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Growth Inhibiting Effects of Three Different Plant Extracts on *Tribolium castaneum* (Herbst) (Tenebrionidae: Coleoptera)

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GROWTH INHIBITING EFFECTS OF THREE DIFFERENT PLANT EXTRACTS ON *TRIBOLIUM CASTANEUM* (HERBST) (TENEBRIONIDAE: COLEOPTERA)

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ABSTRACT

Petroleum ether, acetone and ethanol extracts of neem, sweet flag, and turmeric were evaluated for their growth inhibiting effects on the *Tribolium castaneum* at the application rate of 1000, 500, 250 and 125 µg/g food media. Petroleum ether extracts showed the lowest number of larvae produced (34.00) in 1000 µg/g neem extract. Acetone extract of sweet flag showed minimum and significantly lower number of larvae produced (8.00) at 1000 µg/g, whereas ethanol extracts of turmeric showed minimum numbers of larvae (106.30) at 1000 µg/g application rate. Maximum inhibition ratio of pupation was found as 81.18 % at 1000 µg/g dosage of petroleum ether extract of sweet flag resulting into 8.75 pupae. Acetone extract of sweet flag showed minimum numbers of pupae produced (8.00) at 1000 µg/g of sweet flag, whereas ethanol extract of sweet flag showed minimum numbers of pupae (41.75) at 1000 µg/g. Petroleum ether extract of sweet flag showed a maximum inhibition ratio (40.00 %) resulting into 5.25 adults produced at 1000 µg/g. Acetone extract of sweet flag showed the lowest numbers of adults produced (8.00) at 1000 µg/g. Ethanol extract of neem showed lower numbers of adults (35.25) at 1000 µg/g dosage, followed by 38.50 in sweet flag and 52.75 in turmeric at the same application rate.

Keywords: Sweet flag, Neem, Turmeric, Red Flour Beetle, Plant Extract, Growth Inhibition.

INTRODUCTION

The control of insect pests is primarily dependent upon continuous application of insecticides such as pyrethroids, methyl bromide, phosphine etc. (White and Leesch, 1995). Although effective, their repeated uses for several decades has disrupted the natural biological system and led to development of resistance to various types of insecticides, undesirable effects on non-target organisms, environment and human health (Champ and Dye, 1977; Desmarchelier, 1994; Irshad and Gillani, 1989, 1991; Irshad *et al*., 1992; Irshad and Iqbal, 1994).

In Pakistan, during storage periods, wheat suffers a loss of about 3.5% in public sectors and 4% at the farm level (Baloch *et al.*, 1986; FAO, 1995). This loss is caused mainly by stored-product insects, namely the Red flour beetle *(Tribolium castaneum* (Hbst.), the Lesser grain borer *(Rhizopertha dominica* (F.), the Rice weevil *(Sitophilus oryzae* (L.), theKhapra beetle *(Trogoderma granarium* Everts), and the Angoumois grain moth *(Sitotroga cerealella* (Oliv.).

The adverse effects of insecticides have highlighted the need for development of selective insect control alternatives. Plant materials may provide potential alternatives to currently used insect control agents as they constitute a rich source of

bioactive chemicals (Mahulikar and Chavan, 2007). Many plant extracts are known to possess insecticidal activities against various stored-product and field insect-pests (Jacobson, 1983; Saxena, 1989; Schmutterer, 1995; Balakumbahan, *et al*., 2010;). A number of indigenous Pakistani plants have been studied for their repellent effects on stored-grain insect pests (Jilani and Haq, 1984; Jilani and Saxena, 1988; Jilani *et al.,* 1989, 1991, 1993).

This paper describes a laboratory study to assess the potential of three plants (namely neem, sweet flag, and turmeric) extracted with three solvents (petroleum ether, acetone, and ethanol) for using as growth inhibiting agents against one of the major storage pests, *T. castaneum*.

MATERIALS AND METHODS

Tribolium castaneum was reared in the laboratory of the Insect Pest Management Program, National Agricultural Research Centre, Islamabad, Pakistan under controlled temperatures (27±1°C and 55 ± 5 % R.H) on food media consisting of fine flour: corn flour with a brewer yeast in the ratio of 7:2:1).

