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**Research Article**

# EVALUATION OF PHYTOCHEMICAL PROFILE, BIOEFFICACY AND REPELLENT ACTIVITY OF *PARMELIA PERLATA* AGAINST SITOPHILUS SPECIES

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## ABSTRACT

It is estimated that, need for food will surge by 60 to 98% in the coming years. To meet the escalating demand for food in our densely inhabited world, safeguarding grains from insect pests has become a trending subject. To overcome these issues, there is an urgent need to develop green, environment friendly, cost effective and long-lasting techniques to protect grains from insect pests. The main objective of the current investigation is to analyse phytochemical constituents, and to evaluate bioefficacy and repellent activity of *Parmelia perlata* against *Sitophilus* species. Different concentrations of ethanol and methanol extracts are investigated for its insecticidal and repellent activity. The phytochemicals found include phytosterol in ethanol extract, saponins and phytosterols in the methanol extract. The mortality rate rose proportionally with the extension of the time interval following the treatment. The $LC_{50}$ value of ethanol extract after 72 hours of exposure is 153.152 mg/L and $LC_{50}$ Value of methanol extract after 72 hours of exposure is 194.919mg/L. The ethanol extract appears to be more potent when compared to the methanol extracts. The average repellent percentage of *Parmelia perlata* ethanol extract is 51.85% at 500mg/L concentration. The P-Value of ethanol extract is $1.1971 \times 10^{-192}$ and for methanol extract the P-Value is $2.5349 \times 10^{-169}$ which is less than 0.01 which shows the results obtained are significant. The bioactive compounds in the extract are likely responsible for its effectiveness. Thus, *Parmelia perlata* is a promising grain protectant, ensuring seed viability.

**Keywords:** Bioactive compounds, Ethanol extract, Methanol extract, Mortality, Stored grain pest.

## INTRODUCTION

Farming serves as the primary livelihood for individuals worldwide. In India, more than 70% population depends on agriculture for their livelihood (Champ & Dyte 1977), (Kiruba *et al.,* 2006). A significant challenge in contemporary agriculture is the incessant pressure to increase production to meet the growing demand for food in a population that continues to expand. A serious problem is the significant loss of cereal grains during storage as a result of insect infestation, particularly in developing nations like India (Dubey NK *et al.,* 2008). *Sitophilus oryzae* (L.), formally known as the rice weevil, is the most common and dangerous major insect pest that damages grains that have been kept. While chemical pesticides have proven effective against stored grain pests but their prolonged use over several decades has led to the widespread development of resistance and raised concerns regarding environmental and human health. Worldwide, storage insect pests contribute to significant losses in both the quantity and quality of stored grain products (Madrid *et al.,*1990), mainly in tropical and sub-tropical countries (Tripathi *et al*., 2000).

Three primary methods are employed to control stored grain pests: physical methods, chemical methods, and the use of natural products. Among these, temperature treatment of stored grain stands out as the most effective physical method, successfully eliminating multiple life stages of insects simultaneously (Mahroof *et al.,*2005). Furthermore, some of the commonly employed fumigants to manage stored insects include carbon disulphide, carbon

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chloride, ethylene dichloride, ethylene oxide, methyl bromide, chloropicrin, trichloroethylene, sulphur dioxide, methyl formate, and trichloro acetonitrile (Brattsten *et al.,* 1986). It has been estimated that as many as 30% people in the industrialized countries suffer from food borne diseases each year. For this reason, there is a scope for new methods of producing safe foods that have a natural or green image and one of such possibilities is the use of plant products in the form of essential oils.

