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Efficacy of Several Plant Extracts as Growth Inhibitors against Red Flour Beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

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Abstract: Ethanol and acetone extracts of *Verbena tenuisecta*, *Parthenium hysterophorus*, *Calotropis procera* and *Azadirachta indica* were tested as inhibitors of the growth of *Tribolium castaneum*. *V. tenuisecta* (at 500 ppm) effectively controlled eggs laying of *T. castaneum*, i.e., the minimum mean number of eggs was 61.00, followed by the standard *A. indica* at 1000 ppm with 77.50 eggs, while the maximum mean oviposition amounted up to 139.67 and 144.67 eggs in flour treated with *P. hysterophorus* at 500 and 250 ppm, respectively. The most effective plant extract as an inhibitor of egg hatching (applied as 500 ppm) was the extract of *P. hysterophorus* (only 18.47% of eggs hatched) while the least effective was the extract of *V. tenuisecta* (52.03% of eggs hatched) 500 ppm each, respectively. The highest larval inhibition was exhibited by *C. procera* at 1000 ppm, which did not differ significantly from any of the other treatments or the control. Pupal inhibition was highest for the extract of *V. tenuisecta*. The acetone extract had more effect on egg laying, while ethanol had the highest growth inhibiting effect as egg hatching inhibitor, larval inhibitor, pupal inhibitor and feeding deterrent. Growth inhibiting effects of plant extracts were not clearly dose-dependent. Ethanol extracts had better growth inhibiting effects than petroleum ether extracts, whereas acetone extracts had some effect on egg laying.

Key words: Plant extract, bio-pesticides, neem plant seed, *Azadirachta indica*

Introduction

Studies around the world have been exploring the use of natural plant products as substitutes to synthetic pesticides aiming to avoid risks for human health and environmen related to their toxicity (Singh & Singh 2005; Azevedo *et al.* 2007; Dimetry *et al.* 2007). Different plant extracts are valuable source of unique natural products that can be used as pest control against various insects (Tandorost & Karimpour 2012). A number of plant aqueous solutions has been tested for their insecticidal activity against several insect pests and proved effective for their control (Isman 2000; Carlini & Grossi-de-Sá 2002; Amanda et. al. 2010). The toxic effects of plant compounds on insect pests was confirmed in several ways including mortality, anti-growth inhibition, suppression of reproductive behaviour and through their impact on fecundity and fertility of eggs (Mulungu et. al. 2007; Abida *et al.* 2010; Begum *et al.* 2011; Susana *et al.*, 2013).

Store grain losses caused by insects, microbes and other pests has been estimated to 10-40% globally, while in Pakistan they have been 10-20% (Khan *et al.* 2010, Rashid *et al.* 2012). Currently, in Pakistan and other developing countries, chlorinated hydrocarbon, organophosphorus, pyrethroid and fumigants are continuously used for the suppressions of store grain insect pests, which may endanger human and animal health as well as agroecosystem (Ahmed

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et al. 2008). On the other hand, repeated application of these chemicals not only disturbs biological control systems, which are mainly maintained by natural enemies, but also results into outbreaks of minor insect pests (Collins *et al.* 2002; Ahmed *et al.* 2008).

More than 10,000 arthropods have been reported to trigger serious damage to store grains products worldwide (Rajendran & Sriranjini 2008). In advanced countries, food grain products contaminated with store grain pests are rejected by food industries because the quality is the main concern in food processing (MBTOC, 2002). Fumigants, such as phosphine gas tablets and methyl bromide, are commonly used for the control of store insect pests worldwide; however, pests have developed resistance against these chemicals. Furthermore, these fumigants are damaging the ozone layer and have been banned in several countries (Collins *et al.* 2002; MBTOC 2002).

Due to the aforementioned problems, many studies are focusing on plant products that have insecticidal properties and aim to replace conventional pesticides for the management of store insects. Plant products are not only safe to warm-blooded animals but also easily biodegradable and less prone to insect resistance (Oparaeke *et al.* 2005). More than 2,400 plants species (their extracts or products) have been reported to possess insecticidal properties (Liu & Ho 1999; Tripathi 2000; Abubakkar *et al.* 2000; Umoetok 2000; Meera & Mann 2002; Kanvil *et al.* 2007).