The test plant materials, i.e. seeds of neem (*Azadirachta indica* A. Juss.*),*and rhizomes of turmeric (*Curcuma longa* L.) and sweet flag *(Acorus calamus* L.*),*were purchased from the local market and were ground to fine powder with the help of an electric grinding machine before use.

Each plant's materials were extracted on Soxhlet's apparatus separately with petroleum ether (60-80 °C), acetone, and ethanol to produce extracts of various polarities, having a range of various naturally effective compounds. The extracts were concentrated with the help of a rotary evaporator and finally made solvent-free in a vacuum desiccator.

Different working concentrations (1000, 500, 250, and 125 µg/g) of each plant extract were prepared by diluting them with acetone. During bioefficay tests, three replications of each concentration were applied.

Fumigated wheat flour in batches of 100 g each was treated with 125, 250, 500, or 1000 µg/g concentrations of plant extract dissolved in 10 ml of acetone. Wheat flour treated with acetone only was used as control treatment. After the complete evaporation of acetone, wheat flour was divided into 4 portions (25g flour/ portion) and placed in glass jars (1 lb in capacity). Then, 20 laboratory reared 10 days old *T. castaneum* adults (starved for 24 hours) were released in each jar. The mouth of the jars were covered with muslin cloth and kept in the controlled environment (at 27±1°C and 55 ± 5 % R.H).

Five days after the treatment, the adult insects were removed from the jars and jars were returned to the same controlled environment. Later, on the 13^{th}, 25^{th}, 30^{th} and 45^{th} day, the number of larvae, pupae, and adults, respectively, in each jar were recorded. The data were statistically analyzed using Analysis of Variance and means values were compared using Duncan's Multiple Range Test at 5 % probability (Duncan, 1951), whereas, the Growth inhibition ratio were calculated on the basis of following equations:

$$LI(\%) = 100 - \left(\frac{\text{Number of larvae converted into pupae}}{\text{Total number of larvae}} \times 100\right)$$

$$PI\ (\%) = 100 - \left(\frac{\text{Number of pupae converted into adults}}{\text{Total number of pupae}} \text{ x } 100\right)$$

Where:

LI is larval inhibition.

PI is pupal inhibition.

RESULTS AND DISCUSSION

Effect of Petroleum ether Plant Extracts on Progeny of *T. castaneum*

The data presented in Table 1 revealed that the petroleum ether extracts of all three test plants had shown significant effects on the reproduction of *T. castaneum*, as the number of larvae produced in treated flour were significantly lower than the control flour (239 larvae). In the case of the neem extract (at 1000 µg/g), the minimum number of larvae produced were 34.00, followed by 38.75 in turmeric, and 46.50 in sweet flag, however, they were not significantly different. The sweet flag extract appeared to be the most effective growth inhibitor, especially at the lowest application rate of 125 µg/g wheat flour producing 49.75 larvae, followed by 71.75 larvae in turmeric, and 75.75 in neem extract. However, the number of larvae produced in flour treated with various doses of different plant extracts were not significantly different, though the flour treated with the sweet flag extract had a comparatively lower number of larvae. In the case of the turmeric extract, the number of larvae produced at 1000 µg/g were significantly lower than that at 250 and 125 µg/g doses. In the case of the neem extract, the number of larvae produced at 1000 µg/g were significantly lower than that at 125 µg/g.

The number of pupae produced in flour treated with petroleum ether extracts of various plants was significantly lower than that in the control flour (216.30 pupae). Maximum inhibition of pupation

Table 1: *T. castaneum* progeny and its inhibition in wheat flour treated with petroleum ether extracts.

