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Repellency effect of leaf powder of Neem (*Azadirachta indica*), Eucalyptus (*Eucalyptus saligna*), Peepal (*Ficus religiosa*), and Lemon (*Citrus limon*) against Rice Weevil *Sitophilus oryzae* (Coleoptera, Curculionidae) in stored rice (*Oryza sativa*)

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ABSTRACT

The effect of leaf powders of four medicinal plants viz. Neem (*Azadirachta indica*), Eucalyptus (*Eucalyptus saligna*), Peepal (*Ficus religiosa*), and Lemon (*Citrus limon*) on Rice Weevil (*Sitophilus oryzae)* was studied in stored rice. The repellency rate and mortality effect were calculated using a completely randomized design with 4 treatments. The data was then projected to statistical analysis and the test significance level was $P<0.05$, the best treatment in terms of repellency rate was 10gm lemon leaf powder causing 83% repellency within 48 hours of application and in terms of mortality the treatment with 10gm Neem leaf powder showed best results causing 78.5% mortality within 3-days. The results show this trend (Repellency=Lemon>Eucalyptus>Neem>Peepal) (Mortality= Neem>Lemon>Eucalyptus>Peepal) The following experiment is an effort to find alternative options for organic biopesticides that can be used in place of chemical pesticides in a more biodegradable, cost-effective, and sustainable manner.

KEYWORDS: Repellency, S*itophilus oryzae*, Biopesticide, Leaf powder

INTRODUCTION

Rice (*Oryza sativa*) is an important crop and a staple food for half of the World's population. It is grown in more than 100 countries around the world and Asia contributes to about 90% of its global production (Akhtar & Raza, 2015)Rice is continually infested by a variety of pests during the post-harvest storage period. One of the major pests that hamper the quality as well as quantity of stored rice is *S. oryzae* commonly known as rice weevils (Prastowo, 2022). The feeding of rice weevils on endosperm cause loss of carbohydrate content and remove ample amount of protein and vitamins, and also results in weight loss of stored grains.(Dal Bello et al., 2000) The rice weevils are found actively breeding in whole grains such as Wheat, Barley, Oats, and Rice, and its infestation is also seen in various legumes and split pulses (Rojasara et al., 2019). Both larval and adult phases deteriorate the stored product. (Dhaniya & Dayanandan, 2016) Seed viability is also reduced in addition to direct losses, thus *S. oryzae* reduces both the nutritional and market value of grains. (Ashamo, 2006)

The rice stored in warehouses reduces by 5%-10% due to certain warehouse pests, especially S. oryzae (Bond et al., 2013)The level of damage for each country varies depending upon its climate, temperature, and humidity and for India it ranges between 20%-25% thus, finding measures to control these pests are of utmost importance (Phillips & Throne, 2010). Traditional practices of grain protection include the use of common salt in red gram and the addition of ash to sorghum and rice to help ward off insects. (Trivedi et al., 2018) Though, agricultural chemicals play a miraculous role in forming the front-line defense their long-term hazards such as cancer, genomic damage, oxidative stress, respiratory, thyroid, and metabolic effects can't be kept at stake.(Hajam & Kumar, 2022) These chemicals lead to a large spectrum of hazard-visualizing resistance, resurgence, and residual hazards including soil, water, and air pollution.

Many synthetic insecticides also show mammalian toxicity and the grains treated with such chemicals are not safe for human consumption (Mohd et al., 2016). Plant products on the other hand show insecticidal and repellent properties to insects with no mammalian toxicity (*Evaluation of Botanical Powders Against Rice Weevil (Sitophilus Oryzae) in Stored Sorghum**, n.d.). Secondary plant chemicals or allelochemicals are found in tissues of higher plants which include alkaloids, saponins, steroids, phenolics, essential oils etc which have insecticidal and deterrent properties. (Said, n.d.) Therefore, present-day

researchers are focussing on the use of biopesticide repellents to prevent the negative effect on food safety. Naturally, plants, herbs, and spices are potent sources of pest repellent as they contain certain bioactive that act against the pest and act as target toxicants for the pest (Ravi et al., 2019; Tlak Gajger & Dar, 2021). Most plant materials are active against certain pests and are non-toxic as well as biodegradable which makes them a priority (S. Singh et al., 2017).

