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Assessment of biocontrol competence of some medicinal leaves against *Sitophilus oryzae* L. using residual film method

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Abstract
Quantitative and qualitative loss of stored grains and their products by different pests is a major problem in India and other countries. As synthetic pesticides impose several environmental and health repercussions, botanical products may be used effectively to reduce the loss. The efficacy of leaf extracts of four plants, namely *Azadirachta indica*, *Ocimum tenuiflorum*, *Justicia adhatoda*, and *Centella asiatica*, was assessed against *Sitophilus oryzae* by using the residual film method. Results depict 73.33% and 66.67% mortality of the rice weevils with *O. tenuiflorum* and *A. indica*, respectively, in a 3 mg/cm^2 concentration, within 72 hours. The calculated LC_{50} values in 72 hours of exposure for *A. indica*, *O. tenuiflorum*, *J. adhatoda*, and *C. asiatica* leaf extracts were 1.50, 1.49, 5.76, and 6.22 mg/cm^2, respectively. Bioassay with mixed extracts gives even better results, with LC_{50} values as low as 0.86 mg/cm^2 in 72 hours of exposure. This could be a simple, cost-effective option against stored grain pests. These leaf products have Ayurvedic properties and should be safe to mix with food grains without adverse effects.

Keywords: *Sitophilus oryzae*, biocontrol, medicinal plants, residual film method

Introduction

Food insecurity is a serious issue in several developing countries. In the coming years, this may become worse as the world's population is predicted to reach 9,700 million in 2050, with an increase of 1,700 million from the present [1]. According to the "State of Food Security and Nutrition in the World, 2021" report, an estimated 720–811 million people will face hunger or cannot eat healthily or nutritiously. Predictions express that in spite of some improvements, the world is far from achieving "Sustainable Development Goal 2: Zero Hunger by 2030" nor meeting global nutrition targets [2]. Hence, with the increase in food grain production and their proper distribution, decreasing post-harvest loss of food grains is one of the top priorities today.

Approximately 10% of food grains are lost post-harvest due to unscientific storage, insects, rodents, microorganisms, etc. Pests not only consume huge amounts of stored seeds, but contamination may cause hydrolysis and oxidation in stored grains/products, influenced by environmental conditions, decreasing the nutrient values, or even making mycotoxins [3, 4]. In India, annual losses in storage are estimated to be 14 million metric tonnes worth Rs. 7,000 crores, with insects alone accounting for about Rs. 1,300 crores. The World Bank stipulates that with these lost grains, one-third of India's poor could be fed. About 2.0–4.2% loss in storage is imposed by insects. Nearly 600 insect species were recorded to be accompanying stored grain products, and about 100 of them impose economic losses [5].

Stored seeds, grains, and other products face substantial quantitative and qualitative loss due to insect pest infestations in India. In India, storing food grains is more problematic due to the hot and humid climate, which boosts insect pest infestations. The rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), is one of the most destructive primary pests of stored grains all over the world. Substantially high losses to stored products by *Sitophilus* were reported from different parts of the globe. *S. oryzae* imposes severe damage to stored maize, rice, sorghum, barley, and wheat [6, 7]. Both adults and larvae can damage the grains, although the larval damage is higher. A single weevil can eat up to 10–25 g of grains in its lifespan [3].

Therefore, there is a constant necessity to guard the stored products from quantitative and qualitative loss during storage. Records of recommendations for the use of botanicals to protect stored grains can be found from the Vedic periods, but they never got popularized. Instead, the use of synthetic insecticides has been boosted in the last few decades, even though these chemicals are problematic for the environment and public health and also develop resistance in the targeted insects [8]. Increasing environmental contamination/pollution adds to the problem of enhanced pest/vector populations, biodiversity decline, and health repercussions [9 - 15].

The concept of biological control using predators [16, 17] and phytochemicals [18 - 20] is becoming popular these days to overcome the disadvantages of synthetic insecticides. Botanical products may be highly effective against storage insect pests. The biocontrol competence of some medicinal plants against *S. oryzae* was assessed in the current study using the "residual film method".

Materials and Methods

Test Insects

Adult *S. oryzae* were collected from infested rice and reared and maintained in the laboratory at optimum conditions of 65±5% relative humidity, 28±2 °C temperature, and 12:12 hours (light: dark) photoperiod with insecticide-free rice food supplements. Proper care was taken to avoid exposure to insecticides and pathogens. Healthy insects, irrespective of sex, were taken for the bioassay.