Plant Extract	Application rate (µg/g)	Number of emerged larvae, pupae & adults and inhibition ratio				
		Larvae		Pupae		Adults
		No.	Inhibition ratio (%)	No.	Inhibition ratio (%)	No.
Neem	1000	34.00^{e}	57.35	14.50^{ef}	13.79	12.50^{ef}
	500	48.25^{cde}	34.35	32.25^{b-e}	06.98	30.00^{b-e}
	250	57.50^{b-e}	33.16	37.75^{bcd}	07.95	34.75^{bc}
	125	75.75^{b}	39.27	46.00^{b}	08.15	42.25^{b}
Sweet flag	1000	46.50^{cde}	81.18	8.75^{f}	40.00	5.25^{f}
	500	42.50^{de}	44.71	23.50^{c-f}	26.60	17.25^{c-f}
	250	58.25^{b-e}	33.48	38.75^{bc}	17.42	32.00^{cd}
	125	49.75^{b-e}	30.15	34.75^{bcd}	17.27	28.75^{b-e}
Turmeric	1000	38.75^{e}	52.26	18.50^{def}	14.86	15.75^{def}
	500	46.75^{cde}	51.34	22.75^{c-f}	28.57	16.25^{def}
	250	68.00^{bcd}	41.18	40.00^{bc}	16.88	33.25^{bcd}
	125	71.75^{bc}	43.21	40.75^{bc}	25.15	30.50^{bcd}
Control		239.00^{a}	09.50	216.30^{a}	07.17	200.80^{a}
LSD value		23.73		17.44		15.63

Larval inhibition = Larvae not becoming pupae; Pupal inhibition = Pupae not becoming adults; each value is mean of 4 replications.
Values having same letters in a column are non-significant ($P \leq 0.05$).

was recorded as 81.18 % at 1000 µg/g in sweet flag extract. The flour treated with sweet flag extract produced 8.75 pupae, followed by 14.50 in neem, and 18.50 in turmeric, showing 57.35 and 52.26% inhibition, respectively, at the same application rate. However, the differences were not statistically significant. Similarly, insignificant differences were observed at all lower corresponding application rates. In case of both the neem and turmeric extracts, pupae produced at 1000 µg/g doses were significantly lower than those produced at 250 and 125 µg/g. In the sweet flag extract, the number of pupae produced at 1000 µg/g were significantly lower than those produced at 250 and 125 µg/g.

The number of adults produced in the treated flour were significantly lower than those in the control flour (200.80 adults). The maximum inhibition of adult emergence was recorded as 40.00 % resulting into 5.25 adults produced at 1000 µg/g of sweet flag followed by 13.79 % in neem (12.50 adults) and 14.86 % in turmeric (15.75 adults), respectively. However, their differences were not statistically significant. In case of turmeric, the number of adults produced at 1000 µg/g were significantly lower than those at 250 and 125 µg/g. In case of neem extract, the adults produced at 1000 µg/g were significantly lower than those produced at 125 µg/g.

Effect of Acetone Plant Extracts on Progeny of *T. castaneum*

The data presented in Table 2 revealed that acetone extracts of all three plants had a significant effect on the reproduction of *T. castaneum* as the number of larvae produced in the treated flour were significantly lower than that of the control (276.80 larvae). In the case of the sweet flag (at 1000 µg/g), the minimum number of larvae produced were 8.0 followed by turmeric (168.80) and neem (193.00). Sweet flag was significantly different from turmeric and neem. The number of larvae produced in flour treated with the sweet flag extract at 500 and 250 µg/g were significantly different at all application rates of the turmeric and neem extract. The sweet flag extract appeared to be the most effective growth inhibitor at all application rates. In neem and turmeric, larvae produced at different application rates were not significantly different from each other.

The number of pupae produced in the flour treated with acetone extracts of all the test plants were significantly lower than that in the control (240.80 pupae). In the case of the sweet flag treated flour at 1000 µg/g, the minimum numbers of pupae produced were 8.00 followed by turmeric (151.80), and neem (174.30) at the comparable application rate. Neem and turmeric were non-significant from each other at all lower corresponding application rates. During pupation, the sweet flag extract in acetone did not inhibit the development. Very little effect was noticed at lower application rate.