Inhalation toxicity or Fumigant toxicity is one of the methods adapted for killing insects (K. D. Singh et al., 2021). It is done in closed containers and the plant products in this case release plant volatile compounds (PVOCs) which penetrate the respiratory system of the insect and induce effects. This activity of plant product products is due to different functional groups attached to these volatile chemicals which enable them to persist in the closed containers for a long time (Choi et al., 2003). Different plant products viz. leave, roots, stem, rhizome, etc. contain different volatile compounds and they can be utilized by various methods such as by using powder of dried parts or by extracting the target compound (S. Singh, 2017). PVOCs also act as repellents and induce the emigration of insects away from the treated product and restrict the immigration of new insects (K. D. Singh et al., 2021)This property of repellency has an important implication in the post-harvest storage system adapted traditionally. Previous researchers have evaluated the repellent and mortality effect of various plant products and have coined PVOCs responsible for the particular effect. I have taken 4 dried leaf powders of Neem, Lemon, Eucalyptus, and Peepal accordingly to evaluate the inhalation toxicity and induced repellency of the concerned plant on *S. oryzae* in stored rice.

Neem (*Azadirachta indica)* contains a phytochemical Azadirachtin which has a broad mode of action as a repellent, IGR (Insect Growth Regulator), feeding deterrent, and chemosterilant. It is effective against storage and sucking pests (S. Singh et al., 2017). Neem leaves also contain alkaloid compounds, nimbidine, tannins, and resins and can thus be utilized as plant pesticides (Ahmad et al., 2017). The leaves of citrus plants also contain PVOCs which are active repellents of storage pests. The research proposed by (Mohd et al., 2016) stated 90% repellency of *C. limon* leaf powder against *S. Oryzae* in stored rice (Mohd et al., 2016). The leaves of *Eucalyptus saligna* (Myrtaceae) contain p-cymene which shows fumigant, repellent, and contact toxicity or inhalation toxicity against *S. oryzae* and *T. castaneum* (K. D. Singh et al., 2021). Peepal *Ficus religiosa* is a medicinal plant and no adverse effect on humans is known so far, it is used as a test product to evaluate its toxic and repellent properties against *S. oryzae.* This experiment aims to find a new way of applying insecticide and evaluating the repellency and mortality-related properties of aforementioned plant products against *S. oryzae* in stored rice. This research is expected to be useful in controlling *S. oryzae* in a more environmentally friendly manner.

MATERIAL AND METHODS

The study was conducted during December 2022 at 22℃ ± 5℃ and 85 ± 5% humidity. The design of the experiment was completely randomized with 4 treatments and 10 replications of each; a control without any treatment was also included. 100gm of rice with 10gm of leaf powder was taken for each treatment. This methodology is followed from (Prastowo, 2022) , though some changes are made.

S. oryzae were reared according to the procedure where 100 adapted *S. oryzae* (approx. 50 males and 50 females) were kept in a plastic jar filled with rice as a source of food and habitat, the plastic jar was covered with a cap and left undisturbed for few days. The adults were removed after 7 days of infestation and waited for 35+ days for the new S. oryzae to emerge from the rice. The new insects were then separated to obtain a uniform population of males and females. The male and female insects can be distinguished based on their body size and length of snout region as females are larger with a long snout length than males.

The leaves were sun-dried and ground in a mechanical grinder to obtain the powder. 10 gm of powder (insecticide) was put in a bundle bag made of muslin cloth and tagged with the respective name of the leaf used. 100gm of rice was taken in a plastic cup and the bundle bag was kept in it. Rice was first disinfected by keeping it in the refrigerator for around 15-20 minutes and then wiped with a clean cloth to ensure that it was free from any kind of product and was also observed keenly for any pests. 20 adapted S. oryzae were then introduced in the cup and this cup was placed into a bigger transparent bowl and covered with a cap, this methodology was used to examine the number of S. oryzae escaping or migrating away from the treated rice as a result of repellency of the product.

