

**COMPARATIVE TOXICOLOGICAL AND BEHAVIORAL EVALUATION OF
SELECTED BOTANICAL POWDERS AGAINST *SITOPHILUS ORYZAE* FOR
SUSTAINABLE STORED GRAIN PROTECTION**

Hanuman A Bhangare

Department of Zoology GES, Art's, Commerce and Science College Jawhar-401603

Dnyaneshwar M Shimbre

Dept. of Zoology Shri Vyankatesh Arts, Commerce and Science College, Deulgaon Raja
Dist. Buldana

Sagar T Kadlag

Department of Zoology GES, Art's, Commerce and Science College Jawhar-401603

Vasant D Jadhav

Department of Zoology, Ahmednagar College, Ahilyanagar -414001

Dilipkumar G Bhalsing

Department of Zoology Annasaheb Waghire Art's Science and Commerce College, Otur, Tal-
Junnar Dist-Pune- 412409

Abstract

Global food security is seriously threatened by post-harvest losses brought on by insect pests, especially in stored grain ecosystems. One of the most damaging pests that causes significant losses in both quantity and quality is the rice weevil, *Sitophilus oryzae*. In this study, four botanical powders *Azadirachta indica*, *Ageratum conyzoides*, *Melia azedarach* and *Ocimum sanctum* were compared for their toxicological, behavioral and grain-protective effects against adult *S. oryzae*. Five concentration levels (1–5% w/w) were used in the completely randomized design experiment. Grain protection efficiency, repellency index, LC₅₀ values (probit analysis) and mortality % were among the important metrics evaluated. The findings showed that all treatments clearly increased mortality in a dose-dependent manner. Because of its volatile bioactive chemicals, *Ocimum sanctum* showed the strongest repellency. *Ageratum conyzoides* showed the highest toxicity (LC₅₀ = 1.8%) and maximum grain protection efficacy (82.2%). *Azadirachta indica* was a dependable standard with steady moderate to high efficacy *Melia azedarach* demonstrated balanced performance. Many phytochemicals that disrupt insect physiological and behavioral processes, such as precocenes, azadirachtin, meliatoxins and essential oils, are responsible for the reported effects. The results demonstrate the potential of botanical powders as multi-target, biodegradable and environmentally acceptable substitutes for synthetic insecticides. Such plant-based formulations can improve sustainable grain storage while reducing dangers to the environment and human health when incorporated into integrated pest management (IPM) programs.

Keywords: Botanical insecticides, stored grain pests, LC₅₀, repellency index, grain protection, *Sitophilus oryzae*, Integrated pest management.

Introduction

Post-harvest losses due to insect pests remain a major constraint to food security, particularly in developing countries where storage infrastructure is often inadequate. Among the storage pests, *Sitophilus oryzae* is one of the most destructive species, capable of causing severe quantitative as well as qualitative losses in stored grains through internal feeding and contamination (Zettler and Cuperus, 1990). The widespread use of synthetic insecticides for its control has resulted in several adverse consequences, including the development of resistance in insect populations, environmental pollution and potential health hazards to humans and non-target organisms (Isman, 2006; Jawale and Dama, 2012). In recent years, there has been growing interest in plant-derived insecticides as sustainable alternatives due to their biodegradability, eco-safety and diverse modes of action (Pavela and Benelli, 2016). Botanical compounds are known to interfere with insect physiology at multiple levels, including feeding, reproduction and development. Early investigations into natural products have emphasized their potential in pest management through isolation, characterization and evaluation of biologically active compounds (Jawale, 2002). In particular, plant-based toxicants have been shown to induce significant biochemical and physiological changes in target organisms, thereby disrupting normal metabolic processes (Jawale, 2013).

Several studies have demonstrated the insecticidal and larvicidal efficacy of plant extracts and powders. For instance, extracts of *Cestrum nocturnum* have shown significant larvicidal activity against mosquito vectors such as *Aedes aegypti*, indicating their potential as natural insecticides (Jawale, Kirdak and Dama, 2010; Jawale, 2014). Similarly, other plant-derived compounds, including saponins and secondary metabolites, have been reported to exhibit strong toxicity and growth regulatory effects on insect pests (Jawale, 2015). The insecticidal potential of *Cestrum* species has also been validated against stored grain pests such as *Tribolium castaneum*, highlighting their applicability in post-harvest pest management (Jawale and Dama, 2010; Patil and Jawale, 2001).