Use of botanical pesticides in an agro ecosystem is now emerging as one the prime source to protect crops and the environment from the pesticide pollution, which plays vital role in integrated pest management (Rajendran, S., & Sriranjini, V., 2008). Among higher plants, there are 17,500 aromatic plant species and approximately 3,000 essential oils have been discovered. They are lipophilic in nature with fundamental metabolic, biochemical, physiological and behavioral function in insects. Lichens are symbiotic organisms consisting of a fungal, an algal partner (Crespo, J. G. 2011). *Parmelia perlata* which is commonly known as stone flower is widely used as spices in various Indian dishes. It exhibits hues ranging from greenish mineral grey to yellowish-white on its upper surface, while its lower surface is black (Kumar, *et al.,* 2016). It contains many chemical constituents like tridecylmyristate, 3-ketooleanane, icosan-1-ol, usnic acid12, parmelanostene permelabdone14, atranorin, lecanoric acid, orcin, erythrolein, azolitmin and spaniolitmint, which shows anti-bacterial, anti-microbial activities. Salazinic acid and stictic acid were the major metabolites detected in *Parmelia perlata*; it also contains atranarin and choroatronarin. The extract demonstrated important anti-microbial activity against eight strains of microbes. The cytotoxic effects of *Parmelia consperia* and *Parmelia perlata* extract towards three cancer cell lines and demonstrated important anti-microbial, anti-oxidant and anti-cancer effects (Manojlovic *et al.,*2021).

The present investigation utilizes *Sitophilus* species as the focal pest. *Sitophilus* is highly damage causing pest of raw stored cereals in the world (Champ, B. R., & Dyte, C. E. 1977). The damage inflicted by the rice weevil led to storage losses totaling 18.30 percent (Adams, J. M. 1976). According to studies, a *Sitophilus oryzae* infestation can cause up to 61.3% damage in 150 days when stored under certain conditions (Venkat Rao, S *et al.,* 1958*)*. The biology of *Sitophilus oryzae* has four life phases for the species: egg, larva, pupa, and adult. Typically, oval-shaped, initially translucent and white eggs were placed singly inside the grain cavity. The larvae developed inside the grain, going through four instars and three moults. Larval duration at ideal temperatures ranged from 25 to 34 days. The pupal duration varied from 8 to 11 days, during which time the pupation took place inside the grain. Adults usually stayed inside the grains for one to two days after emerging. The entire life cycle, from egg to adult, took 34–49 days on average, and was seen at temperatures between 15 and 34°C (Swamy, K. N *et al., 2014)*.

## MATERIALS AND METHODS

### Collection, culture and maintenance of *Sitophilus* species

The *Sitophilus* species utilized in this project were collected from ICAR-JSS Krishi Vigyan Kendra Sutturu and from local stores in Mysuru. The test insect *Sitophilus* species were cultured and maintained in the laboratory following the procedure outlined by Kim and Park (Kim, S *et al.,* 2013).

### Preparation of extract

The *Parmelia perlata* had purchased from a nearby store in Mysuru, it was cleaned to get rid of bark and other debris present in it, then it was dried for 3-5 days at 35 to 40°C in a hot air oven. After drying it was ground into a fine powder by using household mixer and labelled and stored in an airtight glass container. Then, this powdered substance was used in more research. With a few adjustments, the cold solvent extraction procedure was carried out using the approach outlined by Ingle and co-workers (Ingle *et al., 2017)*. Both methanol and ethanol were used to dilute the isolated material. Five distinct dilutions were made for the current study: 100 mg/L, 200 mg/L, 300 mg/L, 400 mg/L, and 500 mg/L.

### Phyto-chemical analysis

Phytochemical analysis of the plant extract was carried out following the methods described by Harborne (Harborne, J. B. 1984). For the analysis, a sample was prepared by dissolving 100 mg of *Parmelia perlata* extract in 20 ml of ethanol for ethanol extract and in 20 ml of methanol for *Parmelia perlata* extracted with methanol. The phytoconstituents like alkaloids, carbohydrates, saponins, phytosterols, phenols, flavonoids, and tannins, were screened using the phytochemical analysis techniques to detect phytoconstituents.

### Evaluation of bioefficacy of *Parmelia perlata* extract against test insect

In order to evaluate the bioefficacy of *Parmelia perlata*, various concentrations of the lichen extract were prepared. 5 ml of each concentration were poured and was incubated for 15 minutes. Then to each petri plate 10 flies were introduced. A control was maintained using 5ml of ethanol. All concentrations, including the control, were maintained in triplicates. The mortality rate of *Sitophilus* flies was observed every 24 hours. These observations were continued until the flies ‘attained 100% mortality at regular intervals of 24 hours. Same procedure was followed for *Parmelia perlata* extracted with methanol as the solvent.

