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Toxic and residual effects of *Azadirachta indica, Tagetes erecta* and *Cynodon dactylon* seed extracts and leaf powders towards *Tribolium castaneum*

Direkte und residuale Giftwirkung von Samenextrakten und Blattpulvern aus *Azadirachta indica, Tagetes erecta* und *Cynodon dactylon* auf *Tribolium castaneum*

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Summary

Direct and residual toxicities of seed extracts and leaf powders of the neem tree (*Azadirachta indica),* marigold *(Tagetes erecta)* and durba *(Cynodon dactylon)* along with two commercial insecticides (malathion and carbaryl, respectively) towards the red flour beetle *(Tribolium castaneum),* a major stored-product pest, were evaluated. All seed extracts and leaf powders showed a certain degree of toxicity towards the insects. Among the tested plant derivatives, neem seed extract (100 µg/insect) showed higher direct toxicity (53.13 % mortality) towards red flour beetles than marigold (46.88 %) and durba (37 %) seed extracts. On the other hand, marigold leaf powder (5 %) showed a higher residual toxicity (57.09 % inhibition ratio) than neem (50.06 %) and durba (43.28 %) leaf powder. Compared with the commercial insecticides (malathion and carbaryl), neem seed extract and marigold leaf powder possess a potential as natural alternative insecticides towards the red flour beetle in stored products.

Key words: carbaryl; direct and residual toxicity; durba; insecticides; leaf powders; malathion; marigold; neem; seed extracts; stored-product pests; *Tribolium castaneum*

Zusammenfassung

Die direkte und residuale Giftwirkung von Samenextrakten und Blattpulvern von *Azadirachta indica* (Neembaum), *Tagetes erecta* (Aufrechte Sammetblume oder Studentenblume) und *Cynodon dactylon* (Hundszahngras) sowie den beiden Insektiziden Carbaryl und Malathion auf den Rotbraunen Reismehlkäfer *(Tribolium castaneum),* einen bedeutenden Vorratsschädling, wurde untersucht. Alle untersuchten Samenextrakte und Blattpulver zeigten eine gewisse Toxizität gegenüber den Insekten. Samenextrakte des Neembaums (100 µg/Insekt) wiesen mit einer Mortalität von 53,13 % eine höhere direkte Toxizität auf als solche der Studentenblume (46,88 %) und des Hundszahngrases (37 %). Blattpulver (5 %) der Studentenblume hingegen zeigten eine höhere residuale Toxizität (57,09 %) als solche von Neem (50,06 %) und Hundszahngras (43,28 %). Der Samenextrakt des Neembaums und Studentenblumen-Blattpulver besitzen im Wirkungsvergleich mit den Insektiziden Carbaryl und Malathion ein Potenzial als alternative Insektizide gegen den Rotbraunen Reismehlkäfer im Nacherntebereich.

Stichwörter: Aufrechte Sammetblume; Blattpulver; Carbaryl; direkte und residuale Toxizität; Hundszahngras; Insektizide; Malathion; Neem; Samenextrakte; Studentenblume; *Tribolium castaneum;* Vorratsschädlinge

1 Introduction

Insect infestation of stored grains and their products is a serious problem throughout the world (Irshed and Gillani 1990; Arther and Zettler 1991). It was estimated that world's cereal production is lost to the extent of 8 % every year due to insect infestation alone in storage. If the losses incurred on farms were included, it may amount to 10 %. Among the important stored-product insect pests, the red flour beetle is one of the major pests (Zettler 1991; Talukder and Howse 1995; Zettler and Arthur 1997; Collins 1998; Haubruge and Arnaud 2001). Though the red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) originated in India, it is found throughout all tropical, sub-tropical and warm temperate regions of the world. It is one of the most common pests of stored products. The larvae and adults feed on a wide range of durable commodities and are important secondary pests of cereals, nuts, spices, coffee, coca, dried fruits and occasionally of peas and beans. Like most other storage beetles, *T. castaneum* can penetrate deeply into the storage commodity. However, the red flour beetle normally attacks the broken grains.

