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Research Article

Efficacy Assessment of Different Botanicals Against Rice Weevil (*Sitophilus Oryzae)* in Stored Rice

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Abstract

Rice weevil (*Sitophilus oryzae*) is the major seed destroyer in stored grain, causing both quantitative and qualitative losses. Traditional approaches utilizing chemical insecticides including fumigants to address this problem present environmental risks and health concerns. Focusing on the scenario, a study was conducted at laboratory condition of entomology division, Bangladesh Rice Research Institute (BRRI) to assess the efficacy of different botanicals for the management of rice weevils in stored rice. Employing a Complete Randomized Design (CRD) with three replications, the experiment comprised four treatments alongside an untreated control, namely Neem (*Azadirachta indica*) oil, Mahogany (*Swietenia macrophylla*) seed kernel powder, Neem (*A. indica*) leaf powder, Biskatali plants (*Polygonum hydropiper*) powder and Control (untreated). The findings unveiled that the highest percent of insect mortality was found in neem oil (85%) and which was followed by biskatali plants powder (75%), neem leaf powder (61.67%) and mahogany seed kernel powder (60%), respectively. The lowest emergence of F_1 progeny was recorded in neem oil treated seeds compared to mahogany seed kernel, Neem leaf powder, biskatali and untreated control indicating the efficacy in managing the reproductive capacities of rice weevils. Additionally, neem oil also exhibited the least weight (%) loss of grain compared to other treatments. Regarding seed germination, biskatali plants powder showed highest germination rate (94%) followed by neem leaf powder (90%), mahogany seed kernel powder (89.67%), neem oil (88%) and control (80.67%), respectively. The research finding indicated that the neem oil and biskatali plant powder has the greatest potentiality to minimize the current use of synthetic insecticides for rice weevil management in stored rice.

Keywords: Eco-friendly, F_1 progeny, Germination, Mortality percentage, Neem oils

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Introduction

Rice (*Oryza sativa* L., Family: Poaceae) holds a critical position as a staple among cultivated cereal crops worldwide, serving as a primary food source in many nations. Rice requires prolonged storage following harvesting and utilized for consumption, seeds or any other purposes (Roy *et al.,* 2023). In a standing rice field, rice crop is infected by various insect pests such as rice stem borer, armyworm, rice bug, rice hispa, fall armyworm and many more (Ali *et al*., 2023; Roy *et al.,* 2024a). However, during storage, stored rice is also damaged by various pests such as the rice weevil (*Sitophilus oryzae* L.), angoumois grain moth (*Sitotroga cerealella* Olivier), saw-toothed grain beetle (*Oryzaephilus surinamensis* L.), red flour beetle (*Tribolium castaneum* Herbst), and indian meal moth (*Plodia interpunctella* Hubner), leading to significant economic losses in production (Roy *et al.,* 2023). The rice weevil is recognized as one of the most destructive pests of stored agricultural products, including rice (Jyothi *et al.,* 2024). Rice weevils diminish both the quantity and quality of rice through their feeding and breeding activities on the grain. Under favorable conditions, a single pair of *S. oryzae* can reproduce approximately one million offspring within a three-month timeframe (Thomas *et al*., 2002) and these adult weevils, being internal feeders, inflict significant quantitative and qualitative damage to stored grains. The activities of this primary pest result in a reduction in grain weight, loss of nutrients, and diminished seed viability. Furthermore, their behaviors render the grains susceptible to contamination by mites and fungi (Costa *et al*., 2016; Zakladnoy, 2018). Hence, effective management of storage pests such as rice weevils is crucial for reducing post-harvest losses. Traditionally, the primary approach to managing weevil populations globally has relied on synthetic insecticides. However, utilizing chemical insecticides and widely used fumigation techniques to combat storage pests' infestations during storage poses significant health risks and residues from these chemicals persist in both the stored grain and the surrounding environment, exacerbating potential hazards (Hossien *et al*., 2018; Roy *et al*., 2024b). Moreover, marginal farmers normally reserve tiny amount of rice for their consumptions as well as seed purposes, making it challenging for them to apply fumigation or chemical insecticides to manage rice weevils. Hence, an alternate strategy are suggested, which are prioritizes to environmental safety, less toxicity, and poses no risk to human health to manage this pest in stored rice. Ashamo & Akinnawonu, (2012) reported that various plant products are commonly used to minimize the storage losses by insect pests. Botanicals can play a crucial role in this regard to the local communities as such insect repellent plant materials served as a readily accessible indigenous sources of insecticides and insect repellents. Although their insecticidal effectiveness may not match that of synthetic insecticides and fumigants, except for natural pyrethrins, they do exhibit prolonged surface persistence. Moreover, they have minimal to no adverse effects on seed germination, cooking quality, milling and easily attainable at lower costs (Roy *et al.,* 2023; Roy *et al.,* 2024b). The bioactive metabolites (Azadirachtin, quircetin, quinolone, gingerols, shogaols, flavonoids, saponins, limonoids, phenylpropanoid derivatives, and sesquiterpenoids etc) present in botanicals are responsible for poisons, repellant and antifeedant effects on insect pests. Powdered, oil, or extract forms derived from various bioactive plants (such as *Azadirachta indica, Ocimum tenuiflorum, Nepeta cataria, Swietenia macrophylla, Persicaria hydropiper, Zingiber officinale* etc) can be used on stored grain for managing storage pests. Therefore, our present investigation aims to assess the efficacy of different botanicals for the management of rice weevils (*S. oryzae*) in stored rice.

