

***Piper longum* as grain protectant: Evaluation of its phytochemical profile, bioefficacy and repellent activity against *Sitophilus* species**

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Abstract

Significant portion of the global population relies on Agriculture as their primary occupation. Especially in the country like India, over 70% of the population directly depends on agriculture for their livelihood. The loss of food grains due to insect pests during storage is a major concern in India. The application of chemical insecticides to control insects has substantial challenges and several drawbacks including the emergence of resistant strains, presence of harmful toxic residues and worries over worker safety associated with their use. To address these issues, there is an urgent need to develop environment friendly, long lasting affordable technique to protect grains from insect pests. Plant-based insecticides can therefore serve a crucial role in integrated pest management. The primary aim of the study is to analyse *P. longum* as grain protectant and to analyse its phytochemical properties and repellent activity. Various concentrations of ethanol and methanol extracts were tested for their insecticidal effects against *Sitophilus* species. The average mortality percentage of *Sitophilus* species indicates the toxic and repellent effect which is proportional to the concentration of the extract. Higher concentrations exhibit a more pronounced effect. Phytochemical analysis of ethanol extract confirms the presence of phytosterols and methanol extract showed the presence of alkaloids and phytosterols. The LC₅₀ value of ethanol extract after 72 hours of exposure was 144.023mg/L and LC₅₀ value of methanol extract after 48 hours of exposure was 89.990 mg/L. The methanol extract appears to be more effective than the ethanol extracts. The average repellence percentage of *P. longum* was 57.69% at 500mg/L concentration. The P-Value of ethanol and methanol extracts were 1.557×10^{-100} and 2.26546×10^{-50} respectively which was less than 0.01 which shows the results obtained are significant. The experimental studies demonstrated *P. longum* as grain protectant against *Sitophilus* species. Its natural insecticidal property and ethno pharmacological properties are the evidence that it does not pose any toxic hazard from the treated grain. So, *P. longum* can be considered as an ideal grain protectant from the point of view of seed viability and safety to mammals.

Keywords: *Sitophilus*, stored grain, *Piper longum*, bioefficacy, repellency

Introduction

Piper longum, commonly known as long pepper, is an aromatic flowering vine belonging to the family Piperaceae. It is native to South and South east Asia, particularly in regions like India and Indonesia. The plant is valued for its aromatic fruits, which are used as a spice and in traditional medicine. The fruit and root are rich in alkaloids and are important. Piperine is the key component, followed by piperettine, asarinine, methyl piperine, pipernonaline, N-isobutyl decadienamide, brachyamide-A, brachystine, pipericide, piperderidine, longamide, piperlongumine, piperlonguminine, retrofractamide A, pellitorine, piperundecalidine, pergumidiene, brachystamide-B, dehydropipernonalinepiperidine, and tetrahydropiperine. The fruit essential oil is a complex mixture. Excluding piperine a volatile, the other three major components of fruit are pentadecane, caryophyllene (both about 17.8%), and bisabolone (11%). Others chemicals include terpinolene, thujone, zingiberene, p-cymene, dihydrocarveol, pmethoxyacetophenone, and vitamins A and E. The major organic acids present in fruit are palmitic acid and tetrahydropiperic acid.

P. longum has been a traditional remedy for curing dyspepsia, it acts as an immunomodulatory, hepatoprotective, and also useful for asthma, hypocholesteremic, lumbarleg pain and arthralgia (BenCao, 1999) [3]. The fruit are usually used to cure bloody diarrhea in traditional Thai practice. *P. longum* also havepesticidal

activities and larvicidalefficiency against larvae of *Aedes aegypti* (Sreedev, 2016) [20]. In the treatment of gonorrhoea, tuberculosis, respiratory tract infections and arthritic condition the *P. longum* is used (Srivastava, 2014 [21], Sreedev, 2016) [20]. Due to the activation of the heat and acidity sensing Transient Receptor Potential Vanilloid (TRPV) ion channel TRPV1 on nociceptors (pain sensing nerve cells) pungency of piperine is sensed (Vasavirama, & Upender, 2014) [24].

