Research Article *http://www.alliedacademies.org/journal-agricultural-science-botany/*
ISSN: 2591-7897

Toxic effects of *Nigella sativa* against *Rhyzopertha dominica* (Coleoptera: Bostrichidae).

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Abstract

This study was conducted at the Institute of Plant and Environmental Protection, Insect Pest Management Program (IPMP), Stored Grain Management laboratory (NARC) Islamabad in 2018. The Insecticidal effects of essential oil i.e. *Nigella sativa* at the concentration of 10, 4, 2 and 1 percent showed insecticidal effect (as high as 44.44%) at concentration (10%) after 1 week. Highest mortality (44.44%) at concentration (10%) was observed on *N. sativa*.

Keywords: *Rhyzopertha dominica*, Insecticidal activity, Mortality, Concentration, Plant essential oil, *Nigella sativa*, Rotary evaporator, Sterilization, Stock solution.

Accepted on October 11, 2018

Introduction

R. dominica (Bostrichidae: Coleoptera) is a serious pest of stored grains. It is found in the warmer regions of the world [1]. It feeds on distinct food crops i.e., cereals and legumes made from wood [2]. It is highly polyphagous. Many cereal crops were infested by the insect pests i.e., maize, rice and wheat [3]. Due to attack of *R. dominica*, the infested grain decreases in value in milled products. Adults have strong mouth parts which are used to bore into grain. The ability of strong flight and widely polyphagy, scattered with the fact that *R. dominica* has been trapped in distinct environments that indicates the pest shifting between natural territory and facilities of grain storage. *R. dominica* are 0.1-inch long. These beetles are cylindrical and dark brown. Approximately, 450 eggs are laid. The larvae molt 2-5 times. Then, pupation takes place in the grain. Larvae are free living. They become scarabaeform. Adults are voracious feeders of grains. Adults remain hidden inner softer part of the seed [4]. It is very important to utilize plant oils for protection of stored grains because they have low toxicity, high bio-degradation and eco-friendly environment when it is used. It should need to pay attention to apply these essential oils of plants in stored grains to control insect pests [5]. The Obeng-Ofori observed the results of *R. dominica* as mortality (100%) in form of protection by plant oils of cotton seeds, Soybean and maize etc. The Xie et al. the suitability of extracts Melia plant as bio-pesticides and 48%-99% control can be achieved by different concentrations. The essential oil of *N. sativa* was estimated for toxic effects on *R. dominica* to show potent dose rate, by keeping at minimum concentration and observation for their direct toxic effects.

Nigella sativa is a conspicuous herb. It has a wide range of medicinal properties. It is a spice yielding plant by commercial significance. *N. sativa* seeds are used in medicine as herbal in all over the world for the prevention of diseases. *N. sativa* oil contains plant sterols, oleic acid (25%), linoleic acid (55%), nigellone, and volatile oils including limonene and thymol. *N. sativa* oil an important role as anti-septic, anti-pyretic, anti-inflammatory, anti-allergic, anti-microbial, anti-oxidant and other desirable properties. It is clarified by some studies that *N. sativa* has positive effects on blood pressure, on blood sugar with diabetes and on memory in people. It is needed more research to understand both the positive and negative effects of *N. sativa* on human health. Some scientific evidences to offer that *N. sativa* help to prevent pregnancy, to reduce swelling, to boost the immune system and lessen allergic reaction by acting as anti-histamine and fight against cancer. But there is no sufficient information in human yet [6-8].

Materials and Methods

The research conducted at the Insect Pest Management Program, NARC, Islamabad in 2018.

Rearing of R. dominica

R. dominica collected from the Insect Pest Management Program (Stored grain Management) laboratory. The cultures formerly preserved in the sterilizer and insects' jars covered with woven cloth, at 30°C and 70% relative humidity. Wheat in jars was given to the *R. dominica* as a diet. *R. dominica* permitted to multiply as *F1* generation (Figure 1).