Materials and Methods

This experiment was conducted from September 09 to December 25, 2023 at laboratory of Entomology division in Bangladesh Rice Research Institute (BRRI), Gazipur, Bangladesh.

Sample Collection and Preparations

Fresh leaves of neem tree (*Azadirachta indica* A.Juss) and biskatali plants (*Persicaria hydropiper* L) were collected from the BRRI, Gazipur campus. They were thoroughly washed with fresh water and then dried at fluctuating room temperature (25±2°C). After drying, the leaves were oven-dried at 50°C for 72 hours to reach a constant weight. Mature mahogany fruits (*Swietenia macrophylla* King) were also collected from the BRRI, Gazipur campus and sundried for 3 days. After sundrying, the carpels were detached from the pods, and the external layers were removed from the seeds. The naked seed kernels were then oven dried at 50°C for 72 hours. The dried neem leaves, biskatali plants, and mahogany seeds were grounded into powder using a mortar and pestle. The powder was then sieved (mesh number 20) and subsequently stored in airtight polyethylene bags within a freezer (Model: IRMCO CHL5 Basic, manufactured in Germany) at 4°C for future use. Neem oil (organic neem oil extracted by pressing, manufactured by Garden BD) was collected from the local market.

Insect Rearing and Treatments

At the beginning of the experiment, the population of rice weevils was sourced from the storage facility at BRRI. Subsequently, these weevils were cultured on rice (Var. BRRI dhan89) in the laboratory. Differentiation between female and male moths was achieved through visual observation of their abdominal tergites and body size, facilitated by a stereo microscope (Olympus Model SZ2-STU2, manufactured in Tokyo, Japan, equipped with a 4x scanning objective lens). The weight of the powder extracts was determined using a KERN ABJ220-4NM weighing balance, manufactured in Balingen, Germany.

Experimental Design & procedure

The experiment was conducted using a Complete Randomized Design (CRD), consisting of four treatments (Table 1) along with one control (untreated), with each treatment replicated three times. Previously collected BRRI dhan87 was kept in freezer (Rice were kept at -20°C temperature for 24 hour) and after then oven dried at 50°C for another 24 hours for sterilized from insect egg and larvae. Additionally, the rice was sieved to remove inert materials, dust and dead insects. Fifteen plastic container ($20*12*8$ cm^3) were used and each container holding 200 g of rice. After then treatment were applied according to the design and the containers were shaken thoroughly for uniform adherence of powders and oil on the rice grain surface. After applying the designated treatments, each replication received 10 pairs (10 male & 10 female) of newly emerged adult insects. The containers were covered with nets (mesh size 25) for proper aeration. The environmental conditions for this process were maintained at 65 ± 2% RH and 25 ± 2°C temperature.

