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Insecticidal Activity of Some Plant Extracts Against *Trogoderma granarium* (E.)

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Abstract

An investigation was carried out to evaluate the insecticidal activity of *Allium sativum*, *Zingiber officinale* and *Nigella sativa* extracts against the larvae of *T. granarium* under laboratory conditions in the University of Agriculture, Faisalabad, Pakistan during 2009-10. The highest concentration of (6 %) *Z. officinale* was found to be comparatively more toxic (16.70 %) than those of *A. sativum* (10.45 %) and *N. sativa* (5.49 %) at 96 hours exposure. Regarding latent effect of the test plant materials, *Z. officinale* gave significantly higher reduction (54.15 %) in F_1 progeny than *N. sativa* (41.97 %) and *A. sativum* (32.19 %). The study reveals that *Z. officinale* had a strong latent effect against the larvae of the test pest. Further investigations on the efficacy of longer exposure in combination with higher concentrations of these extracts can be helpful to reduce the wheat store-insect damage.

Keywords: Allium sativum*, Insecticidal efficacy, larval mortality, latent effect, *Nigella sativa*, *Trogoderma granarium*, *Zingiber officinale

1. Introduction

Wheat (*Triticum aestivum* L.) is the major and staple food of Pakistan (Ahmad, 2009). About 90 percent grains produced for daily consumption of human and animals come from cereals like wheat, rice and maize (McFarlane, 1989) while insect pests are of a great importance for major causes of grain losses during storage (Scotti, 1978). Storage of wheat on large scale is a vital component of food security where stored grains insect are causing severe damage to the stored commodities, and of course, the food insect contamination represents a crucial hurdle for export items and food industries (Rajendran, 2002). During storage, grains are destroyed by many stored-grain insect that are responsible for worldwide loss upto 10-40 % annually (Mathews, 1993). Khapra beetle, *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae) is one of the serious storage insect pests, causing tremendous loss to stored grains in tropical regions including Pakistan (Howe, 1965 and Bell and Wilson, 1995).

Synthetic chemicals are major control measures for agricultural insect pests (Mathewes, 1993) all over the world for both field crops and post-harvest protection of crops. During storage for grain protection, liquid insecticides and gases in the form of phosphine and methyl bromide have been widely used as it is cost-effective and simple method (Shaaya *et al.*, 1997; Fields and White, 2002; Lee *et al.*, 2004 and Islam *et al.*, 2010). However, their constant use has caused pest resistance to insecticides, environmental

pollution and side effects to non-target arthropods due to sub-standard techniques of fumigation that ultimately disrupt the beneficial organisms (Irshad and Iqbal, 1994; Bell and Wilson, 1995; Desneux *et al*., 2007 and Pimentel *et al*., 2009).

Botanical insecticides have long been used as attractive alternatives to synthetic insecticides for insect pest management because botanicals cause little threat to the environment or to human health (Behal, 1998 and Isman, 2006). In order to avoid the adverse effects of synthetic chemicals, recently emphasis has been given on investigating plant derivatives as grain protectants (Yang *et al*., 2010). Plant extracts have proved to be effective, safe, cheap, and easy to process and apply for farmers in developing countries (Belmain *et al*., 2001; Isman 2006 and Regnault-Roger *et al*., 2012). In order to make a new pesticide available and make it more successful (Copping and Menn, 2000), more than 75 plant species of different families have been tested and recorded as insecticides for stored-grain insect control (Rajendran and Sriranjini, 2008).

The ground-up root (tuberous rhizomes) of Ginger (*Zingiber officinale* Roscoe) has essential oils (Regnault-Roger *et al*., 2012) that are used as medicine since early times. Several scientists have reported its antifungal (Hasan *et al*., 2005 and Singh *et al*., 2005), antimicrobial (Sa-Nguanpuag *et al*., 2011) and insecticidal properties (Ukeh, 2008 and Owolabi *et al*., 2009). Several chemical constituents have been reported in *Z. officinale:* α-pinene, camphene, β-pinene, 1,8-cineole, linalool, borneol, γ-terpineol, nerol, neral, geraniol, geranial, geranyl acetate, β-bisabolene and zingiberene. Kalwanjii (*Nigella sativa* Linn.) - also known as black seed or black cumin (Rayan *et al*., 2011) is a member of family Ranunculaceae (Atta, 2003), and the seeds of this annual herbaceous plant are rich essential oils, fixed oils, flavonoids, saponins, alkaloids, 20% proteins and 37% lipids (Burits and Bucar, 2000; Al-Ghamdi, 2001 and Ali *et al*., 2012). The main constituents of essential oils are thymoquinone, dithymoquinone carvacol and anethole 4-terpinole (Bruits and Bucar, 2000).