### Bioefficacy of *Parmelia perlata* extract mixed with rice grains

The efficacy of lichen extract was evaluated by mixing it with rice grains. Initially, 10 grams of lichen powder was dissolved in 100 ml of ethanol and it was soaked overnight.

After stirring and filtering, 5 ml of the filtrate was mixed with 10 grams of rice grains in each petri plate. After that, Sitophilus flies were added, and the experiment was run using powdered lichen dissolved in ethanol at different concentrations of 8%, 6%, 4%, and 2%, and as well as a control that used simply ethanol. Three tests were conducted on each concentration as well as the control.

**Evaluation of repellency activity**

The repellency test followed Peixoto and co-workers (Peixoto M. G *et al., 2015)* protocol. Various concentrations of the dried plant extract (100 mg/L to 500 mg/L) were prepared in a solvent. Whatman's filter paper was divided into halves; each concentration was applied to one half, while solvent was applied to the other. The paper was placed on a petri plate, and 20 *Sitophilus* flies were introduced. Readings were taken every 30 minutes for 8 readings.

The percentage repellency was calculated using the formula given by Abbott:

$$\text{PERCENTAGE REPELLENCY} = \frac{A-B}{A} \times 100$$

A = Average number of insects present on untreated portion.

B = Average Number of insects present on treated portion.