Figure 1. *Rearing of R. dominica.*

Preparation of essential oil

N. sativa seed collected from National Agricultural Research Council, Islamabad. This water content in raw material was 8% and oil content was 50%. Repeatedly, *N. sativa* seeds washed to remove contamination. In Oven, the seeds dried at 50°C. The material was grinded. In flask, 100 g material was taken.

Oil extraction by rotary evaporator

Oil extraction by rotary evaporator, using a solvent i.e., N. sativa seed in the form of powder and solvent taken at 1:5 ratios in flask to collect Ethanol acid at 30°C. The extraction done for about 4-5 days and shaked 3-4 times in a day. The solvent filtered by filter paper. Then heated it and evaporated at 65°C-70°C. The solvent free *N. sativa* oil is obtained by Rotary evaporator. By putting the oil in flask, the remaining solvent traces were removed. Then, it was placed on a water bath at 65°C-70°C. This process done for 12 hours. The oil was weighed and its percentage was calculated. The pure oil put in a refrigerator. The flask heated at 65°C by using electric mantle. In the evaporator, the solvent vaporized and condensed. The obtained solvent and oil kept in a bottle (Figure 2).

Figure 2. *Rotary Evaporator.*

Sieving and weighting of wheat

Took few grains of wheat in a pan and then sieved in 10 number mesh pan. Then placed the grains on white paper. Insects sticked on white paper and removed the extra raw material. Then placed the insects in a jar with the help of camel hair brush. And cover the jar with cotton cloth with rubber septum. This method is used for *Rhyzopertha dominica*.

Collection of R. dominica

1 week new adults of *Rhyzopertha dominica* were obtained from the wheat's jars by sieving method, separate the adults and put into empty jar for starvation (Figure 3).

Figure 3. *Jars having R. dominica.*

Preparation of stock solution

First of all, 1 ml *N. sativa* extract was taken and diluted with 9 ml acetone. This was 10% stock solution. Then took 3 ml from the prepared stock solution and added 5 ml acetone into it, in order to make 4% stock solution of *N. sativa*. Above given step was repeated to make 2% and 1% stock solution of *N. sativa* extract (Figure 4).

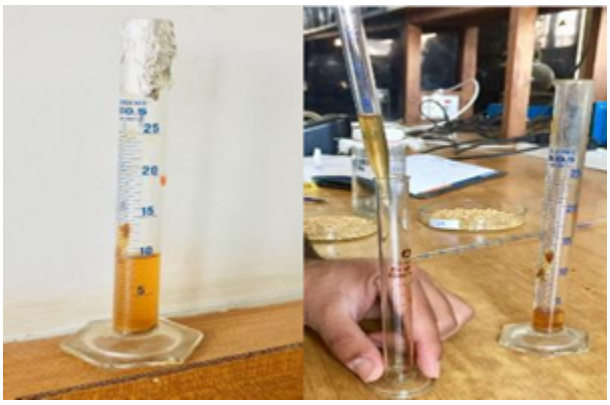

Figure 4. *Stock solution*

Preparation of petri dishes

First of all, 200 grams of wheat variety (galaxy) kept in sterilizer for 24 hours at 60°C-65°C. After sterilization, weighted the wheat (30 g) for 15 petri dishes in *N. sativa* extract and wheat (50 g) for 5 petri dishes in acetone; took 20 petri dishes of 6 mm and 6 petri dishes 16 mm. First, washed all petri dishes and dried in sunlight. After drying, took 5 petri dishes of 16 mm and put 30 g wheat in each. Then, put 50 g wheat in 16 mm petri dish for control replication (Figure 5). Stock solution (10%) of *N. sativa* made in acetone and prepared the solution of concentration (4%, 2%, 1%) from it. The solution (3 ml) on wheat applied in such a way that petri dish (30 g) treated with concentrations by using pipette. On this treated wheat (50 g), the acetone (5 ml) mixed uniformly for control purpose.