The aim of the present study is to assess the efficiency of plant extracts of *Parthenium hysterophorus* L. (Asteraceae), *Verbena tenuisecta* [=*Glandularia pulchella* (Sweet) Troncoso (Verbenaceae)], *Calotropis procera* (Aiton) W.T.Aiton (Apocynaceae) and *Azadirachta indica* A. Juss. (Meliaceae) as growth inhibitors against *Tribolium castaneum* (Herbst) (Coeoptera: Tenebrionidae).

Materials and Methods

Collection and Preparation of Plant Materials

Plant materials of *P. hysterophorus* and *V. tenuisecta* were collected from cultures maintained by National Agricultural Research Centre (NARC), Islamabad. Materials from *C. procera* originated from the region of Multan (Punjab). All parts of each plant species was grounded to fine powder and preserved in jars. Neem oil was extracted from seeds of *Azadirachta indica* with petroleum ether and was used as a standard for comparison. Each plant powder was extracted separately with petroleum ether, acetone and ethanol using Soxhlet's extraction apparatus. These plant extracts were concentrated using rotary evaporator or by removing the excess solvents under vacuum. The concentrated extracts were made solvent free in vacuum desiccators.

Insect Culture

Red flour beetles, *Tribolium castaneum*, were collected from stored grain and were cultured on a medium consisting of 50% wheat flour, 45% corn meal and 5% brewer's yeast. This culture was maintained in the laboratory at constant temperature 29±1ºC, photoperiod 12:12 (L : D) and 65±5% relative humidity (RH).

Inhibition Studies

Wheat flour in lots of 200 g was treated with 1000, 500 and 250 ppm of the test materials in 10 ml of acetone. Untreated flour served as a control. One hundred and fifty-six glass vials (50 ml; 10 cm high; 2.5 cm in diameter) were used in this experiment. We had four main lots (treatments) and each main treatment was divided into 3 sub-lots. Then each sub-lot was divided into three sub-sub-lots and each sub-sub lot was replicated four times. After complete evaporation of the acetone, the wheat flour was poured into the glass vials. *Tribolium castaneum* individuals were randomly picked from a 15-day-old insect cultured colony. In each glass vial, 10 insects were placed, covered with muslin cloth and kept in chambers under controlled conditions (29±1ºC, 65±5% RH and 12 day light). After three days, the adults were removed and data on oviposition, egg hatching, number and weight of the larvae, pupae and adults were recorded. Upon the completion of experiment, the recorded data were analysed using Analysis of Variance and mean values were compared using Duncan's Multiple Range Test (1951) at 5 % probability.

Results

Inhibition Studies

The effects of various concentrations of *V. tenuisecta*, *P. hysterophorus*, *C. procera* extracts and *A. indica* oil, each in petroleum ether, acetone and ethanol, presented as a percent of deterrence of oviposition of *T. castaneum* are given in Table 1. Though the results were non-significant, *V. tenuisecta* at 500 ppm effectively controlled eggs laying of *T. castaneum*, i.e., the minimum mean number of eggs was 61.00, followed by the standard *A. indica* at 1000 ppm with 77.50 eggs, while the maximum mean oviposition amounted up to 139.67 and 144.67 eggs in wheat flour treated with *P. hysterophorus* at 500 and 250 ppm, respectively.

Egg Hatching

The deterrent effect on the percent eggs of *T. castaneum* hatching was examined in wheat flour after treatment with plant products at various dose levels and showed significant differences ($p<0.05$). The most effective plant extract was that from *P. hysterophorus*, while the least effective was *V. tenuisecta*. The mean percent egg hatching in these protectants was 18.47% and 52.03% at 500 ppm each, respectively. The maximum percent inhibition was recorded for ethanol solvent (45.60%) followed by acetone (31.64%), while the minimum inhibition was recorded for petroleum ether (24.15%). We found significant differences for the interaction effect of treatments and solvents (Table 1) with mean percent of eggs hatching at different interaction of treatments and solvents ranging from 5.10 to 74.88% in *V. tenuisecta* at 250 ppm with acetone and in *A. indica* at 250 ppm in ethanol extract, respectively (Table 2).