Table 1. Different plants used as treatment against rice weevil

Treatment	Common name	Scientific Name	Family name	Parts used	Dose /kg
T_1	Neem	*Azadirachta indica*	Meliaceae	Oil	5ml
T_2	Mahogany	*Swietenia macrophylla*	Meliaceae.	Seed kernel powder	10g
T_3	Ghora-neem	*Melia azedarach*	Meliaceae	Leaf powder	10g
T_4	Biskatali	*Persicaria hydropiper*	Polygonaceae	Plants powder	10g
T_0	Control	-	-	Untreated	-

Data Collection

The number of dead insects was counted at 24, 48, 72, 96, and 120 hours after treatment (HAT), and the dead insects were removed after each count. After 120 hours of treatment, no dead insects were observed. The live insects were then allowed to remain for another 7 days to facilitate oviposition and were subsequently removed. The boxes were regularly observed for F1 adult emergence. Adults were removed, and data collection continued until no. F1 weevils were found (about 5 to 6 weeks). Observations regarding the mortality percentage were recorded for each treatment and calculated using the (Henderson & TILTON, 1955) formula.

$$\text{Mortality (\%)} = 1 \times \frac{Ta \times Cb}{Tb \times Ca} \times 100$$

(Where, Ta is the number of weevils after treatment, Tb is the number of weevils before treatment, Ca is the number of weevils in untreated check after treatment, Cb is the number of weevils in untreated check before treatment).

Germination Percentage

One hundred rice seeds were randomly taken from each replication of every treatment and placed in a Petri dish (99mm * 20mm). These seeds were then placed on moist filter papers within a germination box and maintained at room temperature. After completion of germination, the number of germinated seed was counted and recorded. The percentage germination was computed using the following formula.

$$\text{Germination (\%)} = \frac{Number\ of\ germinated\ seeds}{Total\ number\ of\ tested\ seeds} \mathrm{x}100$$

Grain Damage Percentage

Twenty-five damaged grains of rice were taken from each replication of each treatment by using magnifying glasses (5x magnifier). Their weight was determined using a KERN ABJ220-4NM weighing balance, manufactured in Balingen, Germany. Conversely, the weight of 25 undamaged grains from each replication was also measured. The percent weight loss of grains was computed using the following formula given by Mehta & Kumar (2020)

$$\text{Percentage weight loss} = \frac{U.Nd - D.Nu}{U(Nd + Nu)} \mathrm{x}100$$

Where, U is the weight of undamaged grains, Nu is the number of undamaged grains, D is the weight of damaged grains, and Nd is the number of damaged grains.

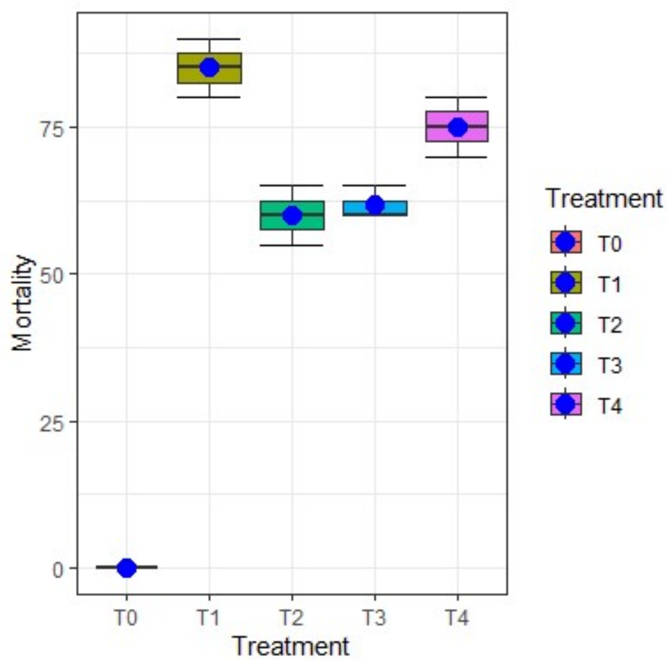
Data Analysis

All data were subjected to statistical analysis separately by using the analysis of variance technique by R software (versions 2024.04.1). The "doebioresearch" package was utilize for ANOVA executed. To discern differences among treatment means, the Least Significant Difference (LSD) test was employed, maintaining a significance level of 5%. The “ggplot2” and “Rmisc” package were used for creating plots.