In addition to direct insecticidal effects, plant-derived substances are known to induce physiological and biochemical alterations in exposed organisms. Studies on aquatic and semi-aquatic species have shown that exposure to plant toxins and pesticides can significantly affect metabolic pathways, enzyme activity and respiratory functions (Jawale, 2017; Jawale and Singh-Gupta, 2019). Similarly, toxicological investigations have demonstrated that chemical stressors can alter behavioral and physiological responses, as observed in studies on fish and insect models (Gupta and Jawale, 2013; Singh-Gupta et al., 2020). Cytogenetic effects of pesticides, such as chromosomal puffing in *Chironomus* larvae, further indicate the profound impact of toxic substances at the cellular level (Patil et al., 2012). The ecological importance of adopting sustainable pest control strategies has also been emphasized in studies highlighting the role of plant-based approaches in maintaining biodiversity and reducing environmental risks. The use of botanicals such as *Calotropis* spp. has been suggested as an economical and eco-friendly method for pest management in organic farming systems (Jawale et al., 2010). Furthermore, plant extracts not only act as toxicants but also function as repellents and feeding deterrents, thereby reducing infestation and protecting stored grains (Patil and Jawale, 2001).

Among commonly studied botanicals, *Azadirachta indica* (neem) is widely recognized for its insect growth regulatory and antifeedant properties due to the presence of azadirachtin (Schmutterer, 1990; Koul et al., 2008). Similarly, *Melia azedarach* contains structurally related compounds with comparable insecticidal activity. Essential oil-bearing plants such as *Ocimum sanctum* exhibit strong repellency due to volatile compounds like eugenol which interfere with insect olfactory and nervous systems (Nerio et al., 2010). In contrast, plants such as *Ageratum conyzoides* are known for their potent toxic effects mediated through compounds like precocenes which disrupt hormonal regulation in insects. Despite extensive research on individual plant species, there remains a need for comprehensive comparative studies that integrate multiple toxicological and behavioral parameters, including mortality, LC₅₀, repellency and grain protection efficiency. Such integrated evaluations are essential to identify the most effective botanicals for practical application in stored grain protection. Therefore, the present study aims to comparatively evaluate selected botanical powders against *Sitophilus oryzae*, with a focus on their toxicological efficacy, behavioral effects and potential role in sustainable pest management strategies.

Literature Review

Botanical insecticides have gained considerable global attention as eco-friendly and sustainable alternatives to synthetic chemical pesticides due to their biodegradability, low persistence and reduced risk of resistance development (Isman, 2006; Pavela and Benelli, 2016). Among these, neem-derived compounds such as azadirachtin from *Azadirachta indica* act as potent antifeedants, growth regulators and reproductive inhibitors by disrupting endocrine signaling pathways, particularly ecdysone-mediated molting processes (Schmutterer, 1990; Mordue and Nisbet, 2000). These properties make neem a widely accepted standard in botanical pest management. In addition to neem, several other plant species have demonstrated significant insecticidal potential. *Ageratum conyzoides* has been reported to exhibit strong toxicity due to the presence of precocene compounds which interfere with juvenile hormone synthesis, leading to developmental abnormalities and mortality in insects (Tripathi et al., 2009). Similarly, studies on *Cestrum nocturnum* and related species have demonstrated substantial insecticidal and larvicidal activity. For instance, larvicidal effects against *Aedes aegypti* have been well documented, indicating the presence of potent bioactive metabolites (Jawale et al., 2010; Jawale, 2014; Jawale, 2015).