Mean larval weight

We found no significant difference in mean weight of *T. castaneum* larvae per treatment, while the data for solvents showed significant difference in mean weight ($p<0.05$). Effects of treatments on mean weight of larvae of *T. castaneum* did not show deterrence of feeding for different treatments (Table 2). However, the plant extract of *V. tenuisecta* at 500 ppm and *C. procera* at 1000 ppm resulted in lower weight gain: by 0.02 g. and 0.03 g., respectively. We found that all three level of concentrations of *V. tenuisecta* produced good results with minimum weight gain. Significant deterrent feeding effect on larvae weight of *T. castaneum* were recorded for all plant products, regardless of the solvents type (Table 2).

Percent of larval inhibition

The mean percent inhibition of the tested grubs was significantly higher for *C. procera* (46.33%) and *V. tenuisecta* (40.58%) at 1000 ppm each, and *A. indica* (39.67%) and *P. hysterophorus* (39.01%) at 500 and 250 ppm, respectively, while lower percent deterrence was observed for *P. hysterophorus* (20.08 and 23.66%) at 1000 and 500 ppm, respectively, and *V. tenuisecta* (25.87%) at 250ppm. The inhibition effect of solvents on the percent deterrence of larvae of the tested insect in wheat flour treated with different plant extracts at various application rates was significantly different between treatments (Table 2).

Mean pupal weight

There were no significant effects of various treatments and interactions on the mean weight of tested pupae, while significant differences were observed among solvents ($p<0.05$). The minimum mean weight of 0.05 g of *T. castaneum* pupae was observed for *C. procera* at 1000 ppm, followed by 0.09 g at 1000 ppm for *V. tenuisecta* and *P. hysterophorus*, while the maximum mean weight of 0.17 g was recorded for *P. hysterophorus* and *A. indica* each at 1000 ppm. On the other hand, the effect of solvents was significantly different from each other. The mean weight of the red flour beetle pupae was 0.05 g. in ethanol, which significantly differed from the other two solvents with 0.12 and 0.15 g (Table 3).

Percent of pupal inhibition

The maximum percent inhibition of the beetle pupae was recorded for *V. tenuisecta* at 500 ppm with 20.83%, followed by *A. indica* at 500 ppm with 17.12%, while the minimum percent inhibition was for *C. procera* at 1000 ppm and 500 ppm (3.34%). Data regarding the effect of different solvents in Table 3 revealed significant differences in the percent inhibition of pupae. We found mean percent inhibition of 13.08% in ethanol, of 10.07% in petroleum ether and of 5.66% in acetone.

Mean adult weight

Effects of treatments were non-significant, however, the lowest mean weight was recorded for *V. tenuisecta* at 500 ppm (0.03 g.), followed by *C. procera* at 1000 ppm (0.06 g.). Higher mean weight of the tested adults was recorded for *P. hysterophorus* at 250 ppm (0.13 g.) and *C. procera* at 250 ppm (0.12 g.). The mean weight of *T. castaneum* adults was significantly different between solvents.

Discussion

The insecticidal properties of several natural plant derivatives as control agents against insect pests have been verified by a number researchers (Meera & Mann 2002; Jilani *et al.* 2006; Mandal *et al.* 2005, Susana *et al.* 2013). The toxic effect of plant extracts and compounds on insect pests can be tested in several ways: like repellents (Kanvil *et al.* 2007), antifeedents, growth inhibitors, impacting on reproductive behaviour, reducing fecundity of insect pests (Papchristos & Stamopoulos 2002; Jilani *et al.* 2003) and causing mortality (Meera & Mann, 2002). Plant extracts can be easily integrated with control measures for the management of pests, as they are pest specific and safe forthe environment (Jilani *et al.* 2003; Mumtaz *et al.* 2013; Susana *et al.* 2013). Hence, various weed plants were selected owing to the various biologically active compounds they had.

