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Evaluation of *Withania somnifera* extracts on a storage pest Rice weevil, *Sitophilus oryzae*

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ABSTRACT

Rice weevil, Sitophilus oryzae is the major stored pest of rice all over the world. Control of this pest using inorganic pesticides leads to even many health hazards, environmental pollution and detrimental to non target organisms. Plant based pest management is an ecologically compatible alternative to synthetics. This study was conducted to evaluate the efficacy of medicinal plant, Withania somnifera (Solanaceae) extracts against adults of Sitophilus oryzae. The plant extracts were applied at five concentrations on green gram which were at 20, 40, 60, 80 and 100%. Adult insects were exposed to treated green gram grains and mortality was calculated after 5 and 10 days of exposure. Damage percentage was calculated after seven days. The results revealed that mortality and damage percentage were proportional to concentrations. Higher concentrations had stronger toxicity and lowered the damage percentage. Maximum mortality registered as 86.07% and 84.17% in five and 10 days after treatment (DAT), respectively. Lower concentration (20%) also checked the population build up of this pest which was 25.41% in 10 DAT. Toxicity was increased with time. In another experiment minimum damage registered in leaf extracts (2.40%), fruit extracts (2.47%) and root extracts (2.93%) at 100% concentration when compared to control (8.13%). There was a clear observation significantly between 80% and 100% of leaf and fruit extracts but not in root extracts at the same concentration. Even though, root extracts reduced damage from lower concentration compared to other two. Overall the root extracts were effective followed by leaf and fruit extracts. The results of this study suggest that various extracts of W. somnifera were effective and can be used to manage stored pests as admixture of integrated pest management tactics.

Key words: *Sitophilus oryzae*, *Withania somnifera*, mortality, damage percentage, extracts

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INTRODUCTION

The rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae), is one of the most destructive insect pest on stored products. Females lay eggs on damaged grain or whole grain by making cavity through its snout; emerging larva damage the kernels by making feeding galleries and pupate inside aggregated grains. Newly emerged adults may spend several days within the grain before chewing and emerge through exit holes [1]. Larval and adults feeding not only decrease nutritional and aesthetic value of the grains but also reduce germination capacity, ends up with low quality of seeds that did not attract a good market price [2].

Application of synthetic insecticides is effective against this pest during storage. But excessive and continuous usage cause residual pollution to the environment, toxicity to consumers and residues on grains. Moreover, *S. oryzae* has been reported to develop resistant to synthetic insecticides [1]. Due to these concerns, it is essential to find resources that effectively protect stored products alternative to synthetics, easily available, less expensive and environmentally safe.

Exploitation of plant extracts are becoming attractive and used in pest management tactics. Plant derived chemicals have been recognized to be antifeedants, repellents, growth inhibitors or as insecticides [3]. Many of the defensive components of plants are biodegradable with non-residual effects on the biological environment and are also serving as sterilizing agents of the stored grains [4], [5].

Withania somnifera Dunal is d commonly known as ashwagandha belonging to the family Solanaceae. It is in wealth position in Indigenous medical practices. Its extracts are variously used and consumed by human beings who are suffering due to various ailments. Numerous studies indicated that ashwagandha possesses antioxidant, antitumor, antistress, anti-inflammatory, immunomodulatory, hematopoietic, anti-ageing, anxiolytic, antidepressive, rejuvenating properties and also influences various neurotransmitter receptors in the central nervous system [6]. In addition several chemicals present in this plant are helpful to exploit this plant. It has strong pesticidal activities which are evident through by several studies and the information about this plant toxicity in animals is lacking [7]. Present investigation was to explore the efficacy of *W. somnifera* extracts employing at different concentrations on *S. oryzae* despite its excellent pharmacological activity.

MATERIALS AND METHODS

Plant collection

Mature plant parts of *W. somnifera* were collected from the Northern Province Government herbal garden at Navakkiri, Jaffna. Laboratory experiments were conducted at department of Agricultural Biology of Faculty of Agriculture, University of Jaffna, Jaffna, Sri Lanka. Samples were brought to laboratory and washed thoroughly using distilled water to discard all the impurities attached with them. They were shade dried for half an hour at room temperature. All plant samples were collected after two weeks of post irrigation. Following to that plant extraction procedure was adopted for each sets of experiment.