The incidence of insecticide resistance is a growing problem. Resistance to one or more insecticides has been reported in at least 500 species of insects and mites (Pasalu and Bhatia 1983). The development of cross- and multi-resistant strains in many important insect species is a serious concern all over the world. Resistance in different insect species against different synthetic insecticides, toxic residues in stored products, increasing cost of application, hazards from handling etc., has been reported worldwide (Khanam et al. 1993; Chan 1992; Kotze and Wallbank 1996). Resistance in *T. castaneum* actually is a worldwide problem. Red flour beetles already show resistance to malathion, dichlorvos, phosphine, chlorpyrifos-methyl, deltamethrin and other synthetic pyrethroids and other insecticides throughout the world (Dhaliwal and Chawla 1995; Haubruge et al. 1997; Collins 1998; Stuart et al. 1998; Zettler and Arthur 1997; Werner 1997; Haubruge and Arnaud 2001). Moreover, the cross-resistance present in the red flour beetle can seriously complicate chemical control (Saxena and Sinha 1995; Werner 1997). The current pest control technology in several tropical regions of the world is based largely on imported synthetic insecticides, which are frequently priced beyond easy reach of smallholder farmers, who constitute a very large proportion of the farming community in developing countries.

The problems associated with conventional pesticides have instigated the need for greater selectivity in environmentally friendly pesticides. This awareness has created a worldwide interest in the development of alternative strategies, including the discovery of new types of insecticides, which must be pest-specific, non-toxic to mammals, biodegradable, less prone to pest resistance, and relatively less expensive (Talukder and Howse 1993; Hermawan et al. 1997). This has led to re-examination of the century-old practices of protecting crops using plant derivatives, which have been known to resist insect attack (Ewete et al. 1996). Plant derivatives of neem *(Azadirachta indica),* marigold *(Tagetes erecta),* durba *(Cynodon dactylon),* pithraj *(Aphanamixis polystachya),* bishkatali *(Polygonum hydropiper),* bankalmi *(Ipomoea sepiaria),* nishinda *(Vitex negundo),* eucalyptus *(Eucalyptus globulus)* etc. have shown their effectiveness in insect-pest management (Mohiuddin et al. 1993; Mordue and Blackwell 1993; Khanam et al. 1993; Malek and Wilkins 1995; Xie et al. 1995; Pascual and Robledo 1998; Sharma 1999; Talukder and Miyata 2002). Malek and Wilkins (1995) tested the effects of *Annona squamosa* seed oil on the larvae of *T. castaneum* and found that larval development was significantly affected by the seed oil. Talukder and Howse (1995) reported the repellent (up to 100 %), antifeedant (up to 69.9 %) and contact toxicity (up to 79.1 % mortality) activities of seed extracts of *A. polystachya* on *T. castaneum.* Sharma (1999) showed that neem seed kernel powder and neem leaf powder, at low dose, protected grains for 5 months from the attack of different storage pests and that neem oil was toxic to red flour beetle. Xie et al. (1995) reported that neem products showed both repellent (behavioural) and toxic (physiological) actions on stored-product insects including red flour beetle. Mohiuddin et al. (1993) reported that among the 12 vegetable oils tested against *T. castaneum,* oil of

A. indica showed 80–100 % repellent activity. Pascual and Robledo (1998) carried out screening of plant extracts from 50 different wild plant species of south-eastern Spain for insecticidal activity towards *T. castaneum* and reported that four species namely, *Anabasis hispanica, Senecio lopezii, Bellardia trixago* and *Asphodelus fistulosus* were found as most bioactive plants. Saxena et al. (1992) reported that the acetone extracts of *Tagetes erecta* had growth-inhibitory and juvenile hormone-mimicking activity to mosquitoe larvae. Mukhopadhyay et al. (1995) described the durba or Bermuda grass *(Cynodon dactylon)* as a medicinal plant to control anti-haemorrhagic, dysentery and nasal bleeding problems.

