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Toxicological and repellent potential of some plant extracts against stored product insect pest, *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae)

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Abstract

Laboratory experimentations were conducted for the evaluation of contact and repellent activities of acetone based plant extracts of *Allium sativum* (Garlic), *Azadirachta indica* (Neem), *Citrus limon* (Lemon) and *Eucalyptus globules* (Safaida) against the insect pest of stored grain commodities, *Tribolium castaneum*. Various concentrations (5, 10 and 15%) of the plant extracts were applied on the filter papers in the bioassay experiments and after the release of 15d old beetles, mortality was evaluated after fixed intervals (24, 48, 72, 96, 120, 144 and 168h). Repellent action was also checked using the area preference method and preference of adult beetles were checked after fixed period (24, 48 and 72h). Contact toxicity experiments revealed that *E. globules* (9.41%) proved to be more effective followed by *A. sativum* (9.20%), *C. limon* (8.39%) and *A. indica* (7.48%). Repellent action confirmed *E. globule* (76.29%) to be more potent and *A. indica* (63.08%), *A. sativum* (59.62%) and *C. limon* (50.12%) were least effective, respectively. Results reflected a positive potential of plant extracts as suitable substitute of conventional synthetic insecticides for the management of insect pest attacking stored commodities.

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Introduction

Stored commodities are vulnerable towards attack of insects (Ukeh *et al.*, 2012) and a possible infestation can deteriorate the quality as well as the quantity of attacked commodity (Nadeem *et al.*, 2012) resulting in significant decrease in volume, substantial weight loss and reasonable germination damage (Phillips and Throne, 2010). Due to insect attack, there occurs a considerable increase in humidity (Keskin and Ozkaya, 2013) as well as temperature of attacked commodity (Semeao *et al.,* 2012) which in turn supports the development of fungus and partial germination of grains (Padin *et al.*, 2013). Use of chemicals is the conventional scheme in IPM and it causes many worse effects on environment which in turn (Perez *et al.*, 2010) have a definite impact on health of living organisms including humans (Meena *et al.*, 2006; Hashim and Davi, 2003). Many plant extracts are reported to have insecticidal properties (Isman, 2000). Essential oils of plant origin are rich in mixtures of complex volatiles and various metabolites. Secondary metabolites are natural defensins that protect plants from any possible external threat (Bakkali *et al.*, 2008). Natural plant extracts have proved to be effective against various stored grains insects especially *Tribolium castaneum* (Sagheer *et al.*, 2011). Present research was focused on the evaluation of some indigenous plant as toxicants and repellents against *T. castaneum* and also to screen out the most effective natural plant extracts for inducing the quick knockdown effect towards the insect pests of intact and processed stored commodities.

Materials and methods

Experiments were performed in the Stored Grain Research, Training and Storage Management Cell of Department of Agricultural Entomology, University of Agriculture, Faisalabad, during the year 2012-13.

Insect collection

Mass Collection of Red Flour Beetle, *Tribolium castaneum*, was made from the markets and stores of District Faisalabad.

Insect rearing

Collected insects was being kept in the sterilized plastic jars under optimum conditions in an incubator (SANYO incubator MIR-254) having uniform temperature and relative humidity of 27±3°C and 70±5%, respectively. Wheat flour was utilized as culture media for *T. castaneum*. Adults were sieved out and hundred adults were released in each of the plastic jars having 300gm of sterilized flour and covered with muslin cloth. Adults were allowed to mate and lay eggs. After oviposition phase of 5 days, beetles were sieved out of the flour. The flour, having the eggs, was kept under the uniform conditions inside the incubator. Homogenous population was achieved after a time period of 28-35 days as illustrated by Islam and Talukder (2005).

Plant materials

Leaves of *Azadirachta indica* (Neem) and *Eucalyptus globules* (Safaida) were collected from Botanical Garden, University of Agriculture, Faisalabad, while *Citrus limon* (Lemon) and *Allium sativum* (Garlic) were bought from the Vegetable Market, Faisalabad.

Preparation of plant extracts

Shade dried leaves, fruit peel and plant parts were washed and dried under the shade to get the dried form of plant material. Grinder was used to crush the plant material to fine powder and then sieved with a fine mesh sieve. The extraction was made by mixing 50 g of ground sieved sample and 100 ml of acetone and shaking was ensured for 24 hours with the help of Rotary Shaker (IRMICO OS-10), adjusted at 120 rpm. After 24 hours, filtration was made with the help of filter paper. Preliminary extract was subjected to the Rotary evaporator to get 100% stock solution as described by Hasan *et al.* (2005) and Sagheer *et al.* (2013).