Table 1. Effect of four plants extracts on fecundity and egg hatching of *Tribolium castaneum*

Treatment		Effect on fecundity				Percent of egg inhibition			
Plant extract	**Rates (ppm)**	**Petroleum ether S_1**	**Ethanol S_2**	**Acetone S_3**	**Mean* $\bar{X}$**	**Petroleum ether S_1**	**Ethanol S_2**	**Acetone S_3**	**Mean* $\bar{X}$**
A. indica	1000	113.50	30.50	88.50	77.50	6.82 hi	44.83 a-f	13.85 f-i	21.83 d
	500	99.25	75.50	103.50	92.75	6.32 i	32.92 b-i	30.59 b-i	23.28 d
	250	100.50	91.75	86.75	93.00	16.67 e-i	74.88 a	41.61 b-g	44.39 a-c
V. tenuisecta	1000	100.00	112.75	116.00	109.58	35.35 b-i	57.96 ab	45.43 a-f	46.25 ab
	500	63.50	61.50	58.00	61.00	48.27 a-e	74.69 a	33.14 b-i	52.03 a
	250	91.25	68.00	101.75	87.00	30.90 b-i	59.44 ab	5.10 i	31.81 b-d
C. procera	1000	79.50	55.75	80.25	71.83	50.02 a-d	38.50 b-h	56.27 a-c	48.27 ab
	500	126.50	80.00	94.75	100.42	11.66 g-i	30.60 b-i	34.20 b-i	25.49 d
	250	85.00	98.25	123.75	102.33	25.59 c-i	36.91 b-i	44.22 a-f	35.57 a-d
P. hysterophorus	1000	114.00	90.25	37.75	80.67	27.60 b-i	29.07 b-i	47.34 a-e	34.67 b-d
	500	88.00	263.75	67.25	139.67	9.03 hi	22.08 d-i	24.28 c-i	18.47 d
	250	103.50	240.75	89.75	144.67	16.69 e-i	43.27 b-g	25.83 c-i	28.60 cd
Control	0	108.75	99.50	77.50	95.25	28.96 b-i	47.62 a-e	9.40 hi	28.66 cd
Mean (%)		97.94	105.25	86.58	-	24.15 c	45.60 a	31.64 b	-

Hatching inhibition = eggs not becoming larvae;
*Each value is mean of three solvents (petroleum ether, ethanol and acetone)
LSD value at 0.05% for solvents = 7.13
LSD value at 0.05% for treatments =14.84
LSD value at 0.05% for interaction =25.71

Table 2. Effect of four plants extracts on larvae of *Tribolium castaneum*

Treatment		Mean larval weight				Percent of larval inhibition			
Plant extract	**Rates (ppm)**	**Petroleum ether S_1**	**Ethanol S_2**	**Acetone S_3**	**Mean***	**Petroleum ether S_1**	**Ethanol S_2**	**Acetone S_3**	**Mean***
A. indica	1000	0.06	0.02	0.08	0.06	11.56	60.47	28.90	33.64 a-c
	500	0.28	0.02	0.08	0.13	18.00	76.10	24.92	39.67 ab
	250	0.10	0.02	0.21	0.11	13.90	47.85	26.30	29.35 a-c
V. tenuisecta	1000	0.05	0.01	0.04	0.04	37.95	41.11	42.70	40.58 ab
	500	0.03	0.00	0.03	0.02	25.04	53.58	35.69	38.11 ab
	250	0.07	0.01	0.10	0.06	16.09	47.08	14.42	25.87 bc
C. procera	1000	0.04	0.01	0.04	0.03	38.73	54.75	45.50	46.33 a
	500	0.32	0.01	0.05	0.13	19.79	52.06	46.77	39.54 ab
	250	0.14	0.15	0.04	0.11	24.34	49.09	44.49	39.31 ab
P. hysterophorus	1000	0.08	0.03	0.02	0.04	10.00	32.76	17.46	20.08 c
	500	0.07	0.19	0.04	0.10	20.63	39.09	11.24	23.66 bc
	250	0.13	0.01	0.07	0.07	12.67	76.18	28.17	39.01 ab
Control	0	0.08	0.07	0.08	0.08	12.85	61.30	29.16	34.44 a-c
Mean (%)		0.11 a	0.04 b	0.07 ab	-	20.12 c	53.19 a	30.44 b	-

LSD value at 0.05% for solvents = 0.045. Means within columns followed by the same letter are not significantly different (P>0.05) using Duncan's Multiple Range test.
LSD value at 0.05% for solvents = 7.056, LSD value at 0.05% for treatments = 14.6

