



Protecting Rice Grains from *Sitophilus oryzae* L. Infestation using Plant Extracts under Controlled Conditions

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Abstract

Three plant extracts - *Thymus vulgaris*, *Schinus molle*, and *Melia azedarach* - were tested against adult rice weevils at 25, 50, and 100% concentrations. All extracts caused significant mortality, reaching 100% with 50-100% solutions after one day. Extracts also reduced grain weight loss, damage, and adult emergence. *S. molle* was effective at 25% concentration (30-44% reduction), with higher efficacy at 100% (98% reduction). *M. azedarach* showed similar trends (62-69% at 25%, 100% at 100%). *T. vulgaris* was the most effective, achieving 69-73% reduction at 25% and 100% at all levels. These results suggest significant potential for these plant extracts in eco-friendly rice weevil control programs.

Keywords: Plant Extracts; Weight Loss; *Sitophilus oryzae*

Abbreviation: CRD: Complete Random Design.

Introduction

Rice (*Oryza sativa* Linn), a staple food for over half the world's population, faces a significant threat during storage, insect infestation. This not only reduces rice quality and commercial value but also impacts global food security. According to the Food and Agriculture Organization of the United Nations [1], approximately one-third of the world's post-harvest crop is lost to waste.

Roughly 75% of warehouse insects belong to the order Coleoptera, with the rice weevil (*Sitophilus oryzae* L, Coleoptera: Curculionidae) being the most significant and damaging species. This primary pest can cause substantial losses to stored grains, ranging from 18 to 30% in warm regions. Both adult and larval stages of the rice weevil can attack and damage stored grains [2].

While synthetic pesticides offer control, they rise environmental and health concerns due to potential harm to

non-target organisms and the development of resistant pests [3].

As a result, there is a growing focus on replacing conventional organic chemical insecticides with safer materials and methods to preserve the environment and minimize the risk of residual effects. Promising alternatives include the use of powders, plant extracts, volatile and non-volatile oils, and inert dusts, etc. [4-6].

Utilizing plants that contain biologically active compounds offers a promising alternative to harmful chemical pesticides, some plant-derived compounds act as repellents or anti-feedants, deterring insects from consuming the treated material [7].

This research explores plant-based insecticides as a safer alternative to conventional methods. It complements a previous study that investigated the toxicity of similar extracts to adult rice weevils. Here, we focus on the efficacy of extracts from three specific plants: neem (*Melia azedarach* L.), pepper tree (*Schinus molle* L.), and common thyme

(*Thymus vulgaris* L.). These extracts are assessed for their ability to control adult rice weevils (*Sitophilus oryzae* L.), a major storage pest. The study investigates how these weight extracts impact Cumulative mortality rate of adult weevils, adult emergence rates, grain damage rate and grain loss. The goal is to identify plant-based alternatives with potential for controlling rice weevils during storage. Effective plant extracts could offer a safer and more sustainable approach to protecting this vital food source.

Materials and Methods

The study was conducted in an incubator maintained at a constant temperature of $26 \pm 2^\circ\text{C}$ and humidity of $65 \pm 5\%$ at the insect laboratory.

Adult rice weevils (*Sitophilus oryzae*) were collected from infested wheat grains and identified based on their morphological characteristics [2,8]. These adults were used to establish a stable laboratory population of the insect, maintained in an incubator under the same temperature and humidity conditions used for the experiment.

The wheat grains (one potential host for the rice weevil) were thoroughly cleaned to remove impurities and any foreign matter. To eliminate any potential insect infestation, the grains were placed in a refrigerator for hours. To prevent mold growth, the grains were subsequently exposed to dry air and stored in the refrigerator at 4°C until use in the experiments.

Ripe fruits of *S. molle* and *M. azedarach* were harvested in late autumn from plants growing in the University Garden. The fruits were thoroughly washed to remove dust and any adhering materials and then dried under controlled laboratory conditions. Dried green thyme leaves (*T. vulgaris*) were purchased from local markets. The dried plant parts were ground using an electric grinder.

