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Bioactivity of *Nigella sativa*, *Syzygium aromaticum* and *Trachyspermum ammi* extracts against *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae)

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ABSTRACT

Acetone based plant extracts of *Nigella sativa*, *Syzygium aromaticum* and *Trachyspermum ammi* was assessed against the stored insect pest of processed commodities, *Tribolium castaneum*. Seed extracts of *N. sativa* and *T. ammi* and flower bud extracts of *S. aromaticum* were utilized for their contact toxicity against the stored beetle adults. Periodic analysis for the contact toxicity, by impregnating filter paper discs and at various concentrations, revealed a significant effect of these extracts on biology of stored product beetle pest, as *T. ammi* (7.17%) unveiled greater knock down effects followed by *S. aromaticum* (7.00%) and *N. sativa* (3.56%), respectively. Fallouts suggest a safer potential of these natural extracts toward disturbing the biology and invasion of stored product pest, *T. castaneum*. Effects indicate a definite potential of these extracts through their utilization as bio-pesticides towards optimizing food storage.

Keywords: *Nigella sativa*, *Syzygium aromaticum*, *Trachyspermum ammi*, *Tribolium castaneum*, bioactivity, contact toxicity, repellency.

1. Introduction

Storage of agricultural production is much needed to sustain food security of any country but on the other hand, these commodities provide perfect breeding places for a variety of pests, particularly storage beetles (Ukeh *et al.*, 2012). The quality and quantity of the commodities attacked by the stored grain insect pests is reduced in severe infestation that results in the reduction of volume, germination damage in grains, and ample weight loss (Phillips and Throne, 2010; Nadeem *et al.*, 2012) ^[15, 11]. Humidity is significantly increased at the place of infestation and the temperature of the infested commodity is enhanced (Semeao *et al.*, 2012; Keskin and Ozkaya, 2013) ^[18, 7]. This increase in temperature and humidity causes fungus development and the incomplete germination of grains in storage house (Padin *et al.*, 2013) ^[12]. A number of uncontrolled ecological aspects and reduced storage technologies support the magnification of insect infestation (Upadhyay and Ahmad, 2011) ^[20]. Chemical control methods are a basic need of the optimal pest management measures. For the management of insect pests, chemical control measures are still being used even the adverse and toxic effects of synthetic insecticides are well known. Environmental pollution is the main hazard for the sustainability of the ecological biome owing to the uncontrolled use of synthetic pesticides and therefore, plant extracts presents a safer solution to all these issues (Sagheer *et al.*, 2013). Insecticide resistance is causing the development of resistant biotypes in insects. The predominant toxicity of synthetic chemicals is causing troubles for the bio-control agents of insect pests (Perez *et al.*, 2010).

The negative ecological changes caused by these chemicals is resulting negative health impacts on human beings and other living organisms (Hashim and Davi, 2003; Meena *et al.*, 2006). The necessary step at recent times is to give consideration to find unconventional substitutes for the control of storage insect pests. Utilizing the plant products and secondary metabolites present in various plants as natural control materials is one of the methods for management (Isman, 2000). It is reported by Bakkali *et al.*, (2008) that secondary metabolites that defend plants from external hazard are natural defensive products. Some plants extracts have evidenced as effective against several storage insects especially *Tribolium castaneum*, the red flour beetle (Sagheer *et al.*, 2011; Khan *et al.*, 2013) ^[16, 17].

2. Materials and Methods

All the experiments were performed at Grain Research Training and Storage Management Cell, Department of Entomology, University of Agriculture, Faisalabad, Pakistan, during the year, 2013-14.

2.1 Insect Collection

Collection of red flour beetle was made from Faisalabad. Grain market and local merchant shops were visited for this purpose.

2.2 Insect Rearing

Rough collection was allowed to remain in sterilized flour kept in plastic jars. The optimum conditions were kept uniform at (SANYO incubator MIR-254). Uniform temperature and relative humidity was kept at 27 ± 3 °C and $70\pm 5\%$, respectively. After a period of 5 days, the adult beetles were sieved out and the remaining flour, having the insect eggs, was kept in 300 ml plastic jars. Homogenous population was achieved after 28-35 days as given by Islam and Talukder (2005) [6].

2.3 Plant Material

Seeds of *N. sativa* and *T. ammi* and flower buds of *S. aromaticum* were obtained from the Spice Market, Clock Tower Faisalabad, Pakistan.

2.4 Preparation of plant powder and extracts

The dried material was grinded in an electric grinder and then sieved to get fine particles. 50 grams of ground sample was allowed to mix with 100 ml of acetone in a conical flask. The covered flasks were then allowed to shake on Rotary Shaker

(IRMICO OS-10), adjusted at 120 rpm for 24 hours. After this period, the initial filtrate was obtained by using filter paper. Concentrated stock solution was obtained by subjecting the filtrate to rotary evaporator, as devised by Hasan *et al.* (2005) and Sagheer *et al.* (2013) [4,8].