Results and Discussion

Insect Mortality Percentage

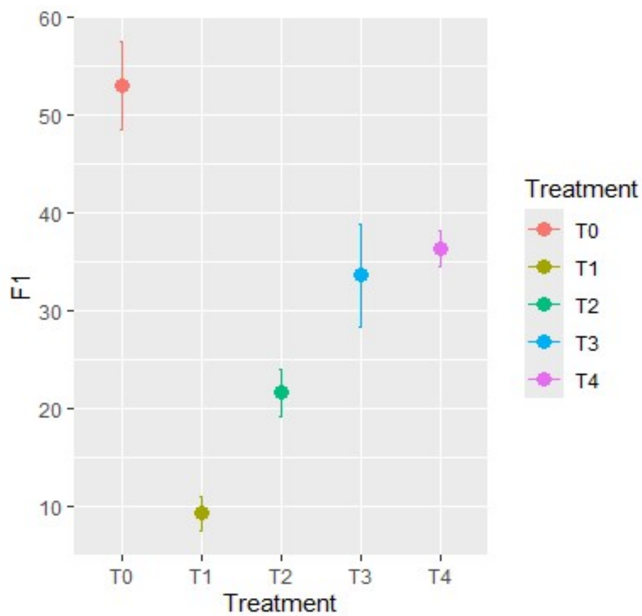
The highest percent mortality of rice weevils was recorded in T_1 i.e., neem oil (85%) which was statically different from other treatments, followed by T_4 (Biskatali plant powder, 75%). Treatment T_3 (Neem leaf powder, 61.67%) and T_2 (Mahogany seed kernel powder, 60%) showed statically identical results (Figure 1). No mortality of rice weevils was observed in T_0 (untreated control). Our findings indicated that T_1 (neem oil) showed highest mortality due to containing most bioactive compound including azadirachtin (Roy *et al.,* 2024b). But neem leaf powder (T_3) also contains bioactive compounds like azadirachtin but the concentration is lower than neem oil, resulting in lower mortality of rice weevils compared to neem oil (Roy *et al*., 2023). However, Jibrin & Mohammed (2020) demonstrated that the neem tree contains active ingredients that make the leaves bitter and bioactive ingredients including azadirachtin in neem, are effective as insect repellents and for causing mortality. The concentration of bioactive compounds might escalate during the conversion of neem leaves or seeds into powder or oil, potentially due to the elimination of inert materials and the expansion of surface area and which is why neem oil causes higher mortality than neem leaf or seed powders (Roy *et al.,* 2024b). According to Zanuncio *et al*., (2016) neem oil has demonstrated to possess lethal effects, the intensity of which varies based on the concentration used and this natural insecticide exhibits potency compared to neurotoxic pesticides and growth inhibitors, establishing its efficacy in pest control. On the other hand, T_4 (biskatali plant powder) showed second highest mortality of rice weevil due to its insecticidal properties Bhattacharjee *et al*., 2020. Both the extract and powder form of *Persicaria hydropiper* (biskatali plant) contains flavonoids as well as drimane-type sesquiterpenes, sesquiterpenoids and phenylpropanoids which exhibit antioxidant, antibacterial, antifungal, antihelminth, antifeedant, cytotoxicity, anti-inflammatory, antifertility, antiadipogenicity, and neuroprotection propertics which contribute to insect mortality (Huq *et al.,* 2014). *Swietenia macrophylla* plants also exhibits antioxidant, anti-inflammatory, antimicrobial, antimutagenic, antifeedant activities (Borah *et al.,* 2023) which are responsible for insect mortality.



T_0=Untreated control, T_1=Neem oil, T_2=Mahogany seed powder, T_3=Neem leaf powder and T_4=Biskatali plant powder

Fig. 1. Effects of Plants on mortality percentage of rice weevil

Number of F_1 progeny emergence: The highest number of F_1 adults was emerged in T_0 (untreated control), which was significantly different from the other treatments, followed by biskatali plants powder, neem leaf powder and mahogany seed powder. Biskatali plants powder and neem leaf powder showed significantly similar results (Figure 2). The lowest number of F_1 emerged in T_1 (neem oil), likely due to the highest mortality of rice weevils. Neem contains bioactive ingredient azadirachtin, which functions by impeding the growth of insects, diminishing their fertility, and reducing the emergence of F_1 populations (Roy *et al.,* 2024b). This compound influences the reproductive system of rice weevils, causing a decrease in fertility and the capacity for egg laying, also inhibiting the production of hormones crucial for molting (Ghazawi *et al*., 2007) consequently leading to a decline in rice weevil populations. According to Alexander *et al.,* (2023) and Roy *et al*., (2023) neem oils possesses insecticidal, anti-oviposition, and ovicidal characteristics, which decrease the emergence of F_1 generation of insects. Roy *et al*., (2023) also demonstrated that neem oil acts as a deterrent to feeding, thereby slowing down insect development and growth, diminishing fecundity and fertility, altering behavior, and inducing abnormalities in the eggs, larvae, and adults of storage insects like anguimous grain moth (*Sitotroga cerealella* Olivier). Although, T_2 (mahogany seed kernel powder) showed 2nd less F_1 progeny emergence but it's showed comparatively lower weevils mortality. While, this could be attributed to the presence of bioactive compounds in mahogany that may inhibit the oviposition, egg laying capacity, or egg hatching capacity of rice weevils.