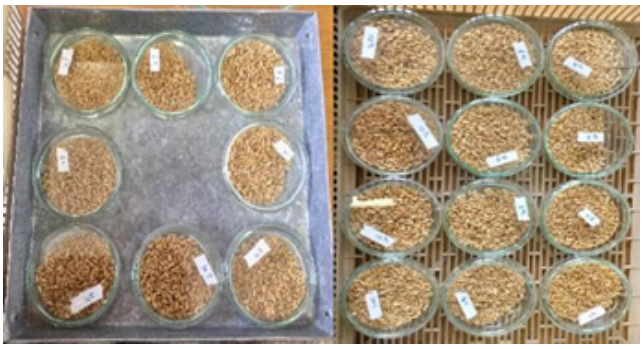

Figure 5. *Petri dishes bioassay*

Wheat (30 g) in 3 petri dishes and wheat (50 g) in 5 petri dishes divided for replication purpose. All petri dishes labelled according to its concentration and replication. Every treatment has 4 replications likewise treated and control (3:1). 20 insects of *R. dominica* dismissed in every replication. Then, dried this treated wheat for 1 hour and 20 adults (1 week old) dismissed in petri dishes. 3 petri dishes having concentrations (10%, 4%, 2%, 1%) of *N. sativa* solution (in acetone) were observed for 24, 48, 72 hours and 1 week respectively. The fourth petri dish (acetone) observed for 24, 48, 72 hours and 1 week. All petri dishes kept into growth chamber. All data collected at constant 28°C-30°C and relative humidity 65%-70%. By using utensils, data of mortality took after 24, 48, 72 hours and one week. The dead insects removed. The data noted on paper.

Statistical analysis

All the data was collected for mortality by using Abbott's Formula (1925);

Corrected %=(1-n in T after Treatment/ n in Co after Treatment) * 100

Where; n=Insect population, T=treated, Co=control

Results

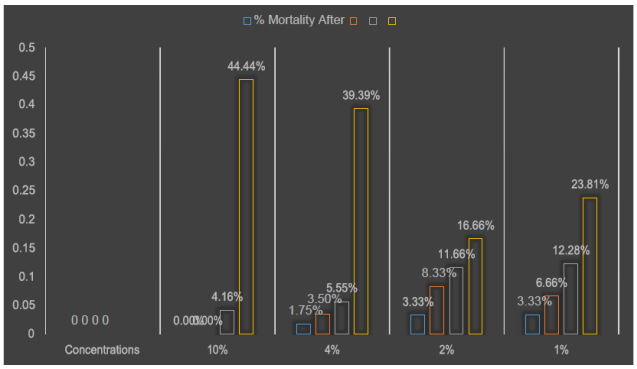

Figure 6. *Graph of Concentration and % Mortality.*

Discussion

Table 1 showed that *N. sativa* had insecticidal impact against *R. dominica* at different concentrations. *N. sativa* applied to check the toxic effect against *R. dominica* at different susceptible periods. After 1 week, highest mortality observed nearly 44.44% in 10% concentration (Figure 6).

Table 1. *Percentage mortality of Nigella sativa on the R. dominica at different exposure time.*

	% Mortality After			
Concentrations	**24 hours**	**48 hours**	**72 hours**	**1 week**
10%	0.00%	0.00%	4.16%	44.44%
4%	1.75%	3.50%	5.55%	39.39%
2%	3.33%	8.33%	11.66%	16.66%
1%	3.33%	6.66%	12.28%	23.81%

N. sativa has some insecticidal properties according the above results. Further, it should be tested for its use. So that it could prove as safe grain protectant. In humans, *N. sativa* oil can cause allergic rashes if applied to skin. It can cause stomach upset, vomiting and constipation when taken by mouth. It can unsafe during pregnancy and breast- feeding. Sometimes, it can stop the uterus from contracting. *N. sativa* can slow blood-clotting and increase the risk of bleeding.

Acknowledgements

I acknowledge with thanks to my training advisor and Program Leader Dr. Ehsan-ul-Haq (PSO), for providing all the facilities, expert advice and perceptive comments during the training course to carry out the training. A big share of thanks goes to my affectionate and loving friend Urooj Mazhar for her big contribution, kind help and their full cooperation in the laboratory. I pay my heartiest thanks to my sweet parents for their moral and financial support and also for their love, amiable attitude, and prayers for my success.

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