may cease the enzymatic secretion or hormonal secretion may causes the antifeedant tendency and the ultimate result is death.

The efficacy of nishinda stem bark in control of *T. castaneum* is truly established, with methanol extracts of the plant providing the most potent and reliable for further work on isolation and identification of bioactive compounds.

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