Where, T_0=Untreated control, T_1=Neem oil, T_2=Mahogany seed powder, T_3=Neem leaf powder and T_4=Biskatali plant powder

Fig. 2. Effects of botanicals on number of F_1 adult emergence of rice weevil

Percentage Weight Loss of Grains

The percentage of grain weight loss showed statistically significant differences. The highest weight loss (%) was recorded in T_0 untreated control (32.97%) which was significantly different from other treatments (Table 2). Since, the highest F_1 emergence and no mortality of rice weevils were found in T_0, live weevils laid eggs without disturbance and fed on grains for meet up their starch, vitamin and protein requirements. The feeding and growth activities of rice weevils' grubs result in the hollowing of grains, ultimately leading to significant grain damage characterized by extensive powdering. It was reported by Okpile *et al.,* (2021), that weevils require both starch and protein for growth and egg laying. The study conducted in in India reported that damage caused by rice weevils has been approximated to 30% weight loss under normal circumstances. The lowest weight loss was observed in T_1 (neem oil) and which was also significantly different from other treatments. Since, the mortality of neem oil treatment was highest and neem oil contained bioactive compound thus reduced feeding on rice grains by rice weevils.

Table 2. Effects of botanicals on weight loss of grains (%) and seed germination (%) against rice weevil

Treatments	Percentage weight loss of grains	Percentage of germinated seeds	Seed germination percentage over untreated control
T_0 (Untreated control)	32.97 a	80.67 c	-
T_1 (neem oil)	11.03 d	88.00 b	9.08
T_2 (mahogany seed powder)	19.14 c	89.67 b	11.16
T_3 (neem leaf powder)	21.81 b	90.00 b	11.56
T_4 (biskatali powder)	21.41 bc	94.00 a	16.52
Level of significance	***	***	-
LSD	2.54	3.79	-
CV%	6.56	2.35	-

Seed Germination (%)

Germination (%) of seeds showed statistically significant differences among treatments (Table 2). The highest seed germination percentage was observed in T_4 i.e., biskatali plant powder (94%), which was statically different from others, followed by T_3 (90%), T_2 (89.67%) and T_1 (88%). The lowest seed germination (%) was observed in T_0 (80.67%) due to absence of weevil's mortality, the highest F_1 progeny emergence, and their feeding and breeding habits, which depend on rice grains, including damage to the seed embryo. As per Roy *et al.,* (2023) and Salam, (2018), the effectiveness of seeds treated with neem leaf powders and neem oil is contingent upon the concentration of bioactive compounds, notably azadirachtin. Lower concentrations of azadirachtin have been observed to positively influence seed germination, while higher concentrations tend to hinder it. According to Kéita *et al*., (2001); Roy *et al.,* (2024b), mixing stored grains with leaf, neem seed powder, or oil extracts from plants led to a decrease in the rate of egg-laying and inhibited the emergence of adult bruchids, consequently lowering the rate of seed damage. Thus, T_1 treated with neem oil showed comparatively lower seed germination than others.

Conclusion

The research was undertaken to comprehensive investigate the effects of various treatments on managing rice weevils in stored rice grains. It specifically focused on factors such as mortality of rice weevils, emergence of F_1 progeny, percentages of weight loss and percentages of seed germination rates. Our findings showed significant differences in insect mortality (%), number of F_1 emergence, weight loss (%) and seed germination (%). Interestingly, the highest insect mortality was found in T_1 (neem oil) with a mortality rate of 85%, followed by T_4 (biskatali plants powders) with a mortality of 75%. Our study indicated that highest insect mortality (%) reduced the number of F_1 progeny emergence, with the lowest F_1 progeny was emerged in T_1, followed by T_2. The lowest percent weight loss of grain was found in T_1 followed by T_2, T_4, T_3 and T_0. The germination percentage of seeds varied significantly, with the biskatali plant powder showing the highest germination percentage. The germination percentage of T_1, T_2 and T_3 showed statically similar results. Therefore, it might be concluded that T_1 (neem oil) showed best performance among the treatments for both the highest mortality and the lowest emerged of F_1 progeny, followed by T_4.

Conflicts of Interest

The authors declare no conflict of interest.

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