The Importance of Plant-Based Pesticides in Agriculture

In recent years, Plant-based pesticides especially essential oils, have gathered more significant attention as environment-friendly and safer substitutes for synthetic chemicals. Use of synthetic chemicals had resulted number of challenges such as increased application costs, the development of resistance to chemicals, and non-target organism toxicity including humans. Moreover, the indiscriminate use of chemical pesticides had given rise to environmental concerns, and long-term effectiveness of these chemicals is questionable as many insects develop resistance against the chemical fumigants. Thus, novel chemotherapeutics for pest management are being investigated, which are natural compounds obtained from higher plants (Mishra., & Dubey, 1994) [14].

P. longum is unique because of its rich content of volatile oils and distinct aromatic properties among the vast plant kingdom, which contains over 17,500 aromatic species and

around 3,000 known essential oils (Brattsten, *et al.*, 1986)^[4]. Strong fumigant and insecticidal activities have been shown by *P. longum*, making them promising candidates as plant-based pesticides. The purpose of the study was to explore *P. longum* fruit its phytochemical profile and to check its efficacy as grain protectant.

Agricultural Challenges and Food Security

Agriculture serves as the backbone of economic growth in many developing countries including India and also faces many challenges, including the increase in food demand to meet the rapidly growing population needs (Chandrasekhar *et al.*, 2005^[5]; Kiruba, *et al.*, 2006)^[12]. One of the most important concern is the loss of food grains during storage due to pest attack, pest infestation severely impacts food security. This problem is particularly acute in India, where inadequate storage and pest infestations result in significant quantitative and qualitative losses of stored food grains (Madrid, *et al.*, 1990)^[13]. In addition, the careless and indiscriminate use of synthetic pesticides has led to a number of cases of other known issues. Some of them are contamination of food, soil, groundwater, rivers, lakes, oceans, air, etc. and increasing application costs (Ahmed, & Grainge, 1986).

Chemical pesticides are commonly used but they come with significant drawbacks like development of pest resistant strains, toxic residues accumulation in the food has prompted the scientific community to seek safer green alternatives (Srivastava & Kumar 2008)^[15]. Among plant-based pesticides, *P. longum* derivative may offer a potential solution by addressing both efficacy and safety concerns.

Sitophilus species and the threat to stored Grains

The most damaging stored grain pest is *Sitophilus oryzae*, commonly known as the rice weevil from the family Curculionidae is notorious and destructive for infesting various cereals like rice, wheat, oats, maize and barley. The damage caused by *Sitophilus* not only leads to significant quantitative loss but also it reduces grains qualitatively, making them unfit for human consumption (Tripathi, *et al.*, 2001)^[23].

In tropical and subtropical regions, such as India, stored grain losses can range from 10-40% in the absence of modern storage technologies (Shaaya *et al.*, 1997)^[19]. Given the pest's adaptability and resilience, finding effective and sustainable methods to control *Sitophilus oryzae* is critical. The purpose of the study is to assess *P. longum*, a plant-based insecticide as grain protectant against most destructive *Sitophilus* sp.

The current study offers more environmental friendly and sustainable alternative for control of stored grain pests as alternative to synthetic pesticides by utilizing the natural qualities of *P. longum*.

Material and methods

a. Selection and extraction of plant material

P. longum is a plant species which was selected to determine the grain protectant activity in the present investigation. *P. longum* was selected by reviewing the phyto-chemical constituents and anti-bacterial, anti-microbial and anti-fungal properties of *P. longum* from various papers. The solvent extraction process was carried out according to Ingle *et al.*, (2017)^[8] with some modifications.

b. Phytochemical analysis

The extracted material is diluted in ethanol and methanol. In the present investigation, 5 different dilutions (100-500mg/L) for both ethanol and methanol extracts are used. Phytochemicals were analysed in the plant extract according to Banu and Cathrine (2015)^[2] methods. For the phytochemical analysis sample was prepared at a concentration of 5mg/ml. Phytochemical analysis was conducted to detect alkaloids, carbohydrates, saponins, phytosterols, phenols, flavonoids and tannins.