Bioassay for percent mortality

Different concentrations (5, 10 and 15%) of acetone based extracts were applied on the filter paper and were allowed to dry for a reasonable time period. Control was maintained by treating the filter paper with acetone only. For examining the percent mortality, 30 adults (15 days old) were taken in the Petri dishes embedded with Whatman's filter paper, covered with lid and tightened with scotch tape on both sides. Small amount of wheat flour was provided to decrease chances of mortality due to starvation. Mortality of adult beetles was being recorded after period of 24, 48, 72, 96, 120, 144 and 168 hours. Experiment was replicated three times and Completely Randomized Design (Factorial) was followed.

Bioassay for percent repellency

Repellency of the plant extracts was checked against the T. castaneum by using the area preference method (Mohana and Fields, 2002) in which filter paper was cut into two equal halves. Different concentrations were made on the one half. After drying, the treated paper was stapled together and was adjusted in the Petri dishes. Twenty adult beetles of T. castaneum were released in the center of both halves. Repellency data was taken after a period of 24, 48 and 72 hours. Diet will be provided on both sides (treated and untreated end of filter paper) to decrease mortality due to starvation.

Results

Experiments proved a definite impact of plant extracts on the overall mortality and repellency of the test insect. Table 1 demonstrates the effect of various concentrations of different plant extracts on the adult beetles. Quick knockdown effect was highest in the case of 15% concentration of *E. globules* (14.51%) while the lowest was observed at 5% concentration of *C. limon* (3.70%) . Overall all the concentration tended to affect the adult stage and by increasing the concentration the increase in mortality was also visible in all the extracts used in the experiment (15%>10%>5%).

Table 1. Contact toxicity of plant extracts at various concentrations (5, 10 and 15%) against *Tribolium castaneum*.

	% Mortality ± S.E			
Conc.	*A. sativum*	*A. indica*	*C. limon*	*E. globules*
5	7.75±0.96 bcd	5.17±0.82 de	3.70±0.53 e	5.01±0.82 de
10	9.05±1.33 bc	7.43±1.15 bcd	6.77±0.65 cde	8.71±1.07 bc
15	10.82±1.54 b	9.86±1.46 bc	14.69±1.75 a	14.51±1.34 a

Table 2. Contact toxicity of plant extracts at various exposure periods (24, 48, 72, 96, 120, 144 and 168h) against *Tribolium castaneum*.

	% Mortality ± S.E			
Time	*A. sativum*	*A. indica*	*C. limon*	*E. globules*
24	2.96±0.37hi	1.85±0.97i	3.70±1.03ghi	3.33±1.11hi
48	3.70±0.37ghi	3.33±0.96hi	4.82±0.97efghi	5.92±1.54defghi
72	5.62 ±1.12defghi	4.49±0.79fghi	5.99±1.18defghi	6.74±1.48cdefghi
96	10.11±1.37abcdef	6.74±1.12cdefghi	8.98±1.86bcdefgh	9.73±1.92bcdefg
120	12.88±1.61ab	10.23±1.27abcdef	10.61±2.71abcde	11.36±1.60abcd
144	13.26±1.51ab	11.36±1.50abcd	12.12±2.94abc	12.88±1.89ab
168	15.91±1.70a	14.39±1.92ab	12.50±2.89abc	15.91±2.12a

Table 2 demonstrates the effect of various time intervals on the overall mortality of the test insect. Highest mortality (15.91%) was observed in the case of E. globules and A. sativum after a period of 168h and lowest (1.85%) was observed in the case of A. indica. Time interval affected the knockdown ability of plant extracts and a delay after the treatment was found to be effective as the mortality tended to increase with the passage of time.

Repellence against the plant extracts was also observed and it varied with the type of extract used in the bioassay. As indicated by Table 3, highest repellence (82.96%) was observed in the case of *E. globules* at 15% of concentration while the lowest (42.96%) was induced by *C. limon* extract at 5% concentration. It was observed that the repellency tended to increase as the level of concentration increased. At 5% repellence was lower while at 15% a greater deterrence forced the insects to remain in the untreated half.

Table 3. Percent Repellency of plant extracts at various concentrations (5, 10 and 15%) against *Tribolium castaneum*.