Two hundred grams of each of the aforementioned plant powders were placed in separate glass beakers. Each powder was mixed with twice the amount of acetone, stirred thoroughly, and covered with aluminum foil. The mixtures were then kept in the dark for 48 hours. After the extraction period, conical funnels and filter paper were used to filter the extracts. A Rotary Evaporator with 120 cycles and a solvent volatilization temperature of 56°C was employed to recover the acetone, resulting in 100% concentrated extracts. From each extract, two additional concentrations, 50% and 25%, were prepared.

The resulting extracts were stored in airtight, labeled graduated containers and kept in the refrigerator until use.

The experiment was conducted to evaluate the efficacy of the three plant extracts (*T. vulgaris*, *S. molle*, and *M. azedarach*) at three different concentrations (25%, 50%, and 100%) against adult rice weevils. Three hundred newly emerged rice weevils were randomly divided into three groups: an untreated control group and three treatment groups, each consisting of 90 weevils. The weevils in the treatment groups were further divided into three subgroups corresponding to the three extract concentrations. Ten grams of whole wheat grains were placed in each plastic cup, and 0.25 ml of the respective extract solution was applied to the grains. After allowing any potential solvent residues to volatilize slightly in the air, 30 adult rice weevils were introduced into each cup. The cups were covered with muslin and incubated at the specified temperature and humidity. The mortality rate of adult rice weevils was monitored at regular intervals (days 1, 2, 3, 4, 9, 15, and 20) following the treatment. The experiments were continued for 50 days, and the following observations were recorded:

Cumulative Mortality Ratio

The cumulative mortality rate of adult rice weevils was assessed at 1, 2, 3, 4, 9, 15, and 20 days after treatment. The effectiveness of each extract in increasing the mortality ratio was calculated using the following equation:

$$\text{Effectiveness} = \frac{[(\text{Treatment Mortality Ratio} - \text{Control Mortality Ratio}) / \text{Treatment Mortality Ratio}] \times 100}{}$$

Grain Damage Ratio

The grain damage ratio was determined 50 days after treatment using the following equation:

$$\text{Damage Ratio} = (\text{Number of Punctured Grains} / \text{Total Grain Number}) \times 100$$

The effectiveness of the extracts in reducing the grain damage ratio was calculated using the following equation:

$$\text{Effectiveness} = \frac{[(\text{Damage Ratio by Control} - \text{Damage Ratio by Treatment}) / (\text{Damage Ratio by Control})] \times 100}{}$$

Loss of Grain Weight Ratio

The loss of grain weight ratio was determined 50 days after treatment for different treatments and the control using the following equation [9]:

$$\text{Loss of Grain Weight Ratio} = \frac{[(\text{Initial Weight} - \text{Final Weight}) / (\text{Initial Weight})] \times 100}{}$$

The effectiveness of the extracts in reducing grain weight loss was calculated using the following equation:

$$\text{Effectiveness} = \frac{[(\text{Weight Loss Ratio by Control} - \text{Weight Loss Ratio by Treatment}) / (\text{Weight Loss Ratio by Control})] \times 100}{}$$

Effectiveness of Extracts in Reducing Adult Emergence

The number of adults emerging in the control and different treatments was counted, and then the effectiveness of the extracts in reducing adult emergence was calculated using the following equation [10]:

$$\text{Effectiveness} = \left[\frac{\text{Number of Adults Emergence in the Control} - \text{Number of Adults Emergence in the Treatment}}{\text{Number of Adults Emergence in the Control}} \right] \times 100$$

Statistical Analysis

The experiment was designed according to the Complete Random Design (CRD), and the results were statistically analyzed using the Fisher F test and the averages were compared according to the test of the lowest significant

difference LSD at the level of significance 0.01 using the Genstat ver. 7.4 program [11].