Table 3. Effect of four plants extracts on pupae and adults of *Tribolium castaneum*

Treatments		Mean pupal weight				Percent of pupal inhibition				Mean adult weight			
Plant Extracts	**Rates (ppm)**	**Petroleum ether S_1**	**Ethanol S_2**	**Acetone S_3**	**Mean (g)**	**Petroleum ether S_1**	**Ethanol S_2**	**Acetone S_3**	**Mean (%)**	**Petroleum ether S_1**	**Ethanol S_2**	**Acetone S_3**	**Mean (g)**
A. indica	1000	0.23	0.04	0.14	0.14	8.71 c-f	33.12 a	9.51 c-f	17.12 ab	0.18	0.01	0.11	0.10
	500	0.19	0.04	0.28	0.17	6.23 c-f	3.57 d-f	9.00 c-f	6.27 c	0.16	0.07	0.11	0.11
	250	0.12	0.10	0.09	0.10	13.67 b-f	0.00 f	1.78 d-f	5.15 c	0.13	0.03	0.08	0.08
V. tenuisecta	1000	0.11	0.07	0.08	0.09	5.42 c-f	23.81 a-c	2.46 d-f	10.56 bc	0.09	0.05	0.10	0.08
	500	0.06	0.01	0.06	0.04	19.79 a-e	30.00 ab	12.71 b-f	20.83 a	0.04	0.00	0.05	0.03
	250	0.13	0.03	0.16	0.11	11.65 b-f	6.66 c-f	11.23 b-f	9.85 bc	0.11	0.03	0.18	0.11
C. procera	1000	0.08	0.03	0.03	0.05	2.69 d-f	0.00 f	7.23 c-f	3.31 c	0.07	0.03	0.08	0.06
	500	0.26	0.06	0.04	0.12	2.98 d-f	6.12 c-f	3.57 d-f	4.23 c	0.21	0.05	0.07	0.11
	250	0.15	0.07	0.10	0.11	2.70 d-f	23.83 a-c	1.66 d-f	9.40 bc	0.11	0.17	0.09	0.12
P. hysterophorus	1000	0.16	0.06	0.05	0.09	14.70 b-f	16.71 a-f	0.89 ef	10.77 bc	0.13	0.07	0.05	0.08
	500	0.13	0.11	0.26	0.17	7.02 c-f	23.53 b-f	4.52 c-f	8.02 bc	0.13	0.08	0.09	0.10
	250	0.18	0.04	0.13	0.12	20.98 a-d	4.41 c-f	5.99 c-f	10.46 bc	0.13	0.15	0.11	0.13
Control	000	0.13	0.04	0.12	0.10	14.40 b-f	9.30 c-f	3.00 d-f	8.90 bc	0.11	0.03	0.10	0.08
Mean		0.15 a	0.05 b	0.12 a	-	10.07 a	13.08 a	5.66 b	-	0.12 a	0.06 b	0.09 a	-

LSD value at 0.05% for solvents = 0.04LSD value at 0.05% for solvents = 4.32. LSD value at 0.05% for treatments = 8.99. LSD value at 0.05% for interaction = 15.58. LSD value at 0.05% for solvents = 0.03. Hatching inhibition = eggs not becoming larvae; each value is mean of 3 solvents (petroleum ether, ethanol and acetone). Means within columns followed by the same letter are not significantly different (P>0.05) using Duncan's Multiple Range test.

We demonstrated that plant products used in this experiment had insecticidal properties that could be used for the control against insect pests of stored grain. Petroleum ether extracts of *V. tenuisecta* exhibited the highest growth inhibiting effects against *T. castaneum* adults, whereas, *C. procera* had the highest larvicidal properties. Among organic solvents used for extraction, petroleum ether extracts had generally better oviposition deterrence, while ethanol extract had more effect on egg hatching, larval and pupal inhibition, and as feeding deterrent. Growth inhibiting effects of plant extracts were not clearly dose-dependent.

