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Studying the insecticidal activity of Bay leaf (*Laurus nobilis*) powder and clove (*Syzygium aromaticum*) powder on rice weevil (*Sitophilus oryzae*).

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ABSTRACT

Insecticidal Activity of Clove (*Syzygium aromaticum*) and Bay Leaf (*Laurus nobilis*) on rice weevil, *Sitophilus oryzae* (L.) - a major stored grain pest. Post-harvest losses inflicted by rice weevil, *S. oryzae* (L), still constitute one of the most significant barriers to grain storage in India and the increasing prevalence of resistance to the chemical fumigants of this pest warrants further investigation of botanical alternatives. The present study was undertaken to assess the insecticidal efficacy of powder of bay leaf (*L. nobilis*), clove (*S.aromaticum*) and a mixture thereof against the rice weevil under laboratory conditions. Treatments were incorporated into 35 g of rice at increasing concentrations 0.5,1.0 and 1.5 mg in a completely randomized design with 3 replications, and the number of twenty exposed rice weevils found dead was recorded at the end of days 1, 3, 5 and 7. Bay leaf powder had a minimal insecticidal effect on the weevil, and mortality observed at different doses was not significantly different from that in the control ($p>0.05$) and showed no dose-response relationship. Clove powder, however, caused a marked and dose and time-dependent mortality on the rice weevil, with a high of 86.1 per cent on the 7th day at the concentration of 1.5 mg/100 g rice, while the median lethal concentration (LC_{50}) value was 1.61 g/100 g rice on the first day but was reduced to 0.61 g/100 g rice on the 7th day. Clove and bay leaf combined treatment showed maximum mortality of 91.1 %, on the 7th day, with a dose of 1.0 mg/100g rice, but the trend of LC_{50} for the combined treatment did not differ much from the trend for only clove treatment, suggesting that it may be the presence of clove that contributed to significantly higher mortality in mixed treatment, more than a synergy. Thus, this study has identified clove powder as a promising, cost-effective and eco-friendly botanical material to protect stored rice from weevil, *S. oryzae*, though it needs to be explored through various formulation studies for its effective use in the field.

Key Words - *Sitophilus oryzae*; *Syzygium aromaticum*; *Laurus nobilis*; botanical insecticide; LC_{50}; stored-grain protection

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INTRODUCTION

Effective reduction of storage loss of cereal grain is as important to food security as raising production, because that loss never reaches the consumer. In particular, in the developing world and even many parts of developing and developing countries, rice is the principal food for about one-third of the world population, especially people living in Asia and Africa. Despite the fact that India is the second largest producer of paddy rice in the world, significant amounts of harvested rice are lost during storage, transport and handling owing to a lack of adequate storage facilities. One of the important causal factors is the infestation of storage insect pests; the overall storage losses in India are estimated between 6-10%, 4-6% of which are caused by insect pest infestation. Of these pests, the rice weevil, *S. oryzae* (L), is the most serious pest of stored rice. This insect bores into rice grains and lays its eggs; the development of larvae takes place inside the grain without external indication till adults come out from the grains, giving exit holes. Its high multiplication rate can make a population that could consume up to 30% of grain in one storage season, and it is estimated to cause 30-40% loss of rice during storage in India. For the last over six decades, synthetic fumigants (like phosphine), and insecticides (like organo-phosphates and pyrethroids) have been widely used to control the weevils. However, the continued and non-specific use of chemical pesticides leads to the development of resistance in the target pest. High levels of phosphine resistance have been reported in rice weevil populations in many regions where rice is cultivated. In addition, the risk of pesticide residues in stored grain has raised consumers' concerns over food safety, leading to strict maximum residue limits. These challenges hinder the adoption of conventional pest management methods, increasing production costs, especially for smallholder farmers. As an alternative approach, plant-derived pesticides (botanicals) have emerged as an environmentally friendly option with low human toxicity. Unlike chemical pesticides that often have a specific mode of action, botanicals usually act through a multi-target approach, including contact toxicity, fumigation, feeding deterrent and anti-ovipositional activities, which delays the development of insect resistance. Many aromatic plant oils, like essential oils extracted from clove (*S. aromaticum*) and bay leaf (*L. nobilis*), have shown good insecticidal properties against stored product insects, mostly due to their main constituents such as eugenol. Most studies, however, used the extracted essential oil instead of dried plant powder, which is easy to handle and relatively cheap for use by farmers. In the present study, we evaluated the insecticidal activity of clove powder, bay leaf powder and their mixture against adults of *S. oryzae* on different dose levels and exposure times and the median lethal dose (LD_{50}) was determined using probit analysis.