The repellency observations were calculated after every 6 hours for 2 days. Repellency was observed as the number of insects escaping the treated cup to the outside of the transparent bowl. Repellency was evaluated by the emigration index, calculated as the percentage proportion of the mean number of insects that escaped to the total insect population. The following formula was used: -

$$\textbf{Repellency effect} = \frac{\textbf{Mean no. of insects escaped}}{\textbf{No. of insects treated}} \times \textbf{100}\%$$

The percentage of mortality was evaluated by observing the no. of insects dead after every 24 hours until 100% mortality was observed which took 36 hours i.e., 3 days. The mortality index was calculated as the proportion of mean no. of insects dead to the total number of insects treated.

$$\text{Mortality index} = \frac{\text{Mean no. of insects dead}}{\text{No. of insects dead}} \times 100\%$$

Statistical Analysis- The data was projected for statistical analysis to find significant differences between different Neem, Lemon, Peepal, and Eucalyptus treatments. The insect emigration rate (Repellency) and Mortality is the dependent variable. The analysis of Variance (ANOVA) was applied. The test significance level was P< 0.05. The calculations were performed using IBM SPSS statistical software and graphs are plotted using the GraphPad Prism 9 software for Microsoft.

OBSERVATIONS

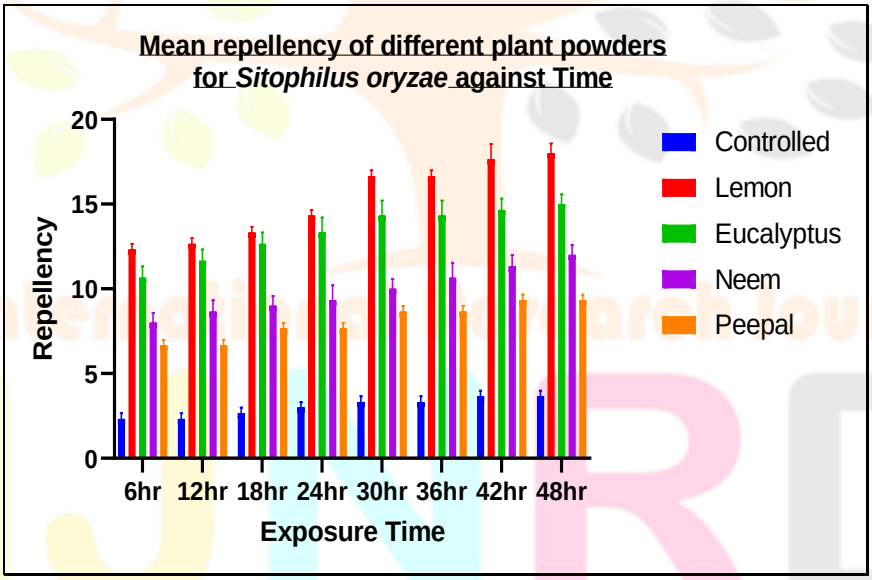
Repellency Effect of different leaf powders on *Sitophilus oryzae*

S. No.	Treatment	N	Repellency (Mean ± *SE*)							
			6hr.	12hr.	18hr.	24hr.	30hr.	36hr.	42hr.	48hr.
1	Control	20	2.33±0.33	2.33±0.33	2.66±0.33	3.0±0.30	3.33±0.33	3.33±0.33	3.66±0.33	3.66±0.33
2	Lemon	20	12.3±0.33	12.6±0.33	13.3±0.33	14.3±0.33	16.6±0.33	16.6±0.33	17.6±0.88	18.0±0.57
3	Eucalyptus	20	10.66±0.66	11.66±0.66	12.66±0.66	13.3±0.88	14.3±0.88	14.3±0.88	14.6±0.66	15.0±0.57
4	Neem	20	8.0±0.57	8.66±0.66	9.0±0.57	9.33±0.88	10.0±0.57	10.66±0.88	11.33±0.66	12.0±0.57
5	Peepal	20	6.66±0.33	6.66±0.33	7.66±0.33	7.66±0.33	8.66±0.33	8.66±0.33	9.33±0.33	9.33±0.33