Regarding the growth inhibition, significantly lower progeny of *T. castaneum* was recorded when different concentration of the tested plants was added to the flour. The extracts of *V. tenuisecta* were the most effective growth inhibitors, especially as oviposition deterrent, egg hatching inhibitor, pupal inhibitor and as a feeding deterrent, while *C. procera* was found to be having insecticidal effects. The growth inhibiting effect of *V. tenuisecta* was likely owing to the phytochemicals found in it: Khaled *et al.* (2009) reported 13 compounds in *V. tenuisecta*, among which 1-octen-3-ol is a major constituent that arrests grubs molting. The extracts of *C. procera* contain alkaloids and phenols phytochemicals which act as larval insecticides. This plant has been used also as a medicine against toothache; cough and subcutaneous diseases, therefore it has been proved safe for humans and other animals. The results are in conformity with those of Begum *et al.* (2011) who conclude that leave products of *C. procera* and *Annona squamosa* could be effective against the maggots of *Musca domestica*.

Ethanol had the highest growth inhibiting effect as an egg hatching inhibitor, larval inhibitor, pupal inhibitor and feeding deterrent. Ethanol is used for dissolving higher polarity compounds or compounds with long chains. We found ovicidal effect for acetone, likely owing to its non-polar nature. Our results confirmed the findings of Jilani *et al.* (2006) who reported significantly higher mortality in fruit flies when fed on diet containing ethanol extract of *Verbena officinalis* for ten, 15 and 20 days, as compared to petroleum ether extracts. Jilani *et al.* (2003) collected neem oil from various localities of Pakistan and tested it as a growth inhibitor against *T. castaneum*. They found that the neem oil from Karachi was the best growth inhibitor when fed to test insects in wheat flour at 250 or higher concentration, resulting in significantly lower numbers of larvae, pupae and adults. We used neem oil as a standard and recorded similar effects on the progeny of *T. castaneum*. Most of the previous studies have tested either plant powder, oil or extracts in one organic solvent, thus they only studied a portion of the plant chemicals. In the present study, the whole spectrum of chemical profiles of the tested plants was examined in different solvents with diverse polarities. Our results on the growth deterrence of the tested beetles are in compliance with the finding of the aforementioned researchers.

Mordue & Nisbet (2003) demonstrated that Azadirachta, a product of *Azadirachta indica*, had direct and indirect influence on the behaviour and physiology of insect pests. It reduced reproduction, slowed down development, induced abnormal and delayed molting in insect pests. Furthermore, neem seeds are known to contain many related triterpenoids in addition to Azadirachta, such as 3-triglyol-azadirachtol (Azadirachta B), nimbin and salanin. Azadirachta is one of the most important plant derived compounds used in insect pest control; it has very low mammalian toxicity and is relatively safe to beneficial insects (Schmuttere 1990). It has mainly antifeedent effects (Zehnder & Warthen 1988; Schmutterer 1990) and is an insect growth regulator (IGR) properties. Neem interferes with many life processes in adults (Saxena *et al.* 1993) during larval development (Saxena *et al.* 1981; Sieber & Rembold 1983) and during metamorphosis of insects (Koul *et al.* 1987). In the present studies *V. tenuisecta* had similarly a strong oviposition deterrence, hatching inhibition, pupal inhibition and feeding deterrent effects.

Plant products cannot replace pesticides completely; however they can reduce their quantity, and thus reduce the toxic residue in store grains. Insect pests are unlikely to develop resistance against plant extracts in the way they do against synthetic chemicals. Food products are usually stored in bags, while lesser amount is preserved in farms around Pakistan. Phosphine gas tablets are mainly used for the management of pests. Several insect pests have developed resistance to conventional pesticides used as grain protectant. Therefore, currently studies focus on replacing synthetic chemicals with natural plant products. Such products have been successfully used in the past as insect growth inhibitor for the control of store pests. It can be easily done through the use of insect proof bags treated with plant extracts as demonstrated by Jilani & Amir (1985). In the case of long-period storage there is a possibility of re-infestation of the pests which can be managed through combined use of neem oil and fumigants (Jilani & Saxena 1990).

Active ingredients derived from plant extracts have different mode of action against insects, such as oviposition deterrence, repellent and growth inhibi-

tor. Therefore, the present work provides a base line for the management of the store grain insect pests in government granaries and farmer storehouses. Plant derived products can also be used successfully for the control of field insect pests, such as mealy bugs, tomatoes insects and fruit flies.

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