MATERIALS & METHODS

Insect Culture and Grain

Rice weevil, *S. oryzae* was obtained from naturally infested rice supplied from the local market, Patna, Bihar, India, and were reared in laboratory stock culture. The species was identified based on morphological characters such as an elongated rostrum, geniculate antennae and the presence of reddish spots on the elytra as per the keys of standard taxonomic descriptions. The stock culture was maintained in the Entomology Laboratory, P.G. Department of Zoology, Patna University, on whole, uninfested grains of rice. The culture was maintained in the sealed plastic jars at laboratory conditions at 28.2–36.5°C temperature, 45-55% relative humidity and 8h light:16h dark. Adult weevils of uniform age were selected from the stock culture for all bioassays to ensure experimental consistency. Rice grains used in the experiments were purchased from the local market and thoroughly cleaned by removing broken grains and other impurities before use.

Plant Material and Powder Preparation

Preparation of plant powders: Hundred grams (approx.) of dried clove buds (*S. aromaticum* L.) and dried bay leaves (*L. nobilis L.*), respectively, were purchased from a local market of spices of Patna. Both plant materials were authenticated using their macroscopic characters like shape, colour, size, and their odour, and were examined for their lack of moisture, absence of mould, insect-damaged pieces, etc. The authenticated samples were stored at 4°C until further processing. Each dried plant material was then ground separately into a fine powder using a mixer grinder GX15 (410516) for (1-3) minutes. The powders were preserved in plastic containers, and stored at 4°C in a refrigerator till use.

Experimental Design

The experimental design was conducted using a completely randomized design (CRD) with three replications for each treatment. Each replicate consisted of a ventilated plastic jar containing 35 g of rice grains. The rice grains were treated with either clove powder (*S. aromaticum*), bay leaf powder (*L.nobilis*), or a 1:1 mixture of both powders. Clove powder and the combined treatment were applied at concentrations of 0.5, 1.0, and 1.5 mg/100 g of rice, whereas bay leaf powder was evaluated only at 1.0 and 1.5 mg/100 g of rice. An untreated control containing only rice grains was also included. Subsequently, 20 rice weevil, *S. oryzae* of different age were introduced into each plastic jar. The plastic jars were covered with mesh lids to allow adequate ventilation while preventing the insects from escaping.

Mortality Assessment

Rice weevil mortality was recorded at 1, 3, 5, and 7 days after treatment. Weevil that showed no movement or response when gently touched with a fine hair brush were considered dead. The number of dead weevils in each treatment was counted manually, and the percentage mortality was calculated for each observation period. To account for natural mortality in the untreated control, the observed mortality in each treatment was corrected using Abbott's formula (Abbott, 1925): Corrected mortality (%) = T-C /100–C X 100, where T is the observed percentage mortality in the treated group and C is the observed percentage mortality in the untreated control.

Statistical Analysis

Mortality data were expressed as mean ± standard deviation (SD) for each treatment at each observation interval. Differences in mortality between each treatment and the untreated control were analysed using a paired *t*-test. Corrected mortality percentages were transformed into probit values and plotted against the base-10 logarithm of the treatment concentration to obtain the regression equation: Probit = $a + b$ log10 (Concentration) where a is the intercept and b is the slope of the regression line. The median lethal concentration (LC_{50}) for each exposure period was estimated by substituting a probit value of 5 into the regression equation, solving for the corresponding concentration, and back-transforming the value from the logarithmic scale.