Plant extraction

210g of fresh leaves, 230g of fresh fruits and 250g of fresh shoots were weighed to prepare the fresh extract for the purpose of investigation. Plant materials were ground in the absence of water with the help of electric grinder and squeezed to get the fresh extract. In case of root, 60g was boiled in 400ml of distilled water at 80^0C.The resultant aliquot was transferred to conical flask and allowed for cooling under room temperature. Thereafter, all the extracts were preserved in an air tight glass bottle prior to use with proper labels. The extracts were prepared in different concentration levels and mixed with the diet of adult to treat them.

Test Organism

Initial stock of *S. oryzae* was collected from naturally infested green gram from Local market. They were reared in the original substrates at the rearing unit under ambient laboratory condition. Plant extracts with 20%, 40%, 60%, 80% and 100% concentrations were formulated. Shoot extracts were mixed with uninfested sterilized green gram (20g) to assess the mortality of rice weevil. To evaluate the damage percentage caused by *S. oryzae*, extracts of leaf, fruit and root were applied on counted (125), uninfested and sterilized green gram. After the application of extracts, the treated grains were shade dried. Prior to the release, insects were exposed to a standard starvation period of 72 hrs in order to facilitate their feeding. Twenty-five newly emerged adults were released into plastic containers having treated diet of *S. oryzae* for both evaluations and were covered firmly using muslin cloth. Normal and control sets were maintained under same condition for comparison and all sets were replicated five times.

Bio assay

There were two different bioassays performed in this investigation. All tests were conducted at optimal laboratory conditions. The observation of mortality was made on 5^{th} and 10^{th} day of the study. The percentage of mortality was calculated by using the formula as given below and the results were corrected by using Abbot's formula [8].

$$\text{Percentage of mortality } (\%) = \frac{\text{Number of dead insects}}{\text{Number of insects introduced}} \times 100$$

$$\text{Corrected percentage of mortality } (\%) = \frac{\text{Po}-\text{Pc}}{100-\text{Pc}}$$

Where Po= *Percentage of observed mortality,* Pc = *Percentage of mortality in Control*.

For the calculation of percentage damage, number of damaged green grams was counted seven days after the introduction (DAI) and the following formula was used [9].

$$\text{Damage percentage } (\%) = \frac{\text{Number of damaged grains}}{\text{Total number of grains}} \times 100$$

Statistical analysis

Results were analyzed by using completely randomized design (CRD) and means were separated by Duncan's multiple range test (DMRT) at 95% confident interval by using SAS statistical package.

Assess the germination percentage

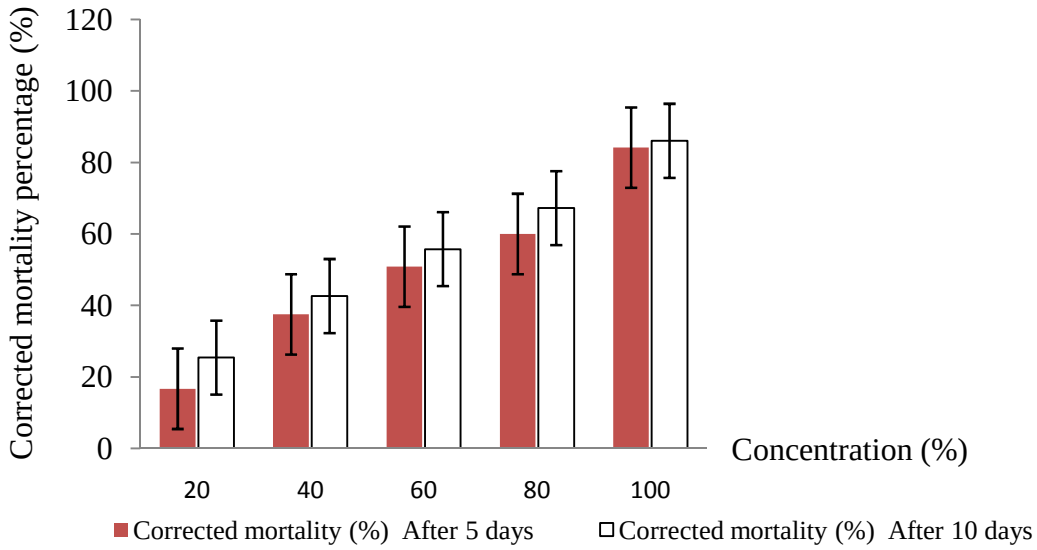
Among the different concentration of *W. somnifera* shoot extract treated green gram bulk, 40 seeds were selected randomly at each concentration. To prepare a standard seed bed, sterile top soil and sand were mixed with cow dung in 3:1:1 ratio. Selected seeds were sown in that medium and normal seeds also sown separately as control to compare with treatments. In each treatment four replicates were maintained. Germination percentage was calculated after two weeks by using the formula described below [10].