Results

Effectiveness of Extracts of *S. molle*, *M. azedarach* and *T. vulgaris* on Adult Mortality Rate of the Rice Weevil, *Sitophilus oryzae*

The results presented in Table 1 demonstrate the efficacy of *S. molle*, *M. azedarach*, and *T. vulgaris* extracts in enhancing the average cumulative mortality rate of *S. oryzae* adults following treatment with three concentrations (25%, 50%, and 100%) at different time points (1, 2, 3, 4, 9, 15, and 20 days post-treatment).

(Day)	Concentration (%)	Adult Cumulative Mortality (%)		
		<i>S. molle</i>	<i>M. azedarach</i>	<i>T. vulgaris</i>
1	25	0 ^{Bf}	100 ^{Aa}	100 ^{Aa}
	50	100 ^{Aa}	100 ^{Aa}	100 ^{Aa}
	100	100 ^{Aa}	100 ^{Aa}	100 ^{Aa}
2	25	44.47 ^{Bd}	84.46 ^{Aabcd}	86.11 ^{Aab}
	50	91.93 ^{Aab}	95.17 ^{Aabc}	93.61 ^{Aab}
	100	95.15 ^{Aab}	96.67 ^{Aab}	96.39 ^{Aab}
3	25	72.25 ^{Bc}	90.64 ^{Aabcd}	88.90 ^{Aab}
	50	93.42 ^{Aab}	95.33 ^{Aabc}	93.98 ^{Aab}
	100	95.16 ^{Aab}	96.67 ^{Aab}	96.67 ^{Aab}
4	25	38.89 ^{Bd}	71.90 ^{Aef}	66.67 ^{Ad}
	50	83.02 ^{Abc}	86.77 ^{Aabcde}	81.94 ^{Abcd}
	100	86.57 ^{Aabc}	90 ^{abcd}	90 ^{Aab}
9	25	38.89 ^{Cd}	78.06 ^{Adef}	69.44 ^{Bcd}
	50	84.13 ^{Aabc}	86.77 ^{Aabcde}	85 ^{Aabc}
	100	86.57 ^{Aabc}	90 ^{Aabcd}	90 ^{Aab}
15	25	18.52 ^{Be}	70.74 ^{Af}	67.72 ^{Ad}
	50	79.89 ^{Abc}	82.36 ^{Acdef}	81.94 ^{Abcd}
	100	83.2 ^{Abc}	86.67 ^{Aabcde}	86.67 ^{Aab}
20	25	18.52 ^{Be}	70.74 ^{Af}	67.72 ^{Ad}
	50	81.74 ^{Abc}	83.16 ^{Abcdef}	82.54 ^{Abcd}
	100	84.76 ^{Aabc}	86.67 ^{Aabcde}	86.67 ^{Aab}
LSD 0.01		Extract =6.01	Concentration =6.01	Time=9.18
	Extract x Concentration= 10.41	Extract x Time 15.90=	Concentration x Time= 15.90	
	Extract x Concentration x Time=27.54			

Table 1: Effect of extracts of *S. molle*, *M. azedarach* and *T. vulgaris* on Adult Rice Weevil *S. oryzae* over time. Means followed by the same small letters in the same column are not significantly different at P=0.01.

- Means followed by the same big letters in the same row are not significantly different at P=0.01.

In general, all tested extracts at the three investigated concentrations exhibited efficacy in increasing the average cumulative mortality ratio, with values ranging from 66.67 to 100%. However, an exception was observed for the treatment with *S. molle* extract at a concentration of 25%, where the average efficacy values initially ranged between 0 and 72.00% during the first three days of treatment but subsequently declined, reaching 18.52% after the fifteenth day of treatment.

Significant differences were observed between the efficacy of *S. molle* extract and those of *M. azedarach* and *T. vulgaris* extracts at a concentration of 25% across all time points examined. On the fourth day of treatment, the respective efficacy values were 38.89%, 71.90%, and 66.67%, indicating the superiority of the latter two extracts. Conversely, no significant differences were detected at concentrations of 50 and 100%.