Preparation of Extracts

Matured leaves of *Azadirachta indica* (Neem), *Ocimum tenuiflorum* (Tulsi), *Justicia adhatoda* (Basak), and *Centella asiatica* (Thankuni) were collected from the outskirts of Gushkara and Raniganj in West Bengal, India. 500 g of each leaf was cleaned properly and crushed separately in an electric mortar (without adding any water). The crushed materials were filtered, dried, and preserved in a refrigerator at 4°C as a stock extract in separate airtight ampules. For the bioassay, the required concentrations of the crude extracts were made by mixing a suitable amount of the stock with distilled water. A fifth type of test solution was prepared by mixing all four extracts in equal amounts to search for better synergistic effects. The tested effective doses used in the experiment were 1 mg/cm^2, 2 mg/cm^2, and 3 mg/cm^2 [21, 22].

Bioassay

The 'residual film method' with some modifications was used for the bioassay [22]. 1 ml of extract of each concentration was applied uniformly in the Petri dishes (9 cm in diameter). Twenty adult weevils were realized in each Petri dish, which was then covered with a fine muslin cloth and tied with an elastic band to protect the insects from escaping. After being kept in optimum conditions, the dead weevils were recorded at 24-, 48-, and 72-hour intervals. The experiments were carried out in triplicates for each concentration and leaf, with a control set as well. When a sharp pin was probed into the abdomen and the insect showed no reaction, it was determined to be dead [23].

LC_{50} and LC_{90} values were calculated by log-probit and regression analysis [21, 24, 25].

Results and Discussion

Crude extracts of the leaves of all the plants under study show low to high toxicity against *S. oryzae* in different concentrations and times. After 72 hours of exposure, the highest mortality was observed in Tulsi and Neem leaf extracts (Figure 1).

In 1.0 mg/cm^2 concentration and 24 hours of exposure, 15.00%, 18.33%, 15.00%, and 13.33% of the weevils died with Neem, Tulsi, Basak, and Thankuni leaf extract, respectively. While in 48 hours, the mortalities were 26.67%, 28.33%, 23.33%, and 20.00%, and in 72 hours, they were 41.67%, 40.00%, 36.67%, and 28.33% with those leaf extracts, respectively.

In 2.0 mg/cm^2 concentration and 24 hours of exposure, 23.33%, 28.33%, 21.67%, and 15.00% of the weevils died with Neem, Tulsi, Basak, and Thankuni leaf extract, respectively. While in 48 hours, the mortalities were 40.00%, 45.00%, 28.33%, and 25.00%, and in 72 hours, they were 53.33%, 53.33%, 46.67%, and 33.33% with those leaf extracts, respectively.

In 3.0 mg/cm^2 concentration and 24 hours of exposure, 31.67%, 35.00%, 26.67%, and 25.00% of the weevils died with Neem, Tulsi, Basak, and Thankuni leaf extract, respectively. While in 48 hours, the mortalities were 53.33%, 56.67%, 38.33%, and 33.33%, and in 72 hours, they were 66.67%, 73.33%, 48.33%, and 41.67% with those leaf extracts, respectively.

When the mixture of all four extracts was subjected to bioassay, an enhanced efficiency was noticed. In 1.0 mg/cm^2 mixture concentration, the mortality rates of the weevils were 23.33%, 36.67%, and 58.33% in 24-, 48-, and 72-hour exposure, respectively. With 2.0 mg/cm^2 concentration, the mortality rates were 30.00%, 48.33%, and 70.00%, whereas with 3.0 mg/cm^2 concentration, the mortality rates were 43.33%, 65.00%, and 88.33% in 24-, 48-, and 72-hour exposure, respectively (Figure 1). No mortality was noticed in the control assay.

Probit analyses of death rates of *S. oryzae* in crude extracts of Neem, Tulsi, Basak, and Thankuni leaves show LC_{50} values as 7.92, 6.69, 14.29, and 20.90 mg/cm^2, respectively, in 24 hours of exposure. Whereas, for 72 hours, the LC_{50} values were 1.50, 1.49, 5.76, and 6.22 mg/cm^2, respectively. The mixed extract shows LC_{50} as low as 4.85, 1.81, and 0.86 mg/cm^2 after 24-, 48-, and 72-hour exposure, respectively. The regression analysis showed a positive correlation between mortality rates (Y) and concentration (X), with regression coefficient value (R^2) mostly close to one in all the extracts (Table 1).

When the surviving weevils in the extract treatments were tried to reared further in normal optimum conditions, they showed impaired survivability and reproductive activity.