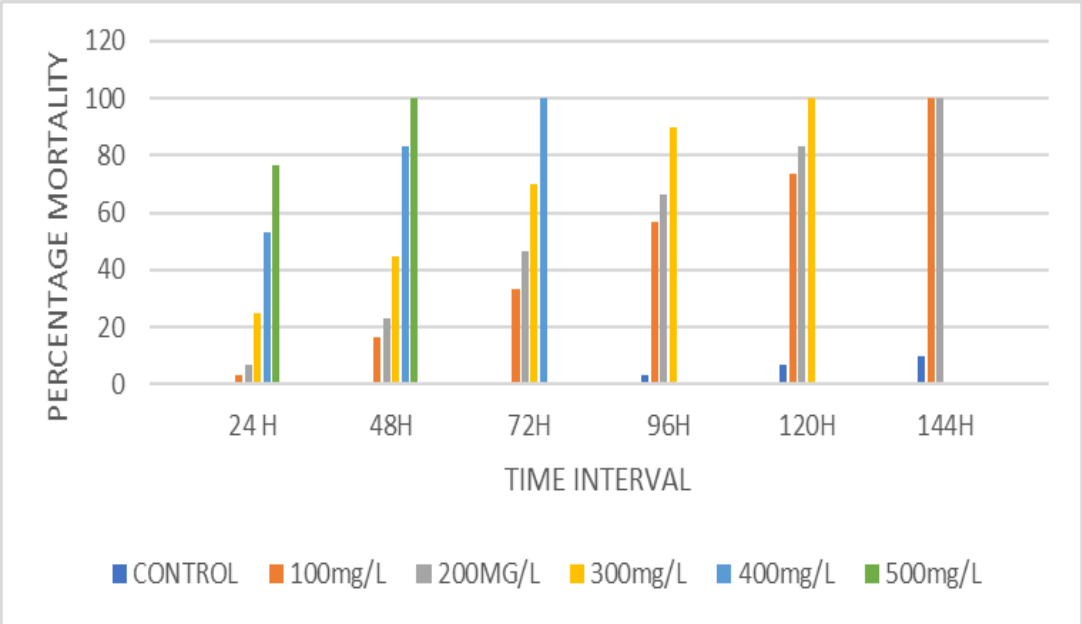
## RESULTS AND DISCUSSION

Phytochemical screening of *Parmelia perlata* ethanolic extract indicated the presence of phytosterols, whereas methanolic extracts showed the presence of saponins and phytosterols. Both ethanolic and methanolic extracts showed negative result for alkaloids, carbohydrates, phenols, flavonoids, tannins, and anthraquinone. The analysis revealed the presence of phytosterols as major phytoconstituents in both the extracts. The average mortality of *Parmelia perlata* ethanol extract at 100 mg/L after 24 hours was 0.34, with an average mortality percentage of 3.4%. This increased to an average of 7.67 flies, with an average mortality percentage of 76.7%, at the 500 mg/L concentration. Complete mortality (100%) was achieved at 48 hours at the concentration of 500 mg/L, whereas it took 168 hours at the 100 mg/L concentration. The time required to achieve complete mortality was reduced to 120 hours at the 300 mg/L concentration and 72 hours at the 400 mg/L concentration compared to the 100 mg/L concentration. Compared to the 100 mg/L concentration, the 500 mg/L concentration was more effective, with the time reduced by 120 hours. In the control group, there was no mortality recorded until 96 hours, at which point 3.4% mortality was observed. By the end of 168 hours, there was a recorded mortality rate of 10% in the control group. At 100 mg/L concentration, no mortality observed within 24 hours, while 6.7% mortality was occurred at 200 mg/L, increasing to 56.7% at 500 mg/L within the same timeframe. The time to achieve 100% mortality was 168 hours at 100 mg/L, which was decreased to 72 hours at 500 mg/L. In control group, there was no mortality observed within 24 hours' timeframe, but it reached 20% at the end of 168 hours. Comparing to ethanol and methanol extracts of *Parmelia perlata*, the ethanol extract looks more effective achieving 100% mortality in 48 hours at 500 mg/L, while the methanol extract required 72 hours for the same to achieve against *Sitophilus* flies.

**Table 1.** *Parmalia perlata* ethanol extract mortality rate.

| | | ***Parmelia perlata* ethanol extract** | | | | | |
|---|---|---|---|---|---|---|---|
| | **Concentration→** | **Control** | **100mg/L** | **200MG/L** | **300mg/L** | **400mg/L** | **500mg/L** |
| 24 H | Average | 0 | 0.34 | 0.67 | 2.5 | 5.34 | 7.67 |
| | Avg. Percent | 0 | 3.4 | 6.7 | 25 | 53.4 | 76.7 |
| 48H | Average | 0 | 1.67 | 2.34 | 4.5 | 8.34 | 10 |
| | Avg. Percent | 0 | 16.7 | 23.4 | 45 | 83.4 | 100 |
| 72H | Average | 0 | 3.34 | 4.67 | 7 | 10 | |
| | Avg. Percent | 0 | 33.4 | 46.7 | 70 | 100 | |
| 96H | Average | 0.34 | 5.67 | 6.67 | 9 | | |
| | Avg. Percent | 3.4 | 56.7 | 66.7 | 90 | | |
| 120H | Average | 0.67 | 7.34 | 8.34 | 10 | | |
| | Avg. Percent | 6.7 | 73.4 | 83.4 | 100 | | |
| 144H | Average | 1 | 10 | 10 | | | |
| | Avg. Percent | 10 | 100 | 100 | | | |

There was no mortality seen at 1g/100ml concentration at 24 hours, at 2mg/100ml concentration the mortality rate was 3.4%, which was increased to 46.7% in 5g/100 ml filtrate. At 3g/100 ml concentration, the mortality percentage increased to 23.4% from 3.4% in 2g/100 ml filtrate, which was 46.7 % in 4g/100ml. In 1g/100ml concentration the 100% mortality was attained on 168 hours, which was completed in just 72 hours in 5g/100 ml concentration. 2g/100 ml took 144 hours, 120 hours in 3g/100ml concentration, 96 hours for 4g/100 ml concentrations respectively to attain 100% mortality. The repellent percentage of *Parmelia perlata* at the 100 mg/L concentration was 2.47%, indicating the least repellency against *Sitophilus*. In 200 mg/L concentration, the repellence percentage was increased to 24.18%, further it increased to 29.03% at 300 mg/L concentration and 41.58% in 400 mg/L concentration accordingly. The repellence of 51.85% was highest repellence observed at 500 mg/L concentration.