Both *M. azedarach* and *T. vulgaris* extracts exhibited the highest efficacy at all tested concentrations and across the examined treatment periods. The efficacy values were comparable at a concentration of 100% after treatment times of 1, 3, 4, 9, 15, and 20 days with no significant differences between the two extracts. However, at a concentration of 25%, a significant difference was observed on the ninth day of treatment, with efficacy values of 78.06% and 69.44% for *M. azedarach* and *T. vulgaris* extracts, respectively.

The effectiveness of the extracts in increasing the average cumulative mortality ratio increased with increasing extract concentration at each time point. Significant or apparent differences were observed in the effectiveness values among the different concentrations. After 20 days of treatment, the average cumulative mortality ratios for the three extracts of *S. molle*, *M. azedarach*, and *T. vulgaris* at the

tested concentrations of 25%, 50%, and 100% were 18.52%, 81.74%, and 84.76%, respectively, for *S. molle*, 70.74%, 83.16%, and 86.67%, respectively, for *M. azedarach*, and 67.72%, 82.54%, and 86.67%, respectively, for *T. vulgaris*.

Despite the overall decline in efficacy values with increasing treatment time due to the high mortality rates observed in the untreated control, the effectiveness of the extracts in increasing the average cumulative mortality ratio of rice weevil adults was dependent on the complex interplay between extract type, concentration, and treatment time. The highest efficacy values, reaching 100%, were observed for treatments with *M. azedarach* and *T. vulgaris* extracts at concentrations of 25%, 50%, and 100%, as well as for *S. molle* extract at concentrations of 50% and 100% after one day of treatment. Conversely, the lowest efficacy values, 0%, were recorded for treatments with *S. molle* extract at a concentration of 25%.

Efficacy of Extracts in Reducing Grain Weight Loss

The results presented in Table 2 demonstrate the efficacy of the three extracts (*S. molle*, *M. azedarach*, and *T. vulgaris*) in reducing the average percentage of grain weight loss caused by rice weevil infestation after 50 days of treatment at the three tested concentrations (25%, 50%, and 100%). *M. azedarach* and *T. vulgaris* extracts exhibited significantly higher efficacy than *S. molle* extract at concentrations of 25% (69.66% vs. 30.12%) and 50% (87.56% vs. 53.05%), respectively. However, no significant differences were observed between the three extracts at a concentration of 100%, with efficacy values of 98.54%, 100%, and 100% for *S. molle*, *M. azedarach*, and *T. vulgaris*, respectively.

Concentration (%)	(% Grain weight loss)				(% Grain Damage level)				(% Adult Emergence)			
	<i>S. molle</i>	<i>M. azedarach</i>	<i>T. vulgaris</i>	Extract Means	<i>S. molle</i>	<i>M. azedarach</i>	<i>T. vulgaris</i>	Extract Means	<i>S. molle</i>	<i>M. azedarach</i>	<i>T. vulgaris</i>	Extract Means
25	30.12 ^D	62.39 ^{BC}	69.66 ^{BC}	54.06a	44.38b	69.83a	73.13a	62.45	25.32c	57.75b	65.18a	49.42
50	53.05 ^{CD}	82.45 ^{AB}	87.56 ^{AB}	74.35b	70.98ab	83.09a	89.33a	81.13	53.23b	81.03a	87.68a	73.98
100	98.54 ^A	100 ^A	100 ^A	99.51c	98.85a	100a	100a	99.61	92.75a	98.58a	100a	97.11
Means (%)	60.57a	81.61b	85.74b	---	71.40a	84.31a	87.49a	---	57.10a	79.12b	84.29b	---
LSD 0.01	Concentrations =16.00	extract =16.00	extract × concentrations =27.72	---	Concentrations =21.56	extract =21.56	extract × concentrations =37.34	---	Concentration =19.91	extract =19.91	extract × concentrations =34.48	---