*Three out of ten replications are considered

*N= No. of insects

* Test Significance level P<0.05



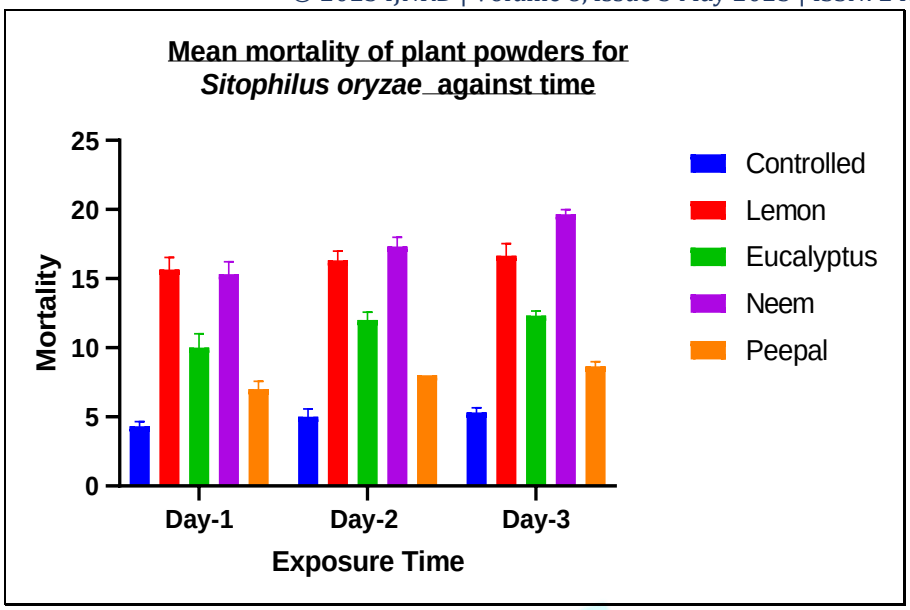
Mortality Effect of Different Plant Powders against *Sitophilus oryzae*

S. No.	Treatment	N	Mortality (Mean ± *SE*)		
			DAY-1	DAY-2	DAY-3
1	Control	20	4.33±0.33	5.0±0.57	5.33±0.33
2	Lemon	20	15.66±0.88	16.33±0.66	16.66±0.88
3	Eucalyptus	20	10.0±1.0	12.0±0.57	12.33±0.33
4	Neem	20	15.33±0.88	17.33±0.66	19.66±0.33
5	Peepal	20	7.0±0.57	8.0±0.00	8.66±0.33

*Three out of ten replications are considered

*N= No. of insects

* Test Significance level P<0.05



RESULTS

The results of the application of leaf powders showed a significant effect on controlling *S. oryzae* in stored rice. Based on observation and results, the leaf powder of *C. limon* showed maximum repellency. Little to no repellence was observed in the controlled treatment. The maximum repellence was 83% in the treatment of *C. limon*, the pattern of repellency was found uniform which increased with time. This was followed by 74% repellency of Eucalyptus. Neem showed 66.5% repellency followed by Peepal which was used as a test product and showed fair repellency of 54.5% and was the least in the experiment. (Lemon>Eucalyptus>Neem>Peepal). According to the results of ANOVA, the F value of treatments at different times of observation varied and the highest was F= 90.56 at the 48th hour while the lowest was F=51.56 at the 12th hour of observation.

In addition to the insect's repellency rate, the mortality effect was also observed and the application of plant leaf powder showed a significant effect on insect mortality though the reason of insect mortality was not included in the experimental study. This data was also projected for analysis of Variance (ANOVA). Based on observation and results, the highest mortality was observed in the treatment of Neem powder i.e., 78%, and maximum mortality was observed within 3 days of application in replications of Neem powder. All the other treatments showed relatively low levels of mortality with Lemon showing 46.5% mortality followed by Peepal and Eucalyptus showing 33.5% and 30% mortality respectively. (Neem>Lemon>Eucalyptus>Peepal). According to the results of ANOVA, the F value for different treatments varied significantly for each day of observation, the highest was F=137.86 for Day-3 and the lowest was F=41.90 for Day-1. The F value for Day-2 was F=83.90