The number of adults produced in flour treated with acetone extracts of all the test plants was significantly lower than that in the control (231.80 adults). The minimum numbers of adults produced were 8.00 at 1000 µg/g of sweet flag followed by neem (146.80) and turmeric (151.30) at the same application rate. The number of adults in neem and turmeric were non-significant from each other at all lower corresponding application rates. During adult formation, the sweet flag extract did not inhibit development at 1000 µg/g and very little effect was noticed in the lower application rates.

Table 2: *T. castaneum* progeny and its inhibition in wheat flour treated with acetone extracts.

Plant Extract	Application rate (µg/g)	Number of emerged larvae, pupae & adults and inhibition ratio				
		Larvae		Pupae		Adults
		No.	Inhibition ratio (%)	No.	Inhibition ratio (%)	No.
Neem	1000	193.00^{bc}	09.69	174.30^{bcd}	15.78	146.80^{bcd}
	500	205.00^{bc}	17.17	169.80^{bcd}	09.72	153.30^{bc}
	250	207.80^{bc}	10.35	186.30^{bc}	11.54	164.80^{bc}
	125	217.30^{b}	09.11	197.50^{b}	08.20	181.30^{b}
Sweet flag	1000	8.00^{e}	00.00	8.00^{g}	00.00	8.00^{f}
	500	95.75^{d}	10.18	86.00^{f}	01.16	85.00^{e}
	250	123.00^{d}	08.13	113.00^{ef}	01.06	111.80^{de}
	125	163.50^{c}	17.43	135.00^{de}	01.26	133.30^{cd}
Turmeric	1000	168.80^{c}	10.07	151.80^{cd}	00.33	151.30^{bc}
	500	178.00^{bc}	08.99	162.00^{bcd}	00.12	161.80^{bc}
	250	195.50^{bc}	14.94	166.30^{bcd}	00.48	165.50^{bc}
	125	195.80^{bc}	11.90	172.50^{bcd}	01.16	170.50^{bc}
Control		276.80^{a}	13.01	240.80^{a}	03.74	231.80^{a}
LSD value		39.11		35.71		33.53

Larval inhibition = Larvae not becoming pupae; Pupal inhibition = Pupae not becoming adults; each value is mean of 4 replications.
Values having same letters in a column are non-significant ($P \leq 0.05$)

Effect of Ethanol Plant Extracts on Progeny of *T. castaneum*

The data presented in Table 3 revealed that ethanol extracts of all three plants had shown significant effects on the reproduction of *T. castaneum* as the number of larvae produced in treated flour were significantly lower than that of the control (213.30 larvae). In the case of the turmeric extract (at 1000 µg/g), the minimum numbers of larvae were produced (106.30) which were followed by neem (110.80) and sweet flag (115.80), however they were not significantly different. Similarly these were non-significant from each other at all lower corresponding application rates of all the plants. The sweet flag extract appeared to be the most effective growth inhibitor, especially at 1000 µg/g as 63.95% larvae died and could not reach to the pupal stage. It was followed by 50.00% inhibition in the sweet flag at 500 µg/g, 41.79 and 36.96% in neem at 1000 and 500 µg/g application rates, respectively. In control, only 7.27% of larvae died.

The number of pupae produced in flour treated with ethanol extracts of all the test plants was significantly lower than that in the control (197.80 pupae). The minimum numbers of pupae produced at 1000 µg/g were in the sweet flag treated flour (41.75), followed by neem (64.50) and turmeric (77.50). Sweet flag was significantly different from turmeric but not from neem. Neem, sweet flag, and turmeric were non-significant from each other at all lower corresponding application rates. Neem extract caused 45.35% inhibition of adult emergence at 1000 µg/g, followed by turmeric (31.94) and sweet flag (7.78) at the corresponding application rate. In all plant extracts, percent inhibition was not clearly dose dependent.

Table 3: *T. castaneum* progeny and its inhibition in wheat flour treated with ethanol extracts.