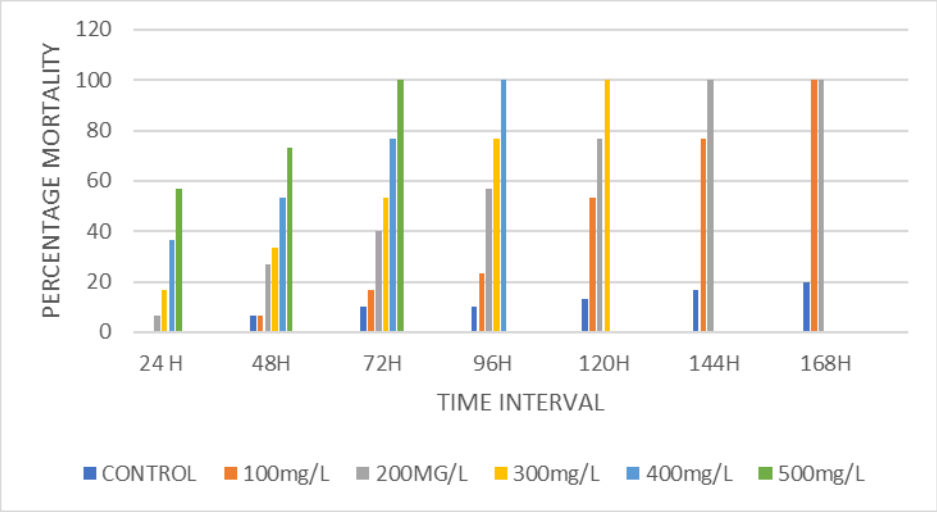

Graph-1 Graph showing Bioefficacy of *Parmelia perlata* ethanol extract against Sitophilus species.

**Table 2**. *Parmalia perlata* methanol extract mortality rate.

| | | *Parmelia perlata* methanol extract | | | | | |
|---|---|---|---|---|---|---|---|
| | **Concentration→** | **Control** | **100mg/l** | **200mg/l** | **300mg/l** | **400mg/l** | **500mg/l** |
| 24 h | Average | 0 | 0 | 0.67 | 1.67 | 3.67 | 5.67 |
| | Avg. Percent | 0 | 0 | 6.7 | 16.7 | 36.7 | 56.7 |
| 48h | Average | 0.67 | 0.67 | 2.67 | 3.34 | 5.34 | 7.34 |
| | Avg. Percent | 6.7 | 6.7 | 26.7 | 33.4 | 53.4 | 73.4 |
| 72h | Average | 1 | 1.67 | 4 | 5.34 | 7.67 | 10 |
| | Avg. Percent | 10 | 16.7 | 40 | 53.4 | 76.7 | 100 |
| 96h | Average | 1 | 2.34 | 5.67 | 7.67 | 10 | |
| | Avg. Percent | 10 | 23.4 | 56.7 | 76.7 | 100 | |
| 120h | Average | 1.34 | 5.34 | 7.67 | 10 | | |
| | Avg. Percent | 13.4 | 53.4 | 76.7 | 100 | | |
| 144h | Average | 1.67 | 7.67 | 10 | | | |
| | Avg. Percent | 16.7 | 76.7 | 100 | | | |
| 168h | Average | 2 | 10 | | | | |
| | Avg. Percent | 20 | 100 | | | | |

In bioefficacy projects, the $LC_{50}$ value is a critical parameter, because it offers a quantitative measure of the concentration of *Parmelia perlata* extract those results in the mortality of 50% of *Sitophilus* species within a specified time interval. To determine the $LC_{50}$ value at 72 hours of mortality of *Sitophilus* against *Parmelia perlata* ethanol extracts, the probit analysis was conducted following the method described by Finney (1971) by using Microsoft Excel. The $LC_{50}$ value obtained from the analysis was found to be 153.152 mg/L. In bioefficacy projects, the $LC_{50}$ value is a critical parameter, because it offers a quantitative measure of the concentration of *Parmelia perlata* extract those results in the mortality of 50% of *Sitophilus* species within a specified time interval. To determine the $LC_{50}$ value at 72 hours of mortality assessment of *Sitophilus* against *Parmelia perlata* methanol extracts, the probit analysis was conducted following the method described by Finney (1971) by using Microsoft Excel. The $LC_{50}$ value obtained from the analysis was found to be 194.919mg/L.