Many plant-derived materials do not cause resistance development in insects, have broad spectrum activity, are safe to natural enemies, compatible with biological control agents for integrated pest management (IPM), and are non-toxic to the environment (Anonymous 1991; Talukder and Miyata 2002). The main advantage of plant products is that they are easily produced by farmers and small-scale industries and are less expensive (Talukder and Howse 1995). Therefore, the present investigation was conducted to determine the direct and residual toxicity of seed extracts and leaf powders of three plants, namely of neem, marigold and durba against a major stored-product insect, the red flour beetle. We also tried to find out the comparative efficacy of these seed extracts and leaf powders in relation to two commercial synthetic insecticides.

2 Materials and methods

2.1 Insect rearing

The test insect *T. castaneum* was reared in a growth chamber in the entomology laboratory, Bangladesh Agricultural University, at 27–30 °C temperature and 70–75 % relative humidity. Wheat flour was used as insect rearing media. Fifty grams of wheat flour were placed in a jar (9.5 × 7.5 cm) and then 50 pairs of adult insects (red flour beetle) were released into the jar for feeding and oviposition. After a week, all adults were removed from the jar and the media was kept in the growth chamber for the next 28–35 days for emergence of the F1 generation. The F1 generation insects were used for the experiments. All tests were carried out at 27–30 °C and 70–75 % relative humidity.

2.2 Preparation of extracts

The leaves and seeds of neem *(A. indica),* marigold *(T. erecta)* and durba *(C. dactylon)* were collected from different areas of Mymensingh, Bangladesh. Collected plant materials (seeds or leaves) were dried in the shade and then crushed separately with the help of a lab mill. One hundred grams of crushed seeds were mixed with 400 ml of solvent (water) and then stirred with a magnetic mixer for 20 minutes. The mixture was then left to stand for the next 24 hours and filtered under reduced air pressure (with the help of water-operated aspirator filter pump). The filtered solution, collected in round-bottomed flasks, was then heated in a water bath to 80–90 °C in order to evaporate the solvent. The resultant extracts were preserved in tightly corked bottles and stored in a refrigerator until used for tests. Stock solutions, from extracts, were prepared by diluting lower concentrations with acetone, prior to the test. Leaf powders were prepared by pulverizing dried leaves in a lab mill.

2.3 Contact toxicity test

Laboratory tests for contact toxicity by topical application were conducted according to the method of Talukder and Howse (1995) with slight modifications. Stock solutions were prepared by dissolving 100 mg of the each seed extract or malathion in 1 ml of the solvent (acetone). Lower concentrations (60 and 40 mg/ml) were obtained by dilution of the stock solution with acetone. Insects were immobilized by chilling for a period of 2–5 minutes and then using a micro-capillary tube, 1 µl of solution (containing 40, 60, and 100 µg extract or malathion/insect) was applied to the dorsal surface of the insect thorax. Fifty unsexed insects in five replicates of 10 adults each were treated with each dose. In addition, the same numbers were treated with solvent only as control. After treatment, insects were transferred into 9 cm diameter Petri dishes (10 insects/Petri dish) containing normal food (wheat flour). Insects were examined daily and those that did not move or respond to a gentle touch were considered as dead. Insect mortalities were recorded at 24, 48 and 72 hours after treatment (HAT).