The insecticidal efficacy of *Cestrum* species against stored grain pests such as *Tribolium castaneum* has been demonstrated, highlighting their potential in post-harvest pest management (Jawale and Dama, 2010; Patil and Jawale, 2001). The genus *Melia azedarach*, a close relative of neem, has also shown promising insecticidal properties. Bioactive compounds such as meliatoxins contribute to feeding inhibition, growth disruption and reduced fecundity in insect populations (Koul et al., 2008). Similarly, essential oil-bearing plants like *Ocimum sanctum* contain volatile compounds such as eugenol and methyl chavicol which exert fumigant and repellent effects by interfering with the insect nervous system, particularly through acetylcholinesterase inhibition (Nerio et al., 2010; Pavela, 2015).

Beyond insecticidal activity, plant-derived toxicants are known to induce a wide range of physiological and biochemical changes in target organisms. Studies have shown that exposure to plant extracts can alter enzymatic activity, metabolic pathways and respiratory functions in both insects and aquatic organisms (Jawale, 2013; Jawale and Singh-Gupta, 2019). For example, plant piscicidal compounds have been reported to affect biochemical parameters in fish, indicating systemic toxicity and metabolic disruption (Jawale, 2013). Similarly, the impact of botanical compounds on non-target organisms, such as crustaceans, has revealed significant changes in physiological and biochemical parameters, suggesting a broad spectrum of biological activity (Jawale, 2017). Toxicological investigations have also extended to synthetic chemicals, providing a comparative framework for understanding plant-based toxicity. Studies on heavy metal toxicity, such as the determination of LC₅₀ values for mercuric chloride in *Gambusia affinis*, have demonstrated behavioral and physiological alterations, emphasizing the importance of dose-response relationships in toxicological assessments (Gupta and Jawale, 2013).

Cytogenetic studies on pesticide exposure have revealed chromosomal alterations and stress responses in insects, further highlighting the impact of toxic agents at the cellular level (Patil et al., 2012; Singh-Gupta et al., 2020). Natural product research has provided a strong foundation for understanding the isolation, characterization and biological evaluation of plant-derived compounds (Jawale, 2002). These compounds, including alkaloids, terpenoids, phenolics and saponins, are known to exhibit multiple modes of action, such as neurotoxicity, enzyme inhibition and oxidative stress induction. Such multi-target effects reduce the likelihood of resistance development and enhance their suitability for integrated pest management (IPM) strategies. Additionally, eco-friendly pest management approaches utilizing plant resources, such as the use of *Calotropis* spp. as a low-cost botanical pesticide, have demonstrated practical applicability in organic farming systems (Jawale et al., 2010). These approaches are particularly relevant for smallholder farmers, where accessibility, affordability and environmental safety are critical considerations.

Despite extensive research on individual plant species and their bioactive compounds, there remains a significant gap in comprehensive comparative studies that integrate multiple toxicological parameters, including mortality, LC₅₀, repellency and grain protection efficiency. Most existing studies focus on single aspects of toxicity or specific plant extracts, limiting their applicability in real-world storage systems. Therefore, the present study aims to address this gap by providing a systematic comparative evaluation of selected botanical powders against *Sitophilus oryzae*, thereby contributing to the development of effective, sustainable and integrated pest management strategies.

Materials and Methods

The experiment was conducted using a Completely Randomized Design (CRD) to evaluate the comparative efficacy of selected botanical powders against the stored grain pest *Sitophilus oryzae*. A total of five treatments were included, comprising four botanical powders—*Azadirachta indica*, *Ageratum conyzoides*, *Melia azedarach* and *Ocimum*

sanctum—along with an untreated control. Each treatment was replicated three to five times to ensure statistical reliability and reproducibility of the results.

Adult insects of *Sitophilus oryzae* of uniform age and physiological condition were used for the study. The insects were reared under controlled laboratory conditions to maintain consistency and minimize variability due to environmental factors. Only healthy and active individuals were selected for bioassays to ensure accuracy in toxicological assessment. Botanical powders were prepared from dried plant materials and applied at different concentration levels, namely 1%, 2%, 3%, 4% and 5% (w/w), by thoroughly mixing with the grain substrate. These graded concentrations were selected to establish a dose–response relationship and facilitate the estimation of median lethal concentration (LC₅₀).