c. Evaluation of *P. longum* as grain protectant

The efficacy of *P. longum* as grain protectant was carried out by preparing 5 different concentrations by using ethanol and methanol solvents separately. 5ml of each concentration was poured to petri plate and kept in incubator for 15 minutes and then 10 flies were introduced to each petri plate. Control was maintained with 5ml of ethanol and methanol as control. All the concentrations including control were maintained in triplicates. The mortality rate of *Sitophilus* flies was observed every 24 hours and the mortal rate of flies in each petri plate was recorded and the average mortality from the triplicate was recorded. The observations were done till the flies attain 100% mortality at the regular interval of 24 hours. The efficacy of plant extract by mixing the plant extract of *P. longum* with rice grains is also evaluated. For that, in a petriplate, 10g of rice grains was taken; 5ml of filtrate was mixed with rice grain and kept in incubator for 15 minutes. 10 *Sitophilus* flies were introduced to petri plate. The same procedure was carried out with 8g, 6g, 4g and 2g of *P. longum* powder. The control was maintained with 5 ml of ethanol mixed with rice grains.

d. Selection and culture of test insect

Coleoptera is the main order which causes considerable damage to the stored grains. The *Sitophilus* species is one of the most important species of stored grains and other stored products. So in the present study, *Sitophilus* species has been selected to evaluate *P. longum* as grain protectant. The identification of male and female *Sitophilus* species carried out as per the morphological characters described by Omar (2012)^[16].

The *Sitophilus* species for the current work is collected from ICAR-JSS Krishi Vigyan Kendra Suttur and from local stores of Mysuru and cultured under laboratory conditions. *Sitophilus* species was cultured and maintained in the laboratory according to the procedure followed by Kim *et al.*, (2013)^[11].

e. Repellent efficacy of *P. longum*

The repellence test was performed according to Peixoto *et al.* (2015)^[17]. The dried plant extract was dissolved with solvent and prepared different concentrations (100-500mg/L) and then marked 2 proportions on Whatman filter paper. 20 adult *Sitophilus* flies were introduced to the centre of the filter paper. The readings were taken every 30 minutes and the percentage repellence was calculated by using the formula given by Abbott (1925).

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

A = Average number of insects present on untreated portion.
B = Average Number of insects present on treated portion.

Results

Phyto-chemical analysis of *P. longum* ethanol and methanol extract

The phyto-chemical analysis was performed to detect the presence of alkaloids, carbohydrates, saponins, phenols, flavonoids, tannins, and anthraquinone. Phytosterol was

detected in ethanol extract and alkaloids and phytosterols were detected in the methanol extract.