	% Repellency ± S.E			
Conc. (%)	*A. sativum*	*A. indica*	*C. limon*	*E. globules*
5	63.33±3.60bcd	55.16±4.12cde	42.96±2.80e	73.33±3.72ab
10	51.11±2.88de	68.15±2.67b	44.81±1.67e	72.59±3.84ab
15	64.44±3.92bc	65.93±3.91bc	62.59±4.50bcd	82.96±2.44a

Table 4 indicates the effects of time on the repellence. Results depicted that the repellence tended to decrease with the passage of time and the highest was observed after 24h in case of *E. globules* (86.67%) while the lowest was observed in the case of *C. limon* (43.70%) after 72 hours of treatments. All the extracts showed lesser repellence after a delayed period of time showing the low persistence of these natural extracts in the environment.

Table 4. Percent Repellency of plant extracts at various exposure periods (24, 48 and 72h) against *Tribolium castaneum*.

	% Repellency ± S.E			
Time	*A. sativum*	*A. indica*	*C. limon*	*E. globules*
24	67.04±3.01bcd	72.22±2.83bc	56.30±4.28def	86.67±1.36a
48	60.00±3.96cde	61.85±3.73cde	50.37±4.24ef	74.81±2.94ab
72	51.85±3.33ef	55.19±3.33def	43.70±3.74f	67.40±2.98bcd

Discussion

Our experiments proved a definite impact of plant extracts towards inducing quick knockdown and repellence against the adults of stored grain insect pest, *Tribolium castaneum*. Garlic has some of the very strong biological properties that are very lethal to insects and pathogens (Corzo *et al.*, 2007) and it proved to be effective as it induced greater repellent effects even at the lower concentrations. Jahromi *et al.* (2012) also conducted experiments to determine the repellent effects of *Allium sativum* in the form of emulsion against the stored pests of primary and secondary nature and our results are somewhat in accordance with their experimentation. They determined the highest repellent effects to be 55.47% against the *T. castaneum* at 10% while our results are 51.11% at the same concentration. Same results indicate a definite impact of garlic extracts as a potential repellent against different insects.

Our experiments proved the effectiveness of the garlic extract as a repellent. Similar studies were conducted by Rahman and Motoyama (2000) and our results are in accordance with their experiments. Ofuya *et al.* (2010) evaluated the impact of the crude crushing of garlic bulb against the stored product pest, *Callosobruchus maculatus* and the fumigant toxicity was found to be associated with the bulb crushing and concluded that 7g of the garlic crushing per 20g of seed was very effective in the egg laying inhibition and the overall mortality of test insect. In our experiments garlic remained effective as a fumigant as it reduced the overall life period of adult beetles. Maximum of 10.82% of mortality was recorded in our experiments and the results are in accordance with them. Iqbal *et al.* (2010) also conducted studies to find out the repellent impact of Neem extract but the results are very much opposite as they reported the repellency ranging from 63% to 75%. They reported a gradual decrease in repellency over 8 weeks but in our case the repellent effects are rapidly decreased and the population was not limited to the treated half rather a free movement was observed with the passage of time. Islam and Talukder (2005) conducted experiments on the toxicity of the neem powder on the mortality of the test insect and 53.13% mortality was observed on an average but in our experiments the mortality at the highest concentration was 9.86% and it was very low as compared to their experiments. This contradiction in the results may be possibly due to the difference of the extracts as Islam and Talukder (2005) utilized the seed extracts and our experiments were based on the leaf extracts of Neem. It may possibly indicate the level of resistance of test insect against the *Azadirachta indica* (Neem). Our results are also in accordance with the experiments of Ahmed *et al.* (2009) who worked on three formulations of Neem and deducted a definite impact on the progeny inhibition in the case of *T. castaneum*. Similar experiments were conducted by Musabyimana *et al.* (2001) and the effect of Neem was significant against the test insect.

As far as *Citrus limon* is concerned, results are in accordance with the Moravvej, *et al.*, (2010). They found a definite impact of two species of *Citrus* on the biology of stored grain insect, *Callosobruchus maculatus*, indicating a definite effect of peel extracts on the biology of insects infesting storage commodities. Our results relating to *Eucalyptus globules* are in accordance with Tunc *et al.* (2000) checked for the fumigant toxicity of vapors of essential oils from five plants against two stored grain insect pests, i.e., *Tribolium confusum* and *Ephestia kuehniella*. Results proved a definite impact of *Eucalyptus* sp., along with other four extracts, on the biology of both insects attacking the storage commodities.

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