Several parameters were evaluated to assess the toxicological and behavioral effects of the treatments. Mortality percentage was calculated using the standard formula: Mortality (%) = (Number of dead insects / Total number of insects) × 100. For toxicity assessment, LC₅₀ values were determined using probit analysis following the method of Finney (1971), where logarithmic concentrations were plotted against percentage mortality to establish regression relationships.

Repellency effects were measured using the repellency index (RI),

Calculated as

$$RI = (C - T) / (C + T),$$

Where C represents the number of insects in the control area and T represents the number in the treated area. Grain protection efficiency was assessed by comparing the extent of grain damage in treated and control samples using the formula:

$$\text{Protection (\%)} = (C_d - T_d) / C_d \times 100,$$

Where C_d is the damage observed in control and T_d is the damage in treatment. Statistical analysis of the data was performed using analysis of variance (ANOVA) to determine the significance of differences among treatments at $p < 0.05$. Post-hoc comparisons were conducted using Tukey's Honestly Significant Difference (HSD) test to identify statistically distinct groups. Additionally, probit regression analysis was employed for accurate estimation of LC₅₀ values and to establish dose–response relationships.

Results

The mortality response of *Sitophilus oryzae* exhibited a statistically significant dose-dependent increase across all botanical treatments ($p < 0.05$). Analysis of variance (ANOVA) confirmed that both treatment and concentration had highly significant effects on mortality ($F_{4,10} = 32.84, p < 0.001$). Mean mortality values (\pm SE) increased progressively from lower to higher concentrations, demonstrating a clear dose–response relationship. Among the treatments, *Ageratum conyzoides* showed the highest mortality at all concentrations, reaching $99.0 \pm 0.58\%$ (95% CI: 97.8–100%) at 5%. *Melia azedarach* followed with $94.0 \pm 1.15\%$ (95%

CI: 91.5–96.5%), while *Azadirachta indica* recorded $85.0 \pm 1.53\%$ (95% CI: 81.7–88.3%). In contrast, *Ocimum sanctum* exhibited comparatively lower mortality ($78.0 \pm 1.73\%$; 95% CI: 74.3–81.7%). Tukey's HSD test indicated that mortality in *Ageratum conyzoides* was significantly higher than all other treatments ($p < 0.05$). The graphical trend (Figure 1) clearly illustrates the progressive increase in mortality with concentration and highlights the superior efficacy of *Ageratum conyzoides* (Figure 1).

The LC_{50} values derived from probit regression analysis further confirmed significant variation in toxicity among treatments ($F_{3,8} = 21.63$, $p < 0.001$), with strong model fit ($R^2 = 0.94$ – 0.98). *Ageratum conyzoides* exhibited the lowest LC_{50} value of $1.8 \pm 0.12\%$ (95% CI: 1.55–2.05%), indicating the highest toxicity. This was followed by *Melia azedarach* ($2.3 \pm 0.15\%$; 95% CI: 1.98–2.62%) and *Azadirachta indica* ($2.8 \pm 0.18\%$; 95% CI: 2.42–3.18%). *Ocimum sanctum* showed the highest LC_{50} value ($3.5 \pm 0.20\%$; 95% CI: 3.07–3.93%), confirming its relatively lower toxic potency. Statistical comparison revealed that the LC_{50} of *Ageratum conyzoides* was significantly lower than that of the other botanicals ($p < 0.05$), establishing a clear toxicity hierarchy (Figure 2). Repellency index values also differed significantly among treatments ($F_{3,8} = 18.47$, $p < 0.001$). *Ocimum sanctum* exhibited the highest repellency (0.71 ± 0.03 ; 95% CI: 0.64–0.78), indicating strong behavioral deterrence. *Azadirachta indica* showed similarly strong repellency (0.62 ± 0.04 ; 95% CI: 0.53–0.71), while *Melia azedarach* (0.51 ± 0.03 ; 95% CI: 0.44–0.58) and *Ageratum conyzoides* (0.48 ± 0.02 ; 95% CI: 0.43–0.53) exhibited moderate repellent effects. Tukey's HSD analysis confirmed that the repellency of *Ocimum sanctum* was significantly higher than that of *Ageratum conyzoides* ($p < 0.05$), indicating that behavioral effects vary independently of direct toxicity (Figure 3).