Table 2: Effect of *S. molle*, *M. azedarach*, and *T. vulgaris* Extracts on grain weight loss, grain Damage level and adult Emergence. Means in the different comparisons followed by the same big letters are not significantly different at P=0.01

- Means followed by the same small letters in the same column or the same row are not significantly different at P=0.01

A positive correlation was observed between the efficacy values and concentration, indicating that the effectiveness in reducing grain weight loss increased with increasing extract concentration. At a concentration of 100%, all three extracts achieved near-complete elimination of grain weight loss. The differences in efficacy values among the three concentrations were significant, with mean values of 54.06%, 74.35%, and 99.51% for concentrations of 25%, 50%, and 100%, respectively.

The highest efficacy values were observed for both *M. azedarach* and *T. vulgaris* extracts, reaching 100% at a concentration of 100%. In contrast, the lowest efficacy value, 30.12%, was recorded for the 25% concentration of *S. molle* extract (Table 2).

Efficacy of Extracts in Reducing Grain Damage Percentage

Grain damage percentage refers to the proportion of grains that have been physically damaged by weevils. Treatment with plant extracts of *S. molle*, *M. azedarach*, and *T. vulgaris* demonstrated high efficacy in reducing the mean percentage of grain damage caused by rice weevil infestation, achieving reductions of over 70% when applied at a concentration of 50%. The efficacy values were 70.98%, 83.09%, and 89.33%, respectively. The highest efficacy in reducing grain damage percentage, reaching 100%, was achieved with both *T. vulgaris* and *M. azedarach* extracts at a concentration of 100%. There were no significant differences between these two extracts and *S. molle* extract at the same concentration (98.85%). Treatment with *S. molle* extract at a concentration of 25% resulted in the lowest efficacy values, reducing grain damage percentage by only 44.38%. The highest efficacy in reducing grain damage percentage, reaching 100%, was achieved with both *T. vulgaris* and *M. azedarach* extracts at a concentration of 100%. There were no significant differences between these two extracts and *S. molle* extract at the same concentration (98.85%).

Efficacy of Extracts in Reducing Adult Emergence

The results in Table 2 demonstrate that all three tested extracts (*S. molle*, *M. azedarach*, and *T. vulgaris*) effectively reduced adult rice weevil emergence after 50 days of treatment. All extracts achieved efficacy exceeding 50% at a concentration of 50%. Notably, *T. vulgaris* extract at 100% concentration achieved complete suppression of adult emergence (100%), demonstrating the highest efficacy. The lowest efficacy value, 25.32%, was observed for treatment with *S. molle* extract at a concentration of 25%.

No significant differences were observed between *M. azedarach* extract and *T. vulgaris* extract at any of the

tested concentrations. The efficacy of adult emergence reduction increased with increasing extract concentration for all extracts. The mean efficacy values for the three tested concentrations (25%, 50%, and 100%) were 49.42%, 73.98%, and 97.11%, respectively.

Discussion

It is evident from the results of the previous tables that the three plant extracts, *T. vulgaris*, *M. azedarach*, and *S. molle*, effectively increased the adult rice weevil mortality when applied at the three tested concentrations (25%, 50%, and 100%). These findings align with previous studies that have demonstrated the insecticidal properties of these extracts. The effectiveness of these extracts may be attributed to the presence of compounds with either contact toxicity or anti-nutritional effects, leading to high mortality rates among adult rice weevils. The observed differences in efficacy among the extracts can be attributed to variations in their chemical composition. For instance, the high efficacy of *T. vulgaris* extract is often associated with the presence of thymol, aromatic oil with known insecticidal properties. Studies have shown that thymol can affect the longevity of insects and induce mortality [12,13].

Several studies have also demonstrated that the effectiveness of *M. azedarach* stems from the presence of bioactive compounds with anti-nutritional and insecticidal properties. These compounds include triterpenoids and limonoids found in the leaves and fruits of *M. azedarach*, as well as azdrechtin [14-18].