RESULTS

Effect of Bay Leaf Powder

In the control the mortality of rice weevil *S. oryzae* varied from 3.8% in day 1 to 38.3% in day 7 which was equivalent to the level of background natural mortality over the exposure days (Fig.1). When applied at 1.0 mg/100 g of rice the mortality of bay leaf powder varied between 11.6% at day 1 and 52.7% at day 7 and which is slightly greater than the control level. On the contrary, the mortality rate at the dose of 1.5 mg/100 g of rice (1.6-32.2%) was significantly lower than that recorded with 1.0 mg/ 100 g of rice and not statistically different from the control rate at any time point (paired t-test, $p > 0.05$). Thus, no clear dose response was evident with this plant-based insecticide.

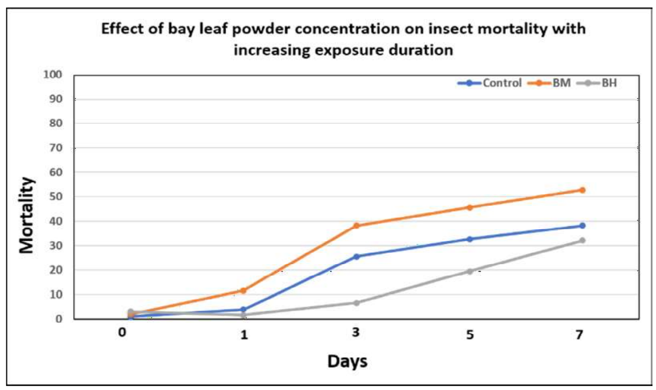

Fig. 1- Effect of bay leaf powder concentration on insect mortality with increasing exposure duration

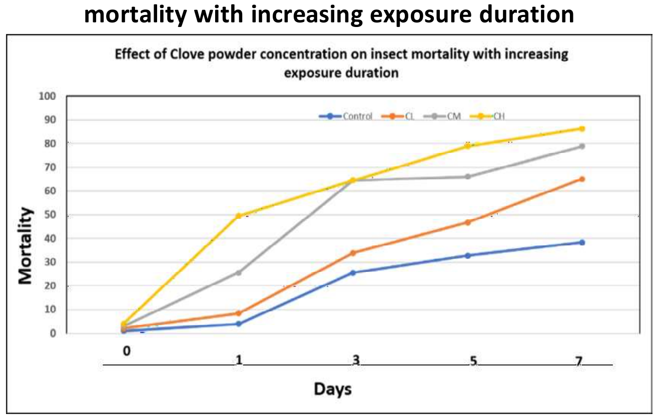

Fig. 2- Effect of clove powder concentration on insect mortality with increasing exposure duration

Effect of Clove Powder

Mortality: Clove powder increased consistently with increased dose and days (Fig.2). Mortality was dose and exposure time-dependent, with concentrations from 0.5 mg/100 g to 1.5 mg/100 g giving mortality between 8.3% and 65% at one day (lowest concentration and lowest day, respectively) up to 86.1% on day 7 (highest concentration and highest day, respectively). Three levels of the clove powder had significantly higher mortality than the control.

Effect of the Combined Clove and Bay Leaf Powder

The combined treatment also induced dose and time-dependent mortalities, although less marked with a pattern less well pronounced than clove treatments only (Fig.3). The combined 1.0 mg/100g treatment produced the highest mortality measured throughout this experiment (33.3% on day 1 and increased until 91.1% by day 7), more than in the combined 1.0mg/100g using clove powder only. With a combined 1.5mg/100g treatment, the mortality reached 80.0% at day 5. No more increase by day 7.