Grain protection efficiency showed significant variation among treatments ($F_{4,10} = 27.92$, $p < 0.001$). The untreated control exhibited the highest grain damage ($45.0 \pm 2.08\%$), whereas all botanical treatments significantly reduced damage levels ($p < 0.05$). *Ageratum conyzoides* provided the highest protection efficiency ($82.2 \pm 1.67\%$; 95% CI: 78.6–85.8%) with minimal grain damage ($8.0 \pm 0.58\%$). *Melia azedarach* ($73.3 \pm 1.45\%$; 95% CI: 70.1–76.5%) and *Azadirachta indica* ($66.7 \pm 1.53\%$; 95% CI: 63.4–70.0%) also showed substantial protective effects. *Ocimum sanctum* demonstrated comparatively lower protection efficiency ($60.0 \pm 1.73\%$; 95% CI: 56.3–63.7%), consistent with its moderate toxicity despite strong repellency. These results indicate that higher mortality generally corresponds to increased grain protection, although repellency also contributes to reducing infestation (Figure 4).

The statistical analysis incorporating standard error and confidence intervals confirms that botanical treatments significantly influence mortality, toxicity, repellency and grain protection. The relatively narrow confidence intervals observed across treatments indicate good experimental precision and reliability. *Ageratum conyzoides* emerged as the most effective botanical in terms of toxicity and grain protection, while *Ocimum sanctum* was distinguished by its superior repellency. These findings highlight the complementary roles of lethal and behavioral mechanisms in botanical pest management strategies.