Images from Fig 1a to 1e represents the different life cycle of *Sitophilus*



Fig 1a: Egg of *Sitophilus*

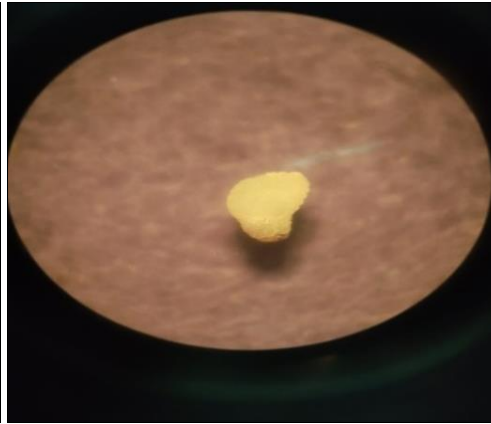


Fig 1b: Grub of *Sitophilus*



Fig 1c: Pupa of *Sitophilus*



Fig 1d: Male *Sitophilus*

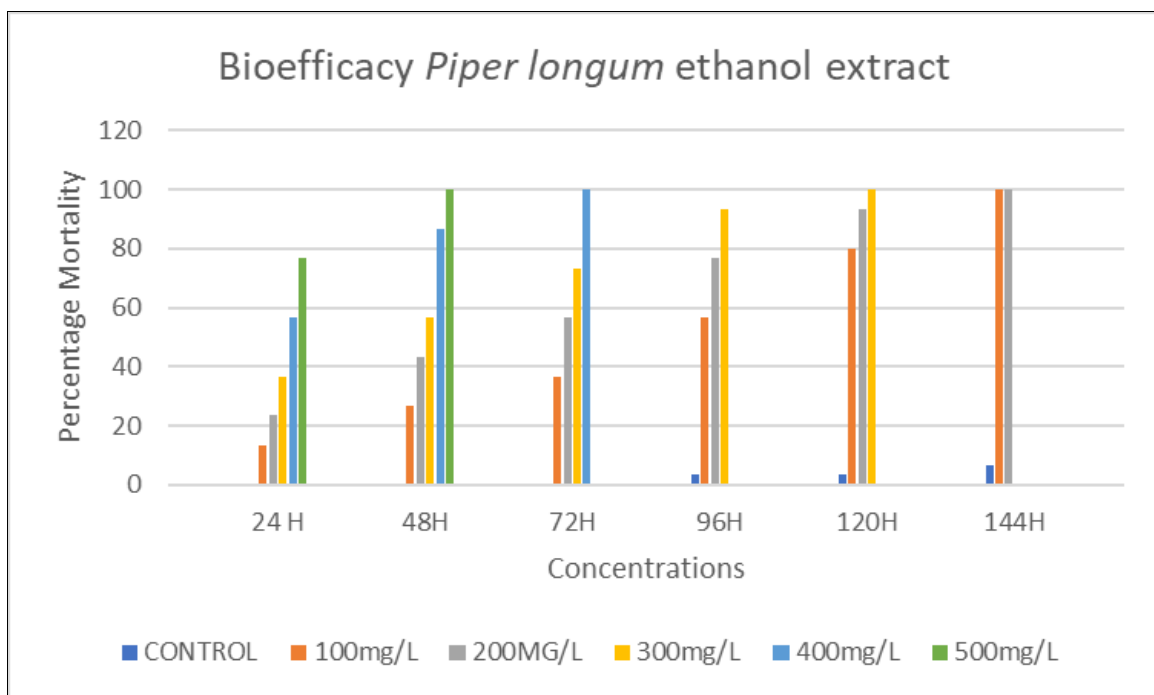


Fig 1e: Female *Sitophilus*

Bioefficacy of *Piper longum* ethanol extract against

Table 1: Bioefficacy of *Piper longum* ethanol extract against *Sitophilus* sp

		<i>Piper Longum</i> Ethanol Extract					
Concentrations →		Control	100mg/L	200mg/L	300mg/L	400mg/L	500mg/L
24 H	Average	0	13.4	2.34	3.67	5.67	7.67
	AVG. Percent	0	13.4	23.4	36.7	56.7	76.7
48H	Average	0	2.67	4.34	5.67	8.67	10
	AVG. Percent	0	26.7	43.4	56.7	86.7	100
72H	Average	0	3.67	5.67	7.34	10	
	AVG. Percent	0	36.7	56.7	73.4	100	
96H	Average	0.34	5.67	7.67	9.34		
	AVG. Percent	3.4	56.7	76.7	93.4		
120H	Average	0.34	8	9.34	10		
	AVG. Percent	3.4	80	93.4	100		
144H	Average	0.67	10	10			
	AVG. Percent	6.7	100	100			
144H	Average	0.67	10	10			
	AVG. Percent	6.7	100	100			