2.5 Bioassay for percent mortality

Different concentrations (5, 10, 15%) were made by diluting the concentrated stock with acetone. These dilutions were then applied on the filter paper and allowed to dry for 60 minutes. Control was maintained by treating the filter paper with acetone. These treated papers were then fitted in Petri dishes. Thirty adults (15 days old) were released and the plate was covered. Small amount of insect diet (wheat flour) was also introduced to reduce the chances of death due to starvation factor. Data was taken at 24, 48, 72, 96, 120, 144 and 168 hours. Experiment was replicated three times and Completely Randomized Design (Factorial) was followed. Statistica 7.0 was used for statistical analysis.

3. Results

Experiment was designed to know the toxic potential of common spices against the stored pests. Table 1 demonstrates the definite quick knockdown effect of the extracts. The highest was noted in the case of 15% concentration of *T. ammi* (10.59%) while the lowest was observed in the case of 5% of *N. sativa* (2.75%). Overall, all the concentrations of plant extracts seem to have toxic effect on adult insect and by increasing the concentration the percentage mortality also increased. The general trend of mortality was $15\% > 10\% > 5\%$.

Table 1: Bio-efficacy of three extracts at three concentrations (5, 10 and 15%) against *T. castaneum*.

Mortality % ± Standard Error			
Concentrations (%)	<i>N. sativa</i>	<i>S. aromaticum</i>	<i>T. ammi</i>
5	2.75 ± 0.59 a	2.91 ± 0.46 a	3.69 ± 0.69ab
10	2.91 ± 0.62 a	4.21 ± 0.87ab	7.22 ± 1.35c
15	5.01 ± 1.03 b	13.89 ± 1.90e	10.59 ± 1.87 d

Table 2 demonstrates the effect noted for different time intervals. The highest mortality was observed in case of *S. aromaticum* (14.40%) after 168h while the lowest one was noted in the case of *N. sativa* and *T. ammi* (0.00) after 24h.

Table 2 Bio-efficacy of three extracts at various exposure periods (24, 48, 72, 96, 120, 144 and 168h) against *T. castaneum*.

Mortality % ± Standard Error			
Time (h)	<i>N. sativa</i>	<i>S. aromaticum</i>	<i>T. ammi</i>
24	0.00 ± 0.00 a	1.85 ± 0.97 ab	0.00 ± 0.00 a
48	0.74 ± 0.48 a	2.22 ± 0.96 ab	0.00 ± 0.00 a
72	2.25 ± 0.49 ab	4.12 ± 0.81 bc	4.12 ± 1.13 bc
96	2.99 ± 0.49 ab	6.74 ± 1.77 cd	7.86 ± 1.77 def
120	2.65 ± 0.82 ab	8.33 ± 1.74 def	10.86 ± 1.69 efg
144	7.57 ± 0.88 cde	11.37 ± 2.95 fg	13.10 ± 1.92 g
168	8.71 ± 1.10 def	14.40 ± 3.70 g	14.23 ± 1.87 g

Table 3 shows the relative effects of all plant extracts as it is evident that *T. ammi* proved to be most effective followed by *S. aromaticum* and *N. sativa*.

Table 3: Comparative bio-efficacy of three extracts against *T. castaneum*

Plant Extracts	Mean Mortality% ± Standard Error
<i>N. sativa</i>	3.56 ± 0.45a
<i>S. aromaticum</i>	7.00 ± 0.93b
<i>T. ammi</i>	7.17 ± 0.86b

4. Discussion

Our experiments proved the effect of commonly used spices' extracts against the insect pest of stored products. Pérez, *et al* 2010^[14] proved a definite impact on plant extracts on biology and life cycle of coleopteran insect pests in stored grains. Our experiments are in accordance with the work of Chaubey (2007)^[11] who investigated effect of *Trachyspermum ammi* (Umbelliferae), *Anethum graveolens* (Umbelliferae) and *Nigella sativa* (Ranunculaceae) essential oils against stored product beetle *Tribolium castaneum*. Insecticidal activity remained different with different plant extracts and different concentrations. Time factor is also important in this regard. Plant extracts are generally containing many metabolites that operate via several modes of actions that have sub-lethal effects on the target insect (Chiasson *et al.* 2004). The results are similar to Mahdi and Rahman (2008)^[9] who tested for the toxicity of spices against the stored beetles. Zeng *et al* (2010)^[21] evaluated the chemical constituents of the clove and evaluated them through GC-MS. 18 active compounds were found and 9 were identified. 2-methoxy-4-(2-propenyl)-phenol was found to be the major constituent that is present as much as 83%. Active form of trans-caryophyllene (12%) was also found to be associated with the insecticidal properties. Stored product insects can be controlled effectively by isolating these main active constituents and then can be used alone or in combination with other formulations. Contact as well as the fumigant action of plant extracts can be used for controlling stored grain insects (Tripathi, *et al.* 2009)^[19] because of their ability to affect different life stages of stored beetles (Han *et al.* 2005)^[3]. Moreover, further research should target the active ingredients as well as to find out the optimum application methods associated with natural plant extracts.

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