The Chi-square analysis reveals that the *Parmelia perlata* ethanol extract shows the significant ($P < 0.05$) insecticidal activity against *Sitophilus* species. By using Microsoft Excel, the expected value and chi-square statistics were computed. The p-value $1.1971 \times 10^{-192}$ provides compelling evidence to reject the null hypothesis and to supports the alternative hypothesis that there is a significant association between the variables. This obtained result underscores the importance of categorical variables relationship and suggests that the observed patterns are unlikely to be due to chance alone. The obtained p-value $2.5349 \times 10^{-169}$ provides compelling evidence to reject the null hypothesis and to supports the alternative hypothesis that there is a significant association between the variables. This obtained result underscores the importance of categorical variables relationship and suggests that the observed patterns are unlikely to be due to chance alone. In developing countries, the age-old tradition of using plants and their derivatives to safeguard stored products is deeply ingrained. There are substantial opportunities for the development and utilization of less refined botanical insecticides for both domestic and agricultural purposes. Against biting insects, the utilization of plants has historical roots dating back to ancient Greek times and it continues to be practiced today. The large number of people employing plants as biopesticides. Before the advent of synthetic pesticides, plant-based products were the primary source of pest management available to farmers worldwide.



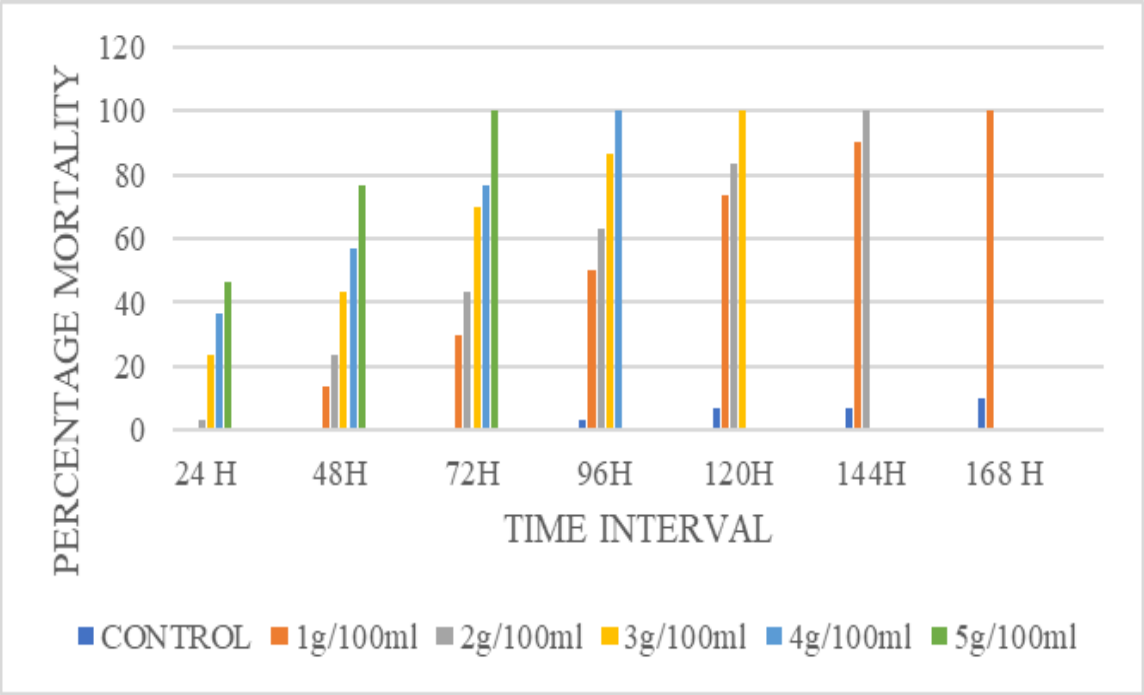

**Graph-2** Graph showing Bioefficacy of *Parmelia perlata* methanol extract against Sitophilus species.

**Table 3.** Insecticidal activity *Parmalia perlata* ethanol extract mixed with rice grain mortality rate

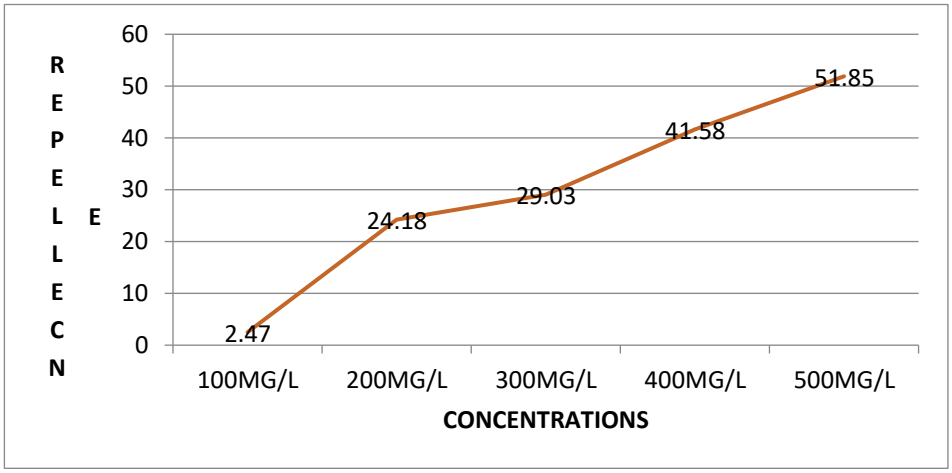
| | | *Parmelia perlata* ethanol extract mixed with rice grains | | | | | |
|---|---|---|---|---|---|---|---|
| | | **Control** | **1g/100ml** | **2g/100ml** | **3g/100ml** | **4g/100ml** | **5g/100ml** |