DISCUSSION

The results of this study showed the highest percentage of repellency of *C. limon* leaf powder against *S. oryzae* in stored rice which was 83% followed by treatments of *Eucalyptus saligna* (74%) and Neem (54.5%). This phenomenon is following the results of the study conducted by (Mohd et al., 2016)where 90% repellency of dried leaf powder *C. limon* was observed, (Author et al., n.d.) also stated efficacy of lemon in controlling the infestation of *S. oryzae* and thus it can be used as an alternative to synthetic pesticides. This effect of leaf powders is due to the active compounds present in the leaf powders which act as repellent against *S. oryzae*, forcing them to leave the container (source of biopesticide).

The leaves of *Eucalyptus saligna* contain the active compound p-cymene (cymol) which has a repellent effect on *S. oryzae* and *T. castaneum* (K. D. Singh et al., 2021). Neem (*Azadirachta indica*), on the other hand, has a greater mortality effect and lower repellency rate for *S. oryzae* and the results of this experiment follow the trend of the experiment conducted by (Mohd et al., 2016) where the lowest repellency of dried Neem leaf powder was observed. The rate of repellency of *S. oryzae* showed an increment with time which must be due to an increase in the concentration of bioactive compounds within the container causing a proportional increase in inhalation toxicity of S. oryzae and compelling them to leave the site of treatment and emigrate to the outside of the container.

The results of the mortality effect were different from the results of the repellency rate. The highest mortality rate of *S. oryzae* was observed in the treatment of dried Neem leaf powder (78.5%). In a similar experiment, (Bakar et al., 2022) found the treatment of Neem leaves caused the highest mortality rate of 45% which stands true for this experiment because the highest mortality rate is 78.5% of the replica treated with Neem leaf powder. This result is also supported by the study conducted by(Ngo et al., 2020) which showed the insecticidal properties of Neem leaves due to the presence of Azadirachtin and other bioactive compounds. Another experiment performed by AD Rojasara (Rojasara et al., 2019) states an 87.08% mortality rate of Neem leaf powder which was the highest in their study thus, it can be mentioned that Neem is a potent product that can be utilized as a biopesticide to control *S. oryzae* in stored rice.

Other treatment showed comparatively less mortality effect and was observed to be less potent as compared to Neem. Considering the 46.5% mortality effect on *S. oryzae* in the treatment with dried leaf powder of *C. Limon* and the 33.5% mortality effect of *Eucalyptus saligna* leaf powder, it can be inferred that these plant leaves also contain some bioactive compounds that induce toxicity in *S. oryzae* causing its mortality. This is confirmed by studies conducted by (K. D. Singh et al., 2021)where it is mentioned that the leaves of *Eucalyptus saligna* contain a bioactive compound p-cymene (cymol) which has contact and inhalation toxicity against stored grain pests *S. oryzae* and *T. castaneum*. (Thomas et al., 2002) mentioned that LC_{50} values of sundried leaves of Eucalyptus and Guava were observed by different researchers as they had a potent effect in suppressing progeny of *S. oryzae* when the powder was mixed with rice.

The leaf powder of *C. limon* is a more potent repellent and shows less mortality effect. Peepal being the test product showed a 30% mortality rate of S. oryzae which stands close to treatment containing Eucalyptus leaf powder. Thus, more research is required to find the bioactive compounds associated with the activity of the fore-mentioned medicinal plants and these plant powders can be used in combinations to form more effective biopesticides to control *S. oryzae* in stored grain.

CONCLUSION

It is concluded that the best treatment in terms of repellency was dried leaf powder of *C. limon* causing 83% repellency within 48 hours and in terms of mortality the treatment with dried leaf powder of *Azadirachta indica* (Neem) showed the best results causing 78.5% mortality within 3 days. Usually, broad-spectrum chemical pesticides are used in stored grains which have long-term serious effects. This outcome is extremely valuable in terms of finding sustainable ways to produce insecticides that do not cause adverse effects on humans and the environment. Since these plants are of high medicinal value and no negative impacts on humans are known, thus, bioactive compounds from these plants can be isolated to produce biopesticide for controlling the infestation of *S. oryzae*. More research is required in this field and is of utmost importance.

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