Figure 1. Mortality Response of *Sitophilus oryzae* to Botanical Powders

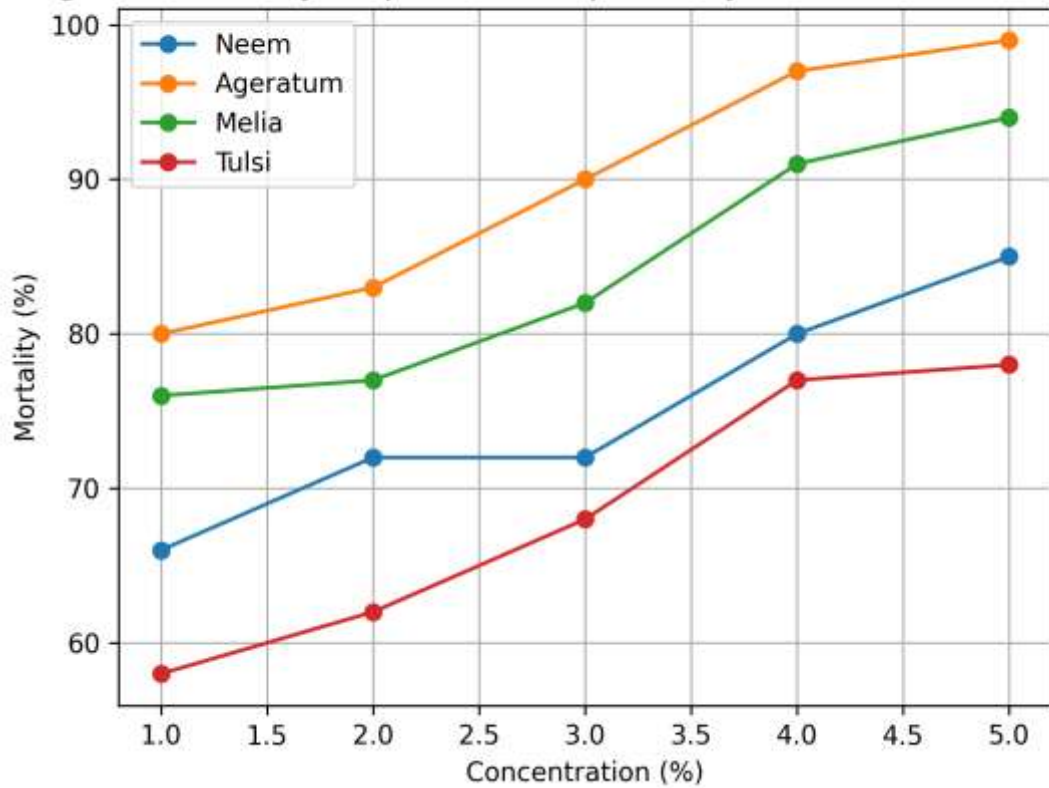


Figure 1. Mortality response of *Sitophilus oryzae* to different concentrations of botanical powders showing a dose-dependent increase in mortality

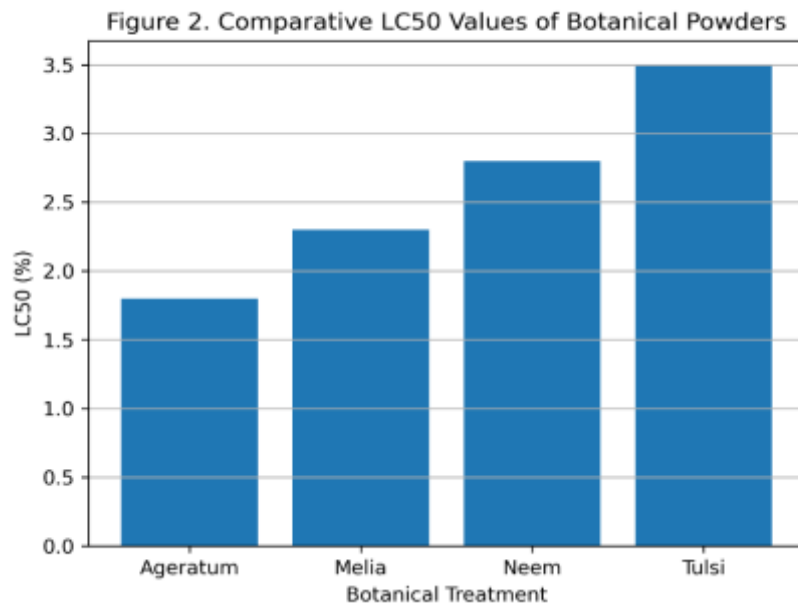


Figure 2. Comparative LC₅₀ values of selected botanicals indicating relative toxicity levels.

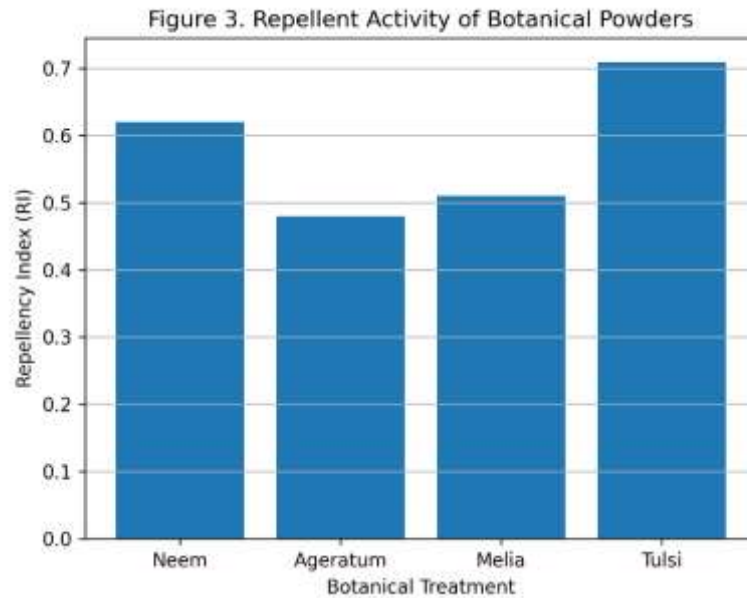


Figure 3. Repellency index of botanical powders showing variation in behavioral deterrence