Adamu *et al*., (2010) reported the presence of nine volatile oils containing 2-methyl-5(1-methyl ethyl)-Bicyclo[3.1.0]hex-2-ene as the major (62.28%) while alphapinene as the minor constituent (2.28%). Several scientists have reported it's medicinal and insecticidal (Dashpande *et al*., 1974) and antimicrobial (Mashhadian and Rakhshandeh, 2005) values. Garlic (*Allium sativum* L.), also known as stinking rose, is a perennial pungent herb with globose bulb (Fenwicka *et al*., 1985). The genus, *Allium* (Alliaceae Family) is a very large and geographically diverse species (Khokar, 2003). It has been reported that the chief constitute of its volatile oils are allicin, 2-propene sulfenic acid, 2-propene thiol, propylene, thioacrolein and ajoene (Gurusubramaniana and Krishna, 1996) while Huang *et al*. (2000) have reported its two major constituents: methyl allyl disulfide and diallyl trisulfide. Several scientists have reported its antifungal and antimicrobial effect (Benkeblia, 2004) and insecticidal properties (Regnault-Roger, 1997).

The present study was conducted to evaluate the insecticidal activity of different doses of bulb extract of *A. sativum*, rhizome extract of *Z. officinale* and seed oil extract of *N. sativa* against *T. granarium* larvae.

2. Materials and Methods

2.1. Insect culture

Heterogeneous population of *T. granarium* was collected from godowns of Food Department located in Fasisalabad district of Punjab, Pakistan for rearing in the laboratory according to the procedure described by Ali *et al.* (2012). The study was conducted at Grain Training and Storage Management Cell, Department of Agri. Entomology, University of Agriculture, Faisalabad, Pakistan during 2007-08. The insects were kept with whole wheat grains in earthen jars (2.5-liter), covered by muslin cloth and

tightened with rubber bands to prevent the escape of insects. The pots were kept in growth chamber in dark room at temperature (T) of 29 ± 2 ^{0}C and relative humidity (RH) of 70 ± 5 %. After 72 hours, adult population was removed from the substrate using a standard 44-mesh sieve (pore size 2 mm) and the sieved wheat grains having pest eggs were shifted to plastic containers (1-liter) covered by muslin cloth and secured by rubber bands. Five hundred g of fresh sterilized wheat grains were placed as food and then these containers were kept in same climatic conditions as described above. In this way, homogeneous population of *T. granarium* was obtained after 28-32 days for further use in the experiment.

2.2. *Preparation of plant extracts*

One kg, each of fresh rhizomes of ginger *Zingiber officinale* (R.), bulbs of garlic *Allium sativum* (L.), and 100 g seeds of kalwanji *Nigella sativa* (L.) were collected from local market in Faisalabad city. All samples were crushed using blender (Model No.WTB-110B, Jiangmen Windtech Electrical Industry Co., Ltd. China), and then electrically ground to fine powder after air drying at room temperature. The samples were stored in a clean and dry place until used. Extracts of garlic and ginger powder were obtained through rotatory shaker. For this purpose, 50 g powder of each sample was dipped in a flask containing 500 ml acetone as solvent. Flasks were closed with cotton plug and aluminum foil. These flasks were placed in rotatory shaker for extraction of ginger and garlic extracts for 24 hours. The extracts were evacuated thereafter in rotatory evaporator and filtered in order to obtain the stock solution. The oil extraction of kalwanji was done by fixing flasks placed in the Soxhlet Extraction Apparatus according to Valladares *et al.* (1997). Ground kalwanji seeds were poured in the filter tube made by making a role of filter paper, closed from one end. There was a flask beneath this glass tube in which 500 ml of acetone was added. This apparatus was placed on burner. The acetone was vaporized and extract was settled at the flask's bottom. Filtration was done after extraction and the extract was concentrated in the rotatory vacuum evaporator thereby getting 100% stock solution. From 100% stock solution, 2, 4 and 6% concentrations were prepared and were marked as T1, T2 and T3, respectively.