2.4 Residual toxicity test

Either 3 or 5 g of ground powders were added to 100 g of wheat flour to prepare 3 % and 5 % mixtures. A commercial synthetic powder insecticide, Sevin 85 WP (active ingredient carbaryl) was also used in this experiment for the comparison of the effect of plant powders. The Sevin was admixed with wheat flour at a rate of 4 or 8 mg per 100 g flour. The food mixture was put into plastic pots (4 × 3.5 cm diameter). The pots were filled with either treated or untreated food (about 3–5 g each). Then five pairs of adult insects were introduced in each pot, to give them the choice for free oviposition for a period of 7 days. Then, they were removed from the food and the pots were kept in a growth chamber for the emergence of the F1 generation. On the subsequent days, the data were recorded on: [1] Number of adults in each small pot (on day 7) and [2] Number of F1 adults emerging from each pot (from day 30 to day 45). The inhibition rates (IR %) were calculated by the following formula:

$$IR\ \% = \frac{Cn - Tn}{Cn} \times 100$$

where, Cn = Number of F1 progeny on control pot and Tn = Number of F1 progeny on treated pot.

2.5 Statistical analysis

For direct toxicity tests, insect mortalities were recorded at 24, 48 and 72 HAT and the data were corrected by ABBOTT's (1925) formula and then analyzed using ANOVA. The mean values were adjudged with Duncan's Multiple Range Test (DUNCAN 1951). The median lethal doses (LD_{50}) were calculated using probit analysis (FINNEY 1971) with a $\log_{10}$ transformation of concentrations of extracts and insecticides. Results were expressed as micrograms per insect (µg/insect). Two LD_{50}s were considered to be significantly different ($P < 0.05$) if their 95 % fiducial limits did not overlap; slopes were similarly considered to be significantly different if their standard errors did not overlap. The data for residual toxicity were transformed by using square root ($Y = \sqrt{X}$) for numbers and arcsin values for percentage values and then analyzed by ANOVA; mean values were compared with DMRT.

3 Results

The results from the direct and residual toxicity are given in Tables 1, 2 and 3. Mortality percentage indicated that malathion at highest dose (100 µg/insect) possessed the highest direct toxic effect on red flour beetles (84.38 %) at 72 HAT. Among the three plant extracts, neem showed the highest insect mortality (53.13 %), followed by marigold (46.88 %) in the same dose. When the total effects of extract/insecticide and time were calculated, the mortality data showed that the malathion possessed the highest toxic effect on red flour beetles (56.12 %) at 72 HAT (data not shown). Among the plant extracts, neem possessed the highest mortality (53.13 %), followed by marigold (46.88 %). Durba showed the lowest (37 %) mortality. The order of toxicity was malathion > neem > marigold > durba.

The probit analysis at 24 HAT for the comparison of LD50 values of three plant extracts along with malathion towards red flour beetle are presented in Table 2. Malathion was the most toxic with lowest LD_{50} values (70.15 µg/insect). Among the extracts, neem showed lower toxicity (LD_{50}= 257.66 µg/insect), followed by marigold (LD_{50}= 357.43 µg/insect). At 48 HAT, comparison of LD50 values showed that malathion was most toxic (59.58 µg/insect) to red flour beetle. Among the extracts, neem was highly toxic (118.33 µg/insect), followed by marigold (165.4 µg/insect). The LD50 values compared at 72 HAT showed a similar trend and malathion continued to possess the highest toxicity (LD_{50}= 52.06 µg/insect) towards red flour beetle. Among the extracts, neem had the highest toxicity (74.27 µg/insect), followed by marigold (98.28 µg/insect). Durba had the lowest toxicity towards red flour beetle (LD_{50}= 152.3 µg/insect).

The residual toxicities of different leaf powders and the commercial insecticide Sevin 85 WP towards the red flour beetle are given in Table 3. The results show that the ovipositing females were deterred from wheat flour treated with leaf powders of neem, marigold and durba (at 3 and 5 % level) or Sevin (4 and 8 mg/100 gm). In case of the 3 % mixture, the lowest number of F1 progeny (55.75) emerged

Table 1. Mean mortality percentages of red flour beetle treated with different seed extracts and malathion through topical application