| 24 h | Average | 0 | 0 | 0.34 | 2.34 | 3.67 | 4.67 |
| | Avg. Percent | 0 | 0 | 3.4 | 23.4 | 36.7 | 46.7 |
| 48h | Average | 0 | 1.34 | 2.34 | 4.34 | 5.67 | 7.67 |
| | Avg. Percent | 0 | 13.4 | 23.4 | 43.4 | 56.7 | 76.7 |
| 72h | Average | 0 | 3 | 4.34 | 7 | 7.67 | 10 |
| | Avg. Percent | 0 | 30 | 43.4 | 70 | 76.7 | 100 |
| 96h | Average | 0.34 | 5 | 6.34 | 8.67 | 10 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Avg. Percent | 3.4 | 50 | 63.4 | 86.7 | 100 |
| 120h | Average | 0.67 | 7.34 | 8.34 | 10 | |
| | Avg. Percent | 6.7 | 73.4 | 83.4 | 100 | |
| 144h | Average | 0.67 | 9 | 10 | | |
| | Avg. Percent | 6.7 | 90 | 100 | | |
| 168 h | Average | 1 | 10 | | | |
| | Avg. Percent | 10 | 100 | | | |

Several plant substances have been explored for their potential as insecticides, antifeedants, or repellents, including terpenes, flavonoids, alkaloids, phenols, and related compounds (Adeyemi, M. H. 2010). Biopesticides encompass secondary metabolites derived from plants, microbes, animals, and certain minerals (Isman, M. B., & Machial, C. M. 2006). In current study, the phytochemical constituents of *Parmelia perlata* were analysed, which reveals the presence of phytosterols in ethanol extract and both saponins and phytosterols in the methanol extract. These findings align with those of Asha *et al.*, who also identified these compounds in *Parmelia perlata*, along with proteins, glycosides, and others phytochemical constituents (Asha C *et al., 2023)*. The insecticidal activity (bioefficacy) of *Parmelia perlata* ethanol and methanol extracts were assessed at various concentrations (100, 200, 300, 400, and 500 mg/L), with mortality of *Sitophilus* as an indicator. Higher mortality of flies typically suggests potential insecticidal activity of *Parmelia perlata* extracts. The data showed that 100% insecticidal activity was achieved at a concentration of 500 mg/L for the ethanol extract taking 48 hours compared to the methanol extract, which required 72 hours. The ethanol extract is more effective than the methanol extract.