Plant Extract	Application rates (µg/g)	Number of emerged larvae, pupae & adults and inhibition ratio				
		Larvae		Pupae		Adults
		No.	Inhibition ratio (%)	No.	Inhibition ratio (%)	No.
Neem	1000	110.80^{de}	41.79	64.50^{ef}	45.35	35.25^{e}
	500	128.50^{cde}	36.96	81.00^{cde}	29.94	56.75^{cde}
	250	157.50^{bc}	25.52	117.30^{b}	33.72	77.75^{bc}
	125	160.30^{b}	18.40	130.80^{b}	26.41	96.25^{b}
Sweet flag	1000	115.80^{de}	63.95	41.75^{f}	07.78	38.50^{de}
	500	129.00^{cde}	50.00	64.50^{ef}	14.73	55.00^{cde}
	250	156.00^{bc}	32.05	106.00^{bcd}	21.70	83.00^{bc}
	125	156.50^{bc}	14.70	133.50^{b}	27.90	96.25^{b}
Turmeric	1000	106.30^{e}	27.09	77.50^{de}	31.94	52.75^{cde}
	500	113.30^{de}	32.48	76.50^{de}	24.51	57.75^{cde}
	250	141.30^{bcd}	25.12	105.80^{bcd}	33.60	70.25^{b-e}
	125	163.80^{b}	33.27	109.30^{bc}	32.07	74.25^{bcd}
Control		213.30^{a}	07.27	197.80^{a}	05.31	187.30^{a}
LSD value		27.63		27.71		31.83

Larval inhibition = Larvae not becoming pupae; Pupal inhibition = Pupae not becoming adults; Each value is mean of 4 replications
Values having same letters in a column are non-significant ($P \leq 0.05$)

The number of adults produced in the treated flour was significantly lower than control (187.30 adults). In the case of the neem treated flour, the minimum numbers of adults produced at 1000 µg/g were 35.25, followed by sweet flag (38.50) and turmeric (52.75). The number of adults produced in all the plant extracts were non-significant from each other at all lower corresponding application rates.

The petroleum ether extract was found as the most promising growth inhibitor. Because of being highly volatile due to the low polarity of compounds, the vapor pressure in the jars containing wheat flour treated of petroleum ether extract affected egg hatching. This also showed that the vapors might have acted as an ovicide, rather than having a lethal effect on other developmental stages. Such action of sweet flag oil (petroleum ether extract) had been recorded by Yadava (1971) and Rahman & Schmidt (1999). Further to this, it was noted that besides the ovicidal or oviposition inhibition, the effect of petroleum ether extract of sweet flag was comparatively more visible on earlier stages (larvae) of metamorphosis. Conversely, the effect of ethanol extract of neem was slightly better shown up on later stages (pupation and adult emergence). This phenomenon may be due to the presence of “asarone” in the petroleum ether extract of sweet flag (Chopra, *et al*., 1965) and “azadirachtin” in the ethanol extract of neem (Warthen *et.al*., 1978), which are the active components. The direct effects of “azadirachtin” are on cells and tissues, and indirect effects are exerted via the endocrine system. Neem seeds contain many related triterpenoids in addition to “azadirachtin,” including 3-Tigloyl-azadirachtol (azadirachtin B), nimbin, and salanin. Their efficacy is directly related to “azadirachtin” content.

However, many other compounds also have biological activities and add to their effects, whereas, the pure "azadirachtin" has been shown to be effective in the field (Mordue *et al.*, 1995). Studies on several insects indicated that IGR effects are induced by "azadirachtin" applications. Neem interferes with many life processes in the adult, during larval development and during the metamorphosis of insects (Schluter *et al.*, 1985; Dorn *et al.*, 1987; Koul *et al.*, 1987, Schmidt and Pesel, 1987).

In Pakistan, the public awareness created against harmful pesticide residues and recommendations made in the Pesticide Ordinance of Pakistan has opened up opportunities for promotion of plant-originated/botanical pesticides in Pakistan. These plants, traditionally used for medicinal and pest control purposes, are therefore expected to be safe for humans and animals. Being volatile or sensitive to light, these are biodegradable and therefore, not expected to leave any harmful residues on treated commodities (Jilani and Su, 1983). The active principles, especially "asarone" and "turmerones," having simple structures can be synthesized for use in commercial preparations and can very well be fitted in future pest management strategies.

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