Seed extract or insecticide	Dose (µg/insect)	No. of insects treated	Average insect mortality (%) at		
			24 HAT	48 HAT	72 HAT
Neem	040	50	12.78^{de}	30.55^{cd}	31.25^{efg}
	060	50	12.50^{ef}	30.55^{cd}	43.75^{bcd}
	100	50	27.88^{bc}	44.44^{b}	53.13^{b}
Marigold	040	50	12.83^{de}	19.44^{ef}	28.13^{fg}
	060	50	17.50^{de}	30.55^{cd}	37.50^{cde}
	100	50	22.77^{c}	36.11^{bc}	46.88^{bc}
Durba	040	50	05.00^{g}	13.89^{f}	18.75^{h}
	060	50	07.5^{fg}	13.89^{f}	25.00^{gh}
	100	50	12.78^{de}	25.00^{de}	37.00^{cde}
Malathion	040	50	20.83^{cd}	22.22^{ef}	34.38^{def}
	060	50	30.83^{b}	41.66^{b}	50.00^{b}
	100	50	76.95^{a}	75.00^{a}	84.38^{a}

Ten insects were treated as a replication.
Original data corrected by Abbott's (1925) formula and then transformed into arcsine percentage values before ANOVA and DMRT test.
Each insect was treated with 1 µl of diluted extract or insecticide.
Values followed by the same letter within a column are not significantly different at the 0.05 level (Duncan's multiple range test).
HAT = Hours after treatment.

Table 2. Probit analysis of different plant extracts and malathion treatments of red flour beetle

Seed extract or insecticide	No. of insects	LD_{50} (µg/insect)	χ^2 value	95 % fiducial limit	Slope ± S. E.
Neem					
24 HAT	200	257.66	00.0913	150.66–440.55	1.419 ± 0.014
48 HAT	200	118.33	00.2204	085.31–164.06	1.051 ± 0.012
72 HAT	200	074.27	00.6027	063.53–86.50	1.300 ± 0.013
Marigold					
24 HAT	200	357.43	00.0139	170.22–750.00	1.326 ± 0.014
48 HAT	200	165.40	01.6368	104.47–261.22	1.086 ± 0.014
72 HAT	200	098.279	00.1180	075.50–127.64	1.140 ± 0.013
Durba					
24 HAT	200	794.04	00.0104	189.23–3349.65	1.261 ± 0.014
48 HAT	200	432.96	02.3521	139.63–133.68	0.093 ± 0.013
72 HAT	200	152.30	00.1535	100.46–230.14	1.27 ± 0.014
Malathion					
24 HAT	200	070.15	18.506*	066.99–73.62	4.075 ± 0.013
48 HAT	200	059.58	07.2228*	055.34–62.66	2.865 ± 0.012
72 HAT	200	052.06	00.9578	048.75–55.46	3.41 ± 0.014

No. of insects were based on four concentrations (including control) and five replicates of 10 insects each.
LD_{50} = Lethal median dose.
HAT = hours after treatment.
χ^2 = chi-square, goodness-of-fit test [the tabulated χ^2 value is 3.84 (df = 1, $p < 0.05$)].
S. E.= Standard error.
* Significant at 5 % level of probability.

Table 3. Residual toxicity of different leaf powders and the insecticide Sevin 85 WP towards *Tribolium castaneum*

Leaf powder or insecticide	Number of F_1 adult emerged		Inhibition ratio (IR %)	
	3 %	5 %	3 %	5 %
Neem	081.25 bc	067.00 b	40.15 bc	50.06 b
Marigold	067.75 cd	058.25 b	50.09 ab	57.09 b
Durba	093.50 b	077.00 b	31.12 c	43.28 c
Sevin 85 WP**	055.75 d	031.75 c	58.93 a	76.61 a
Control	135.75 a	135.75 a	–	–

Values followed by the same letter within a column are not significantly different at the 5 % level of probability (Duncan's Multiple Range Test).
For Sevin 85WP, two different doses, 4 mg and 8 mg/100 g flour were used.
IR % = (Cn – Tn / Cn) × 100 where, Cn = Number of F1 progeny on control and Tn = number of F1 progeny in treated pots.

from the flour treated with Sevin followed by marigold (67.75), though their differences were not statistically significant. Similar trends were observed for the 5 % mixture of leaf powder and Sevin (8 mg/100 gm). In all cases, the highest number of F1 adults (135.75) emerged from control pots. The highest insect inhibition ratio (IR %) of F1 progeny was found in wheat flour treated with Sevin followed by marigold leaf powder. However, their differences were not statistically significant. The durba leaf powder showed the lowest IR (43.28 %).