Graph1: Bioefficacy of *Piper longum* ethanol extract against *Sitophilus* sp

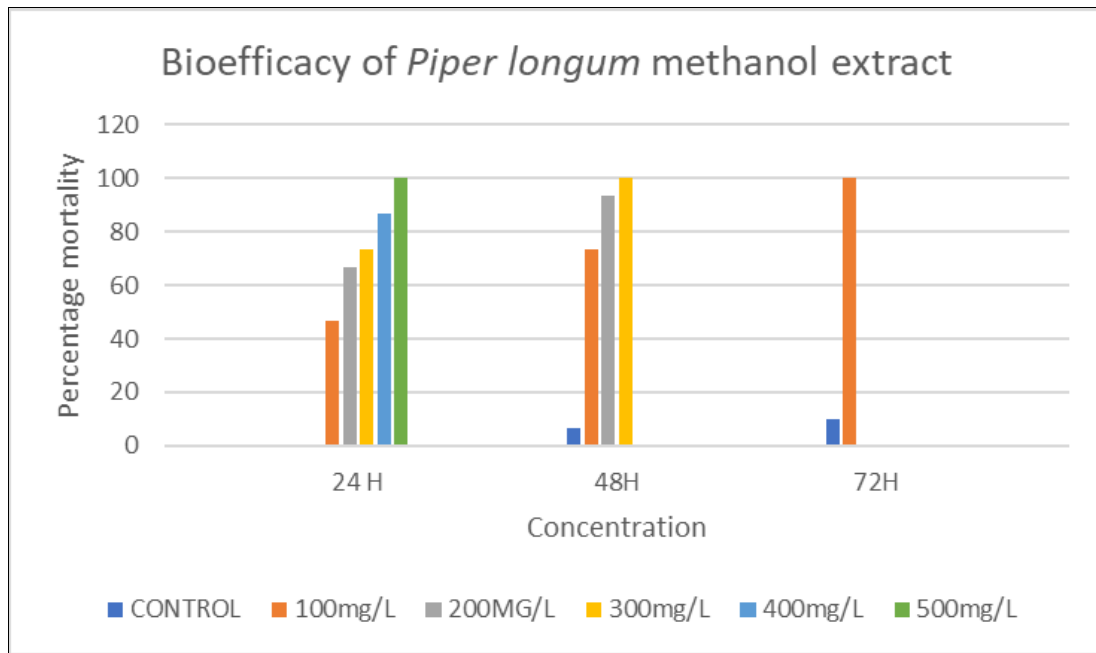
The ethanol extract of *Piper longum* was tested on *Sitophilus* species with different concentrations from 100mg/L to 500mg/L. Triplicates of each concentration was kept with 10 *Sitophilus* flies in each petriplate. Complete mortal rate (100 %) was achieved on 48 hours at the concentration of 500mg/L, which took 144 hours in 100mg/L concentration. There was no mortal rate recorded in control till 96 hours, at 96 hours 3.4% of mortality of

Sitophilus was recorded.10% of mortality was recorded at the end of 144 hours in control. As showed in table 1, both 100mg/L and 200mg/L took 144 hours for completing 100% of mortality, which was reduced to 120 hours in 300mg/L and 72 hours for 400mg/L concentration.

Bio efficacy of *Piper longum* methanol extract

Table 2: Bioefficacy of *Piper longum* methanol extract against *Sitophilus* spp

		<i>Piper Longum</i> Methanol Extract					
Concentrations →		Control	100mg/L	200mg/L	300mg/L	400mg/L	500mg/L
24 H	Average	0	4.67	6.67	7.34	8.67	10
	AVG. Percent	0	46.7	66.7	73.4	86.7	100
48H	Average	0.67	7.34	9.34	10	10	
	AVG. Percent	6.7	73.4	93.4	100	100	
72H	Average	1	10	10			
	AVG. Percent	10	100	100			



Graph 2: Bioefficacy of *Piper longum* methanol extract against *Sitophilus sp*

The methanol extract of *Piper longum* was tested on *Sitophilus* species with different concentrations from 100mg/L to 500mg/L. Triplicates of each concentration was kept with 10 *Sitophilus* flies in each petriplate. The complete mortality was attained at 500mg/L concentration in 24 hours. There is no mortal rate recorded in control at 24 hours. As presented in the table 2, both 100 mg/L and 200 mg/L concentration took 72 hours to attain complete mortality, with different percentages of mortality at 48 hours. Whereas, 24 hours is the time interval took by 500mg/L concentration for attaining 100 percent mortality rate.

Bio efficacy of *Piper longum* ethanol extract mixed with rice grains

5 ml of filtrate was mixed with 10 g of rice, and kept in incubator to evaporate ethanol, after 5-10 minutes 10 *Sitophilus* flies was introduced to each petri plate. Same method was followed by taking 2g, 3g, 4g and 5g of *P.longum* powder.