2.3. Bioassays

2.3.1 Filter paper application

Whatman No.1 filter papers were labeled with lead pencil impregnation. Each filter paper was supported on three common pins inserted into a sheet of soft pith to prevent loss of extract to the substrate during application. Each concentration was applied with the help of syringe as microapplicator. After drying, the filter papers were transferred to 80-mm diameter glass Petri-dishes where these were allowed to remain overnight. Afterwards, third instar larvae were confined under inverted glass funnels on treated filter papers. The deposits on the filter paper were referred to as concentrations of solutions in a volatile solvent.

2.3.2 Insect mortality and population build up

Larval mortality and population build up of test insect pests against plant materials were investigated by using three concentrations as mentioned above along with acetone as control in three replications in a complete randomized design (CRD). Twenty larvae were exposed to each treatment and the same number was also confined on filter papers treated with acetone only as an untreated check. The mortality data were recorded after 24, 48, 72 and 96 hours exposure. The bioassays were conducted under the same climatic conditions as for rearing except for L14:D10 photoperiod supply. The specimens were considered dead if they failed to respond while prodding with fine paint-brush. The survivors were released on fresh grains for recording the latent effect of treatments on the population build up of test pest in F_1 progeny. For this purpose, 200 g of sieved grains were placed in each glass jar (volume: 30 cc) for each

treatment. The mouth of each jar was covered with muslin cloth and secured by a rubber band to disallow the entry of any insect in the jar. It was acclimatized in the laboratory for a period of 35 days. This part of experiment was also conducted under the same climatic conditions as for toxicity assays.

The data regarding insect mortality and population build up were subjected to one way analysis of variance (ANOVA) using statistical software, Statistix version 8.1 2005. Means of treatments were compared by LSD at 5% level of significance.

3. Results and Discussion

The results of the investigation regarding test plant materials, exposure time, interaction of treatments and exposure periods varied significantly. The results regarding mortality under different treatments and exposure times are given in Table 1 and Table 2, while for reduction in population build up are given in Table 3. Table 1 reveals that mortality of *T. granarium* larvae ranged from 3.17-5.49, 7.83-10.45 and 13.01-16.70 % at 2, 4 and 6 percent concentrations in case of *N. sativa*, *A. sativum* and *Z. officinale*, respectively with no mortality in control treatment. The effect of treatments differed significantly from each other but the overall mortality percentage was generally very low. Larval mortality varied with concentration as it was higher in the highest concentration (6 %) of each plant extract. Table 2 shows that the larval mortality varied with exposure time; at 96 hours exposure, these were 6.29 ±1.5, 13.53 ±1.5 and 22.05 ±3 SE % in case of *N. sativa, A. sativum* and *Z. officinale*, respectively. An appreciable decrease in population build up of F_1 progeny of *T. granarium* exposed to different treatments of *N. sativa, Z. officinale* and *A. sativum* for one month exposure was obtained in Table 3. The reduction in population varied with concentration of each plant material; at 6 % concentration it was 41.97, 32.19 and 54.15 % in case of *N. sativa, A. sativum* and *Z. officinale*, respectively.

The results above revealed that mortality and latent effect on population build up of *T. granarium* in the F_1 progeny varied greatly with different extracts concentrations of ginger rhizome, garlic bulb and kalwanji seed oil. Out of the three botanicals, *Z. officinale* gave comparatively higher mean mortality (15.05 %) at the maximum exposure period of 96 hours than *A. sativum* (8.98 %) and *N. sativa* (4.27 %). The maximum concentration (6 %) caused reduction in population build up by 41.97, 32.19 and 54.15 % in case of *N. sativa, A. sativum* and *Z. officinale,* respectively. The results might be useful to control the stored grain insect pests in open and closed spaces for better management. However, certain plant essential oils, like those from garlic may leave a persistent odour and when applied at a high dose could cause food to retain a strong smell and unpleasant taste (Sekou *et al*. 2000 and Benkeblia, 2004). So, this aspect has to be kept in mind while trying higher dose for effective results.