4 Discussion

Though our experimental results show that malathion had the highest toxicity towards the red flour beetle, it is too expensive for many poor farmers in developing countries and at the same time, it is hazardous in nature and has caused insecticide resistance problems (Pasalu and Bhatia 1983; Halliday et al. 1988). On the other hand, the three tested plant species showed moderate toxic effects towards red flour beetle, and among them, neem showed the highest toxicity, followed by marigold and durba. These plants are locally available in many developing countries and preparations of their extracts are easy and cheap. Therefore, they might be useful for the control of red flour beetles in storage. It was found that the insect mortality percentages were directly proportional to the time, as for example, the insect mortality at 100 µg/insect neem extract increased from 27.88 % at 24 HAT to 53.13 % at 72 HAT. The results show that neem extract might be an alternative and cheap measure for the control of red flour beetle, next to the costly malathion, as neem trees are easily available in different developing countries and the preparation of crude neem extract might be within the know-how of the farmers. The differences among their LD50 values indicated the possibility of future use of neem extract for the protection of wheat from the red flour beetle, instead of malathion. Though the neem seed extract caused lower mortality rates (53.13 %) than the conventional insecticides, it is more environmentally friendly, less costly and can easily be accessed by the farmers in developing countries. The higher concentrations of all tested extracts/insecticides caused higher insect mortality rates and they appeared to be an important factor in the degree of control. In case of residual toxicities, all three leaf dusts were effective in increasing the inhibition rates. Significantly fewer parent adults were found in food treated with leaf dusts. The marigold leaf powder showed statistically similar inhibition effects on red flour beetle when compared with the commercial insecticide Sevin 85 WP. Neem *(A. indica)* contains the main allelochemical azadirachtin that is well known for its anti-insect properties (Mordue and Blackwell 1993), but in case of marigold or durba, the main allelochemicals responsible for insect control properties are not known yet.

Though the chemical insecticides (malathion and Sevin) showed higher effectiveness, their use in the storage also creates some problems including insecticide resistance, toxic food residues, health hazards etc. (Osman and Regesus 1981; Pasalu and Bhatia 1983; Halliday et al. 1988). From our current experimental results, it appeared that among three plant species, neem seed extract showed the highest toxic effects in case of direct toxicity test. In case of residual toxicity, marigold leaf powder the showed highest toxicity. Therefore, the present investigations indicate that plant products (extracts and powders) might be useful as insect control agents especially in developing countries.

To minimize the severe damage caused by insect pests, many developing countries have to import a large quantity of insecticides. As the poor farmers in those countries are confronted with the high costs of insecticides, many of them have difficulties to afford synthetic pesticides to protect stored products. On the other hand, developing countries in Asia and Africa have a long history to protect stored grains with locally available herbal substances, where the application of plant materials is simple and aqueous extracts in several cases proved to be highly effective against stored product insects. Moreover, the crude extracts, oil, leaf powder etc. are easy to prepare and handle and very much cheaper in comparison to the imported chemical pesticides. Bomford and Isman (1996) reported that *Spodoptera litura* larvae became desensitized to pure azadirachtin, but did not desensitize to neem extract containing the same amount of azadirachtin after repeated exposures. Therefore, the use of plant products (crude extracts, oil, powders etc.) as insecticides in stored product protection might benefit the farmers by a reduction of protection costs, insecticide resistance development and environmental impact in term of insecticidal hazard.

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