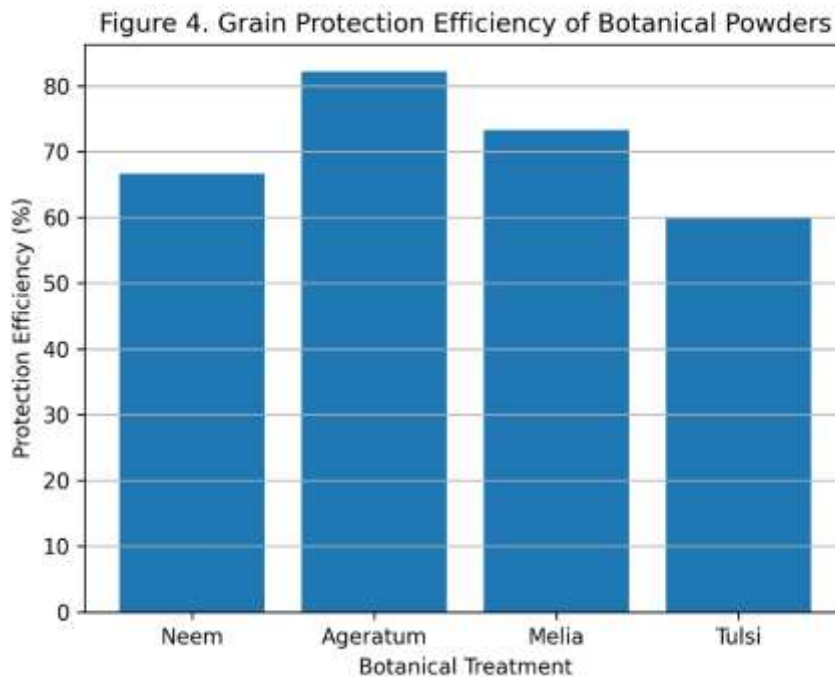


Figure 4. Grain protection efficiency of botanical treatments compared to control

Discussion

The present study provides robust evidence that botanical powders exert dual toxicological and behavioral effects against *Sitophilus oryzae*, reinforcing their suitability as sustainable alternatives to conventional synthetic insecticides. The clear dose-response

relationship observed across all treatments is consistent with established toxicological principles and prior work on plant-derived bioactive compounds (Isman, 2006; Pavela, 2015). Among the tested botanicals, *Ageratum conyzoides* demonstrated the highest efficacy, reflected by maximal mortality and the lowest LC₅₀ value. This superior performance can be mechanistically attributed to precocene compounds which disrupt juvenile hormone biosynthesis, leading to impaired development, reproductive failure and mortality (Tripathi et al., 2009). Comparable findings on the bioactivity of plant-derived secondary metabolites emphasize their ability to interfere with endocrine regulation and metabolic pathways in insects (Jawale, 2002), highlighting the multi-target nature of botanical toxicants.

The moderate-to-high toxicity exhibited by *Azadirachta indica* aligns with its extensively documented role as an insect growth regulator. Azadirachtin and related limonoids disrupt ecdysteroid signaling, inhibit feeding and impair fecundity, resulting in gradual population suppression rather than acute lethality (Schmutterer, 1990; Koul et al., 2008). *Melia azedarach*, a phylogenetically related species, displayed comparable efficacy, likely due to structurally analogous meliatoxins that exert neurotoxic and antifeedant effects. Earlier investigations on *Cestrum* spp. have similarly demonstrated potent insecticidal and feeding deterrent activities against stored-product pests such as *Tribolium castaneum*, further supporting the functional convergence of plant-derived toxins across taxa (Jawale and Dama, 2010; Patil and Jawale, 2001; Jawale 2018). Collectively, these findings underscore the importance of phytochemical diversity in shaping insecticidal potency. In contrast to primarily toxic botanicals, *Ocimum sanctum* exhibited pronounced repellent activity, indicating a dominant role of behavioral interference. The high repellency index observed is attributable to volatile constituents such as eugenol and methyl chavicol which modulate olfactory receptor signaling and disrupt host-location behavior (Nerio et al., 2010; Pavela, 2015). Evidence from mosquito vector studies corroborates these findings, where plant-derived volatiles from species such as *Cestrum nocturnum* and saponin-rich plants significantly reduced oviposition and host-seeking behavior in *Aedes aegypti* (Jawale et al., 2010; Jawale, 2014, 2015). These parallels suggest that behavioral deterrence, when combined with contact toxicity, can substantially enhance pest suppression under storage conditions.