The average mortality of *Sitophilus* in 24 hours in 1g/100

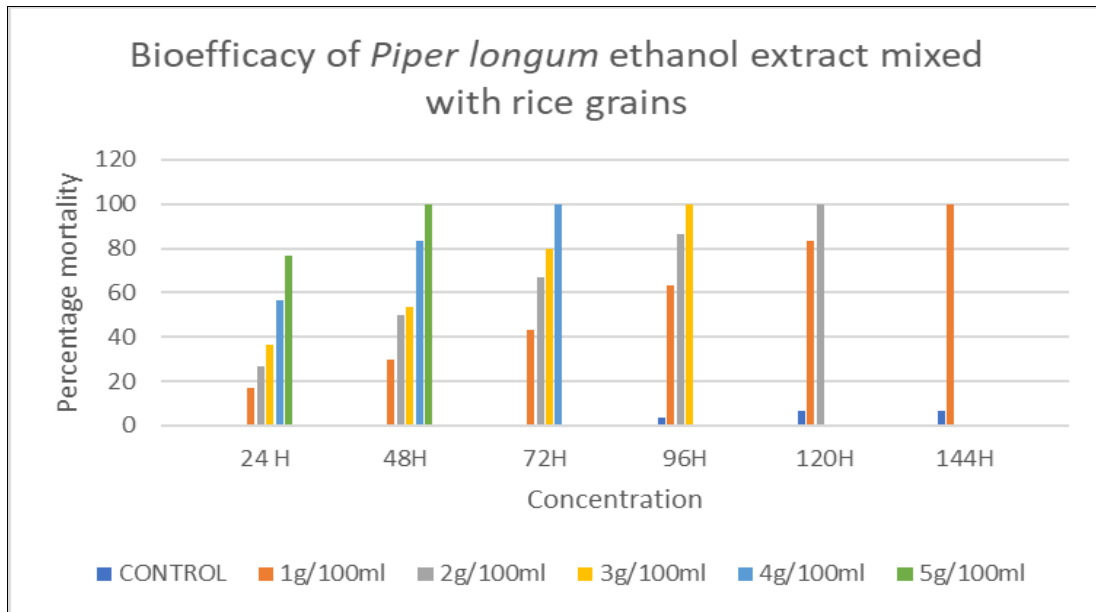
ml filtrate is 16.7%, which was increased to 76.7 % in 5g/100 ml filtrate. In 1g/100ml concentration the 100% mortality was attained at 144 hours, which was completed in just 48 hours in 5g/100 ml concentration. 2g/100 ml took 120 hours, 96 hours in 3g/100ml concentration, 72 hours for 4g/100 ml concentrations respectively to attain 100% mortality. As represented in table 3, in control there is no mortality was seen in 72 hours and in 144 hours the average mortality rate was 6.7% was observed.

Repellence test

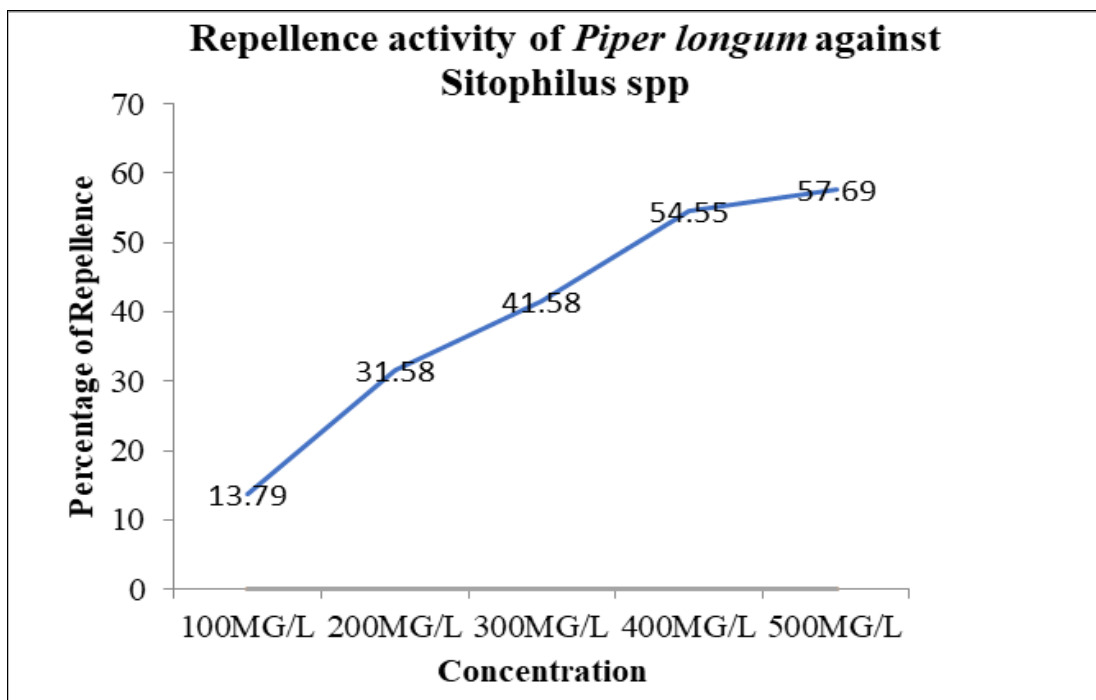
Repellent bioassay of *P.longum* conducted against *Sitophilus* species with different concentrations (100-500mg/L). The filter paper was divided into 2 equal halves; one half is treated with plant extract, which is the treated half, another half is treated with ethanol, which act as control. 20 flies were introduced to centre of the filter paper. The readings were taken at regular intervals of 30 minutes. The percentage repellence percentage was calculated by using the formula from Abbott 1925.