The repellency of *N. sativa, A. sativum* and *Z. officinale* has been studied in the past by many scientists. Shaaya *et al*. (1997) assessed the fumigant toxicity of a large number of essential oils extracted from various species of herb plants against several major stored-product insects. *Tribolium castaneum* (Herbst.) was found to be the most resistant compared with *Sitophilus oryzae* (L.), *Rhizopertha dominica* (F.) and *Oryzaephilus surinamensis* (L.), to most essential oils tested. With the highly active *Labiatae* species, Oil ZP51, a concentration of 1.4-4.5 µl/l air and 24 hr exposure time was enough to obtain 90 % mortality of all the insects in space tests. In columns, 70 % filled with wheat, a concentration of 50µl/L and 7 days exposure period was needed to obtain 94-100 % mortality of the insects. Similar studies carried out by Yanfang *et al*. (1997) showed that oil of *A. sativum* extracted by the petroleum ether repelled adults of the *T. granarium* most efficiently at 0.2 ml per culture dish while repellence after 72 hours was 81%.

Gulati (2007) tested the efficacy of commercially available garlic products like garlic powder, oil and garlic oil emulsion against stored grain insects and reported that all products reduced pest population by 96-100% at concentration of 0.2%. Oparaeke *et al.* (2007) revealed that aqueous garlic bulb extracts significantly reduced the populations of the *Maruca vitrata* and *Clavigralla tomentosicollis* on cowpea compared with the untreated control. Abd El-Salam (2005) reported the garlic oil as the most effective deterrent against *Callosobruchus maculates* on cowpea with 100% oviposition deterrent index.

Table 1. Percent mortality of *T. granarium* larvae at different concentrations of tested plant products

Plants	Extract	Conc. (%)	Mortality (%)	Average mortality (%)
Nigella sativa	Seeds	2	**3.17	
		4	4.17	4.27 c
		6	5.49	
Allium sativum	Bulbs	2	7.83	
		4	8.68	8.98 b
		6	10.45	
Zingiber officinale	Rhizomes	2	13.01	
		4	15.44	15.05 a
		6	16.70	

*T_1= 2, T_2= 4, and T_3= 6% solution of plant extracts.

**Values followed by same letter(s) are statistically identical by LSD at 5% level of significance.

Table 2. Percent mean mortality of *T. granarium* larvae at different exposures periods (4 days) of tested plant products

Exposure	Percent mean mortality ± SE			
hours	*N. sativa*	*A. sativum*	*Z. officinale*	Average
24	2.5	4.39	9.81	5.56
48	3.59	6.53	12.34	7.48
72	4.66	11.47	15.99	10.70
96	6.29	13.53	22.05	13.29

Table 3. Population and percent reduction of *T. granarium* over control in F_1 progeny under different treatments

Plant Extract	Concentrations (%)						
	*2		4		6		Control
	Total population	% reduction	Total population	% reduction	Total population	% reduction	Total population
N. sativa	**335.3bc	20.03	225.0ef	39.18	243.3f	41.97	419.3a
A. sativum	360.7b	15.86	305.3cd	28.78	290.7d	32.19	428.7a
Z.officinale	330.0bc	20.67	281.3de	32.37	190.7g	54.15	416.0a

*T_1= 2, T_2= 4, and T_3= 6% solution of plant extracts
**Values followed by same letter(s) are statistically identical by LSD at 5% level of significance.

4. Conclusions

The results of the present study have shown that the tested botanicals did not give encouraging results in contact toxicity against *T. granarium*. However, the latent effect of exposure to these botanicals gave very encouraging results, showing a significant reduction in population buildup in F_1 progeny. These results clearly indicate the need for further investigations on the efficacy of longer exposure period in combination with higher concentration that will set the stairs to create new biopesticides for stored grain insect pests management. Finally, the essential oils effect on grains quality, taste and risks to human health and environmental pollution would need to be determined before commercialization and at most, to confirm the active and stable compound present inside essential oils in order to protect the flavor of foodstuffs or stored grain from being tainted.

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