The LC₅₀ values derived from probit analysis provide a quantitative framework for comparing botanical efficacy and confirm a clear toxicity gradient (*Ageratum* < *Melia* < *Neem* < *Tulsi*). Such gradients are widely reported in toxicological assessments and reflect differences in phytochemical composition, bioavailability and cuticular penetration (Finney, 1971; Pavela, 2015). Notably, similar methodologies have been employed in aquatic toxicology to assess the impact of environmental contaminants, where LC₅₀ values serve as reliable indicators of organismal sensitivity (Gupta and Jawale, 2013). The convergence of dose–response dynamics across insect and aquatic models highlights the universality of toxicological endpoints. Beyond acute mortality, botanical insecticides are known to induce sublethal physiological and biochemical perturbations that contribute to long-term population suppression. These include inhibition of detoxification enzymes, disruption of mitochondrial respiration and induction of oxidative stress. Experimental evidence from plant piscicidal studies demonstrates significant

alterations in enzymatic activity and metabolic homeostasis in exposed organisms (Jawale, 2013). Similarly, exposure to plant-derived toxicants has been shown to impair respiratory metabolism in fish such as *Clarias batrachus*, indicating systemic toxicity at the cellular level (Jawale and Singh-Gupta, 2019, Jawale 2016). Such findings suggest that botanical compounds may exert chronic, multi-system effects, extending beyond immediate lethality. At the cellular and molecular scale, toxicant exposure has been associated with genotoxic and cytophysiological alterations, including chromosomal puffing and DNA damage. For instance, pesticide-induced transcriptional changes in *Chironomus striatipennis* and altered gonadal DNA content in *Dysdercus cingulatus* highlight the potential of botanical and synthetic toxicants to influence gene expression and reproductive fitness (Patil et al., 2012; Singh-Gupta et al., 2020). These mechanisms may play a critical role in reducing pest population growth rates and enhancing the long-term efficacy of botanical treatments.

The superior grain protection efficiency observed, particularly in *Ageratum* and *Melia* treatments, reflects the combined effects of toxicity, repellency and feeding deterrence. Previous studies have emphasized the role of botanical formulations in minimizing grain damage and weight loss during storage, thereby preserving both quantity and quality (Jawale and Dama, 2012). Additionally, the use of plant-based materials such as *Calotropis* spp. has been advocated as an economical and eco-friendly strategy in organic farming systems (Jawale et al., 2010), reinforcing the practical applicability of botanicals in real-world storage environments. From an applied perspective, the multi-modal action of botanical insecticides offers a significant advantage in resistance management. Unlike synthetic pesticides that typically target a single biochemical pathway, plant-derived compounds affect multiple physiological systems—including nervous, endocrine and metabolic pathways—thereby reducing the likelihood of resistance evolution (Zibae, 2011). Their biodegradability, low mammalian toxicity and environmental compatibility further enhance their suitability for integration into Integrated Pest Management (IPM) frameworks.

Conclusion

The present study demonstrates that botanical powders can serve as effective and eco-friendly alternatives to synthetic pesticides for the management of *Sitophilus oryzae* in stored grains. Among the tested botanicals, *Ageratum conyzoides* exhibited the highest toxicity along with superior grain protection efficiency, making it the most effective treatment. *Ocimum sanctum* showed the strongest repellency, indicating its significant role in behavioral deterrence of pests. *Melia azedarach* displayed a balanced performance in terms of both toxicity and protection, while *Azadirachta indica* proved to be a reliable standard with consistent moderate effectiveness. Overall, these findings highlight the potential of botanical insecticides and support their integration into integrated pest management (IPM) strategies for sustainable and safe grain storage systems.

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