Table 3: Bioefficacy of *Piper longum* ethanol extract mixed with rice grains against *Sitophilus sp*

<i>Piper Longum</i> Ethanol Extract Mixed With Rice Grains							
		Control	1g/100ml	2g/100ml	3g/100ml	4g/100ml	5g/100ml
24 H	Average	0	1.67	2.67	3.67	5.67	7.67
	AVG. Percent	0	16.7	26.7	36.7	56.7	76.7
48H	Average	0	3	5	5.34	8.34	10
	AVG. Percent	0	30	50	53.4	83.4	100
72H	Average	0	4.34	6.67	8	10	
	AVG. Percent	0	43.4	66.7	80	100	
96H	Average	0.34	6.34	8.67	10		
	AVG. Percent	3.4	63.4	86.7	100		
120H	Average	0.67	8.34	10			
	AVG. Percent	6.7	83.4	100			
144H	Average	0.67	10				
	AVG. Percent	6.7	100				



Graph3: Bioefficacy of *Piper longum* ethanol extract mixed with rice grains against *Sitophilus* sp



Graph 4: Repellence activity of *Piper longum* against *Sitophilus* sp

As showed in Graph 4, the average repellence percentage at the concentration 100mg/L was 13.79%, which was least repellent against the *Sitophilus*. At the concentration of 200 mg/L there was 31.58% of repellence, which was increased to 41.58% in 300mg/L concentration. There was increase in the repellence at 400mg/L concentration, where it shows 54.55% of repellence. The 500mg/L concentration has the repellence of 57.69%, which is the highest percentage of repellence recorded.

Probit analysis to find LC₅₀ for bioefficacy of *P.longum* ethanol and methanol extracts

The LC₅₀ value is an essential component in bio efficacy project, which gives numerical representation of the concentration of *P.longum*, results in the mortality of 50% of *Sitophilus* species within a specified period of time interval. The results of an experiment conducted to

determine the LC₅₀ value at 72 hours after exposure for ethanol extract and 48 hours after exposure for methanol extract. The probit analysis was carried out using Microsoft excel according to Finney 1971 method. The LC₅₀ value of ethanol extract after 72 hours of exposure is 144.023mg/L. This value indicates that at a concentration of 144.023mg/L of *P.longum* ethanol extract, causes 50% mortality of *Sitophilus* at 72 hours and LC₅₀ value of methanol extract after 48 hours of exposure is 89.990 mg/L. This value indicates that at a concentration of 89.990 mg/L of *P. longum* methanol extract causes 50% mortality of *Sitophilus* at 48 hours.

Chi- square analysis of insecticidal activity of *P.longum* ethanol and methanol extract

The methanol extract and the ethanol extract from *P.longum* have considerable (P<0.05) insecticidal action against

Sitophilus species, according to chi-square analysis. The chi square statistics, carried out by using Microsoft excel. The p-value of *P.longum* ethanol extract is 1.557×10^{-100} and p-value of methanol extract is 2.26546×10^{-50} which is less than 0.01 provides compelling evidence to reject the null hypothesis and supports the alternative hypothesis that there is a significant association between the variables which suggests that the observed patterns are unlikely to be due to chance alone.

Discussion

Fumigation by conventional chemical pesticides has been the main strategy