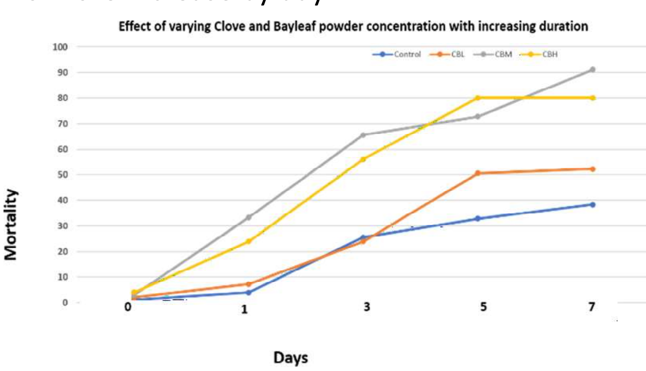

Fig. 3: Effect of clove and bay leaf powder concentration on insect mortality with increasing exposure duration

Median Lethal Concentration (LC_{50})

It was revealed by probit analysis that the LC_{50} value of clove powder gradually reduced with increase in exposure time from 1.61 g/100 g rice on day 1 to 0.61 g/100 g rice on day 7 of storage (Fig.4 and Fig.5). Similarly, it also reduced in combination of clove and bay leaves treatments from 2.54 g/100 g rice on day 1 to 0.74 g/100 g rice on day 7. However, on comparison, it was found that the LC_{50} value of combination treatments was numerically larger than that of alone clove treatments at every exposure period, showing clove being most effective on a per-concentration basis.

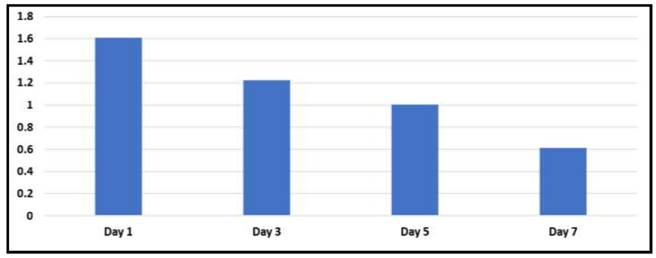

Fig. 4- LC_{50} of clove (g/100 g)

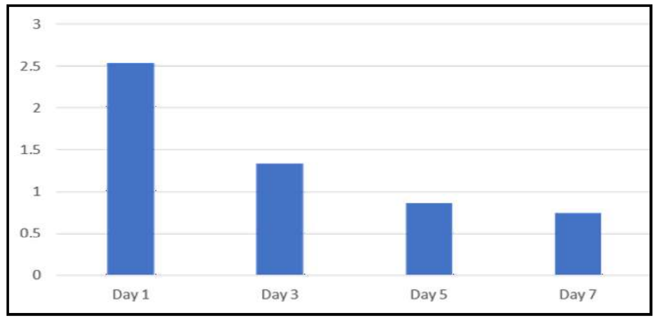

Fig. 5- LC_{50} of clove & bay leaf (g/100 g)

DISCUSSION

The present study demonstrated that the insecticidal activity of clove powder (*S. aromaticum*) against *S. oryzae* was significantly greater than that of bay leaf powder (*L. nobilis*), indicating that clove powder is a promising botanical protectant for stored rice. These findings are consistent with previous studies reporting the contact and fumigant toxicity of clove essential oil and its major bioactive constituent, eugenol, against several stored-product insect pests. Eugenol is known to disrupt the insect nervous system and interfere with respiratory metabolism, resulting in both contact and fumigant toxicity. Unlike previous studies that primarily evaluated purified essential oil, the present investigation employed crude clove powder and achieved a maximum mortality of 86.1% after seven days at a concentration of 1.5 mg/100 g of rice. This finding suggests that the powdered spice contains sufficient quantities of biologically active compounds to provide substantial protection against *S. oryzae* without the additional cost and processing associated with essential oil extraction. In contrast, bay leaf powder exhibited very limited insecticidal activity throughout the experimental period. Mortality in the bay leaf-treated groups did not differ significantly from that observed in the untreated control, and no clear dose-dependent response was evident. Interestingly, mortality at 1.5 mg/100 g of rice was lower than that recorded at 1.0 mg/100g, suggesting that the observed mortality was more likely due to natural variation than to the toxic effect of bay leaf powder. These findings differ from previous reports demonstrating the insecticidal efficacy of *L. nobilis* essential oil against stored-product insects. This discrepancy may be attributed to the higher concentration of volatile monoterpenes in the essential oil. In contrast, these compounds occur naturally in much lower concentrations in dried leaves and are released less efficiently from powdered bay leaves.