$$\text{Germination percentage (\%)} = \frac{\text{Number of seedlings}}{\text{Initial number of seeds}} \times 100$$

RESULTS AND DISCUSSION

Figure 1 shows comparative mortality of the rice weevil at five different concentrations. This difference observed in mortality due to different concentration of shoot extract of *W. somnifera*. Many treatments exhibited significantly ($p<0.05$) to higher mortality. The adult mortality observed to have a direct relationship with the concentration, being maximum and minimum when treated with extract of 100% and 20% of concentration, respectively. Higher mortality recorded at 100% of concentration as 86.07% followed by 80% of concentration as 67.21% in 10 days after treatment (DAT). Even though, lowest concentration was also able to kill the *S. oryzae* in both days. There was no much difference between five and ten days of mortality but the mortality was still higher in 10 (DAT). There was no significant difference observed between 60 and 80 percent of concentration in fifth day of mortality but was not in tenth day of mortality.

Figure (i): Effect of shoot of *Withania somnifera* extract on adult mortality of rice weevil



In case of damage percentage many of the treatments indicated significantly lower damage compare to control. Lower damage (2.40%) was observed in sets treated with 100% extract of leaf followed by 100% of fruit extract (2.47) and 100% of root extract (2.93%). All concentrations were significantly reduced the damage percentage except 20 percentage of leaf extract which was 7.33% but the control was still higher (8.13%). In case of root, 20 percentage also significantly reduced the damage when compared to other two extracts. Moreover, significant difference noticed in 80 and 100 percent of leaf and fruit extract but not in root extract at the same concentration levels. The damage percentage was observed to have an inverse relationship with the concentration irrespective to the source of extracts, was minimum when treated with 100% of concentration and maximum at 20% of concentration. In general, extracts from the roots of the plant caused lower damage followed by the treatments of fruit and leaves comparatively.

Table (ii): Effect of *Withania somnifera* extracts on damage percentage (%) of *Sitophilus oryzae*

Dose Concentration (%)	Mean damage percentage of		
	Leaf	Fruit	Root
20	7.33 ± 0.67 ^{ab}	6.67 ± 0.85 ^{b}	5.20 ± 0.18 ^{b}
40	6.07 ± 1.66 ^{bc}	6.13 ± 1.12 ^{bc}	5.00 ± 0.13 ^{b}
60	5.33 ± 0.53 ^{cd}	5.20 ± 1.02 ^{c}	4.93 ± 0.19 ^{b}
80	4.33 ± 1.11 ^{d}	3.87 ± 0.77 ^{d}	3.40 ± 0.60 ^{c}
100	2.40 ± 0.86 ^{e}	2.47 ± 0.99 ^{e}	2.93 ± 0.76 ^{c}
00 (Control)	8.13 ± 0.99 ^{a}	8.13 ± 0.99 ^{a}	8.13 ± 0.99 ^{a}

Means followed by same letters within each column do not differ significantly based on DMRT test ($p < 0.05$). Values are mean ±standard deviation of five replicates.

Yankanchi and Gadache (2010) [11] were observed that *W. somnifera* extracts effectively caused mortality of *S. oryzae*. In another study leaf powders from *W. somnifera* exhibited 100% of mortality on *Callosobruchus chinensis* [12]. The toxic consituents present in plant extracts penetrating the seed or the accumulation of the toxic metabolites kills insects [13]. Furthermore, *W. somnifera* contains withanolid as the active compound and it acts as antifeedant and repellent of insects [14]. Panwar *et al*. (2009) [15] reported that *W. somnifera* possesses psoralen and isopsoralen which act as antifeedant and insecticidal. These qualities of this plant may be the reasons for the present findings which are mortality of *S. oryzae* and lowered the damage by this pest.

Germination

The results revealed that germination percentage of green gram which was treated with different concentration exhibited more than 96 percentages. Differences between control and treated green gram seeds did not observed. These results support the earlier work which was done by Adedire and Akinkurolere (2005) [16] that plant extracts are effective as protectants without hampering germination.

CONCLUSION

The results from present investigation based on laboratory experiments, can therefore suggest the potential exploitation of *W. somnifera* as admixtures in integrated stored pest management strategies, particularly by small scale farmers who store small amounts of stored products for their consumption and planting purpose. However, further investigations are recommended to determine the possibility of using this extracts, exact mode of action of constituents and their effect on non-target organisms.

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