**Graph 3**. Graph showing Bioefficacy of *Parmelia perlata* ethanol extract mixed with rice grains against Sitophilus species.




**Graph 4.** Graph showing repellence percent of *Parmelia perlata* against Sitophilus species.

The highest mortality observed in present study could be due to the presence of active toxic principles in the higher concentrations of *Parmelia perlata* extracts. The micropyle region of the egg chorion, may be blocked by these compounds, leads to death of embryo due to oxygen depletion for respiration. The plant extract may also interfere with normal embryonic development by suppressing hormonal and biochemical processes alternatively (Chiranjeevi C & Sudhakar T. R. 1996). In the insecticidal activity of *Parmelia perlata* extracts mixed with rice grains, the concentration of 5g/100ml appears to be the most effective among the five concentrations tested. It took 72 hours to achieve complete mortality. Both the *Parmelia perlata* methanol extract and ethanol extract mixed with rice grains also took 72 hours to achieve complete mortality. The solvent ethanol used as a control showed 10% mortality, while methanol showed 20% mortality, and ethanol mixed with rice grains showed 10% mortality. The mortality rates recorded in the control clearly indicate that 100% mortality was not achieved by the solvents alone, but by *Parmelia perlata* extracts.

The results which include repellence percentages, are supported by the observations of Chaubey, who studied the repellent activity of three essential oils against the stored product beetle *Tribolium castaneum* (Chaubey M K 2008). The results section clearly illustrates that the maximum repellent activity caused by *Parmelia perlata* was 51.85% at a concentration of 500 mg/L against *Sitophilus* species. *Parmelia perlata* contains various substances including proteins, tannins, sugars, phenols, vitamins A and C, alkaloids, glycosides, steroids, and terpenes, among others. Additionally, it contains acids such as usnic acid, lecanoric acid, and atranorin (Asha C *et al., 2023*). From the observation of varying activity across different concentrations of plant extract, it suggests that pest-controlling and repellent factors were not uniformly distributed. The present investigation clearly demonstrates that the mortality and repellence percentage differed across different concentrations of *Parmelia perlata* extracts. The *Parmelia perlata* extracts of ethanol and methanol were examined in a series of laboratory experiments, reveals toxicity and strong repellence towards *Sitophilus* species. The evaluation of bioefficacy of *Parmelia perlata* extracts in the present study marks a significant advancement in the field of stored-grain insect pest management. By utilization of botanical pesticides, substantial amount of money could be saved compared to synthetic chemical pesticides with less or no side effects. The extracts of *Parmelia perlata* were found to be highly effective in controlling insect pest populations. Consequently, the use of *Parmelia perlata* could also mitigate health hazards associated with synthetic chemical pesticides.

## CONCLUSION

The experimental studies have demonstrated the bioefficacy of *Parmelia perlata* extract against *Sitophilus* species as grain protectant through its insecticidal and repellent activities. It serves as a natural insecticide without posing any toxic hazard to the treated grain. Among the ethanol and methanol extracts, ethanol extract of *Parmelia perlata* exhibits superior activity compared to the methanol extract. The potential insecticidal and repellent activity of *Parmelia perlata* extracts may be attributed to presence of bioactive compounds within them. Therefore, *Parmelia perlata* could be considered an ideal grain protectant in terms of seed viability and safety for mammals.

## ACKNOWLEDGMENT

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## CONFLICT OF INTERESTS

The authors declare no conflict of interest

## ETHICS APPROVAL

Not applicable

## FUNDING

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## AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

## DATA AVAILABILITY

Data will be available on request

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