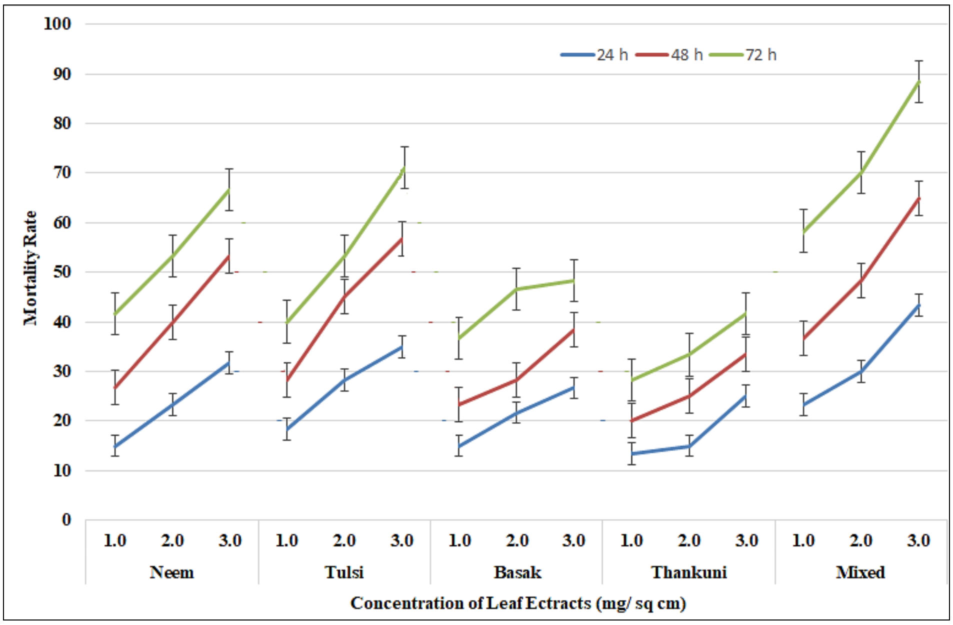

Fig 1: Mortality of *S. oryzae* in exposure to different leaf extracts in different concentrations and time exposure.

Table 1: Results of log-probit and regression analysis for LC_{50} and LC_{90} values of different extracts against *S. oryzae*.

Extract	Period of exposure	LC_{50} (mg/cm^2)	LC_{90} (mg/cm^2)	Regression equation	R^2 value
Neem	24 h	7.92	97.61	y = 1.1733x + 3.9457	0.9857
	48 h	2.77	22.10	y = 1.4192x + 4.3719	0.9845
	72 h	1.50	14.59	y = 1.2971x + 4.7702	0.9515
Tulsi	24 h	6.69	94.56	y = 1.1128x + 4.0813	0.9999
	48 h	2.34	15.13	y = 1.5823x + 4.4129	0.9981
	72 h	1.49	8.24	y = 1.7264x + 4.6989	0.9217
Basak	24 h	14.29	376.63	y = 0.9008x + 3.9597	0.9999
	48 h	7.76	237.70	y = 0.8614x + 4.2332	0.9145
	72 h	5.76	231.32	y = 0.7982x + 4.393	0.9001
Thankuni	24 h	20.90	567.90	y = 0.8925x + 3.8218	0.7802
	48 h	11.56	441.93	y = 0.8089x + 4.1402	0.9451
	72 h	6.22	299.55	y = 0.7608x + 4.396	0.9122
Mixed	24 h	4.85	66.44	y = 1.126x + 4.2279	0.9272
	48 h	1.81	13.86	y = 1.4467x + 4.6281	0.9249
	72 h	0.86	3.90	y = 1.9473x + 5.1282	0.8839

Conclusion

More and more food grains are required to feed the world's rapidly growing population. To meet the "Sustainable Development Goal 2: Zero Hunger by 2030", with the increase in crop production, post-harvest management has also become a necessity to stop huge amounts of food grain loss. The World Bank Group prioritizes investment in agriculture and rural development to enhance food production and nutrition, collaborating with partners to improve food security, encourage climate-smart farming, and reduce food losses [2]. By effectively managing the moisture levels, creating temperature gradients, and oxygen circulation within the storage facility, food grains can be stored securely [26]. In addition, several works are in progress to discover eco-friendly insecticides using modern technologies [16, 27 - 29].

The scope of synthetic insecticide application in stored grains is limited due to its residual and hazardous effects. Botanical insecticides can be a solution to this problem. Leaf of Neem, Tulsi, Basak, and Thankuni are known for their Ayurvedic properties and are used in several health complications without any side effects. So, directly mixing these leaf products with the food grains should not impose any adverse effects [18]. The present study shows that these leaves can be used for effective management of *S. oryzae*. The use of the leaves together can have even enhanced outcomes. This should be a simple, cost-effective option. Concerned organizations should act to increase public awareness of using environment-friendly pesticides, and people should be encouraged to use these types of methods to protect stored products in the absence of proper alternatives.

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