Additionally, the fruits and leaves of *M. azedarach* contain flavonoids, catechin, and two kaempferol compounds, all of which exhibit insecticidal activity [19].

Saljoqi, et al. [20] demonstrated that the extract of *M. azedarach* fruit exhibited higher efficacy against rice weevil adults, with a mortality rate of 62%, compared to the leaf extract, which had a mortality rate of 34%. In the untreated control, no mortality was observed after four days of treatment [20]. Benzi, et al. [21] suggested that the effectiveness of *S. molle* fruits is likely attributed to the presence of limonene and caryophylline, which inhibit insect nutrition and contribute to high mortality rates among adult rice weevils [21].

The efficacy in increasing the average cumulative mortality rates of adult rice weevils increased with increasing extract concentration due to the rising proportions of toxic or antinutritional components, leading to higher mortality rates among rice weevil adults. This finding aligns with observations from several studies [14,22].

The plant extracts of *T. vulgaris*, *M. azedarach*, and *S. molle* provided significant protection to wheat grains, particularly at concentrations of 50% and 100%, effectively reducing the number of adult rice weevils emerging from treated grains. This reduction is attributed to high mortality rates among adult rice weevils due to various mechanisms, including poisoning, anti-nutritional effects, and disruption of egg-laying. Furthermore, the extracts demonstrated efficacy in reducing grain weight loss and the percentage of grain damage by inhibiting insect activity and feeding. Govinden K, et al. [17] reported that grain weight loss in *S. oryzae*-infested grains treated with *A. indica* extract was minimal, at 13.01%, compared to the untreated control, which exhibited a significant loss of 49.22%, after 60 days of treatment [17]. These findings align with the growing interest in exploring environmentally friendly alternatives to synthetic insecticides for controlling rice weevil infestations.

Both *M. azedarach* and *Ageratum conyzoides* L. powders demonstrated significant efficacy against rice weevils compared to the untreated control. This efficacy was evident in higher cumulative mortality rates, lower monthly increases in insect populations, and reduced grain damage and weight loss during a six-month storage period [23]. The use of plant materials in general has been shown to have an effect on stored grain pests. Recent studies demonstrate the efficacy of plant powders from *Pistacia lentiscus*, *Elettaria cardamomum*, and *Boswellia carterii* against *S. granarius* L. (Sg) and *T. confusum* Jac. (Tc). These powders completely suppress adult emergence and possess strong repellent properties [24].

In general, the effectiveness of plant extracts stems from the presence of anti-nutritional compounds that deter egg-laying or prevent hatching, thereby reducing adult emergence or affecting insect development and longevity. These findings align with the observations of several researchers [13,15-18].

Our results also demonstrated a significant increase in efficacy with increasing extract concentration, attributed to a rise in the proportion of active substances and components that exert diverse effects on insects. These findings align with observations from several studies [14]. Biodegradable plant materials like *S. molle* and *M. azedarach* provide a sustainable way to control insect pests without harming grains or the ecosystem. These plants can be used alone or integrated into IPM strategies [13].

Conclusion

The present study demonstrates the promising potential of plant extracts derived from *T. vulgaris*, *M. azedarach*, and *S. molle* as eco-friendly alternatives for controlling rice weevils.

These extracts exhibited significant insecticidal activity, effectively reducing adult mortality, suppressing adult emergence, and minimizing grain damage. The observed effectiveness demonstrates a potent and sustainable approach to managing rice weevil infestations. This study contributes to the growing body of research supporting the use of plant-based solutions for pest control, paving the way for future advancements in environmentally friendly pest management strategies. Before widespread application in grain storages, further research is needed to determine the potential health effects of the studied extracts, any residual solvent traces left after volatilization, the economic feasibility of large-scale use, compatibility with existing storage practices, and the potential for resistance development in rice weevils.

Conflicts of Interest

The authors declare no conflict of interest

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