The combined application of clove and bay leaf powders produced the highest mortality (91.1%) after seven days at a concentration of 1.0 mg/100 g of rice, initially suggesting a possible additive or synergistic interaction. However, the LC_{50} values estimated through probit analysis did not support this interpretation. Across all exposure periods, the combined treatment required higher concentrations than clove powder alone to achieve 50% mortality, indicating that the addition of bay leaf did not enhance clove's insecticidal efficacy. Instead, the mixture required a greater concentration to produce the same level of mortality as clove alone, suggesting that bay leaf contributed little to the overall insecticidal activity. Furthermore, since bay leaf powder alone exhibited negligible toxicity, the improved mortality observed in the combined treatment is more likely attributable to the presence of clove rather than to any true synergistic interaction. These findings emphasise the importance of evaluating the efficacy of individual botanical components before combining them, as mixtures do not necessarily result in enhanced insecticidal activity. The progressive increase in mortality with increasing exposure duration indicates that clove powder possesses cumulative insecticidal activity, making it a promising grain protectant for long-term storage. Owing to its low cost, widespread availability in many Asian countries, and environmentally friendly nature, clove powder has considerable potential as an alternative to conventional synthetic insecticides, which are often associated with insect resistance, environmental persistence, bioaccumulation, and harmful chemical residues in food products. Despite these encouraging findings, the present study has certain limitations. The experiments were conducted under controlled laboratory conditions and evaluated adult mortality for only a single insect generation. Therefore, further studies are required to investigate the effects of clove powder on oviposition, egg hatchability, F progeny development, and its long-term effectiveness under fluctuating storage conditions. In addition, the effects of clove powder on seed germination, cooking quality, and the sensory characteristics of stored rice should be evaluated before recommending its large-scale application as a botanical grain protectant.

CONCLUSION

The present study demonstrated that clove powder (*S. aromaticum*) exhibited significant dose-and time-dependent insecticidal activity against the rice weevil, *S. oryzae*. A maximum mortality of 86.1% was recorded after 7 days of exposure at a concentration of 1.5 mg/100 g of rice, while the LC_{50} decreased from 1.61 to 0.61 g/100 g of rice as the exposure period increased from one to seven days, indicating increased toxicity with prolonged exposure. In contrast, bay leaf powder (*L. nobilis*) showed negligible insecticidal activity under laboratory conditions and failed to exhibit a clear dose-dependent response. Although the combined application of clove and bay leaf powders resulted in the highest mortality (91.1%), the LC_{50} values were not lower than those obtained with clove powder alone, suggesting that the increased mortality was primarily attributable to the insecticidal activity of clove rather than any synergistic interaction between the two botanicals. Overall, the findings indicate that clove powder is a promising, safe, cost-effective, and environmentally friendly botanical alternative for the management of *S. oryzae* in stored rice. However, further studies are required to evaluate its effects on grain quality, seed germination, progeny development, and long-term storage performance under farmers' storage conditions before recommending its large-scale application. Based on the present results, bay leaf powder alone does not appear to be a suitable botanical material for the protection of stored rice against *S. oryzae*.

CONFLICT OF INTEREST

We state that they have no commercial, financial, professional, or any personal relationships with any other people or organisations that may have unfairly influenced this work.

ETHICAL APPROVAL

The current study used Laboratory Rearing and Bioassay of the stored grain pest *S. oryzae* and did not use vertebrates or humans; hence, ethical clearance by the institution is not applicable and has not been sought.

INFORMED CONSENT

Not applicable, as this study did not involve human participants.

AUTHOR CONTRIBUTIONS

Md Ekramul Hoda, Gayatri Arya, Swastika Raj and Mayank Kumar contributed to conceptualization, methodology, investigation, data curation, formal analysis, writing and editing. All authors read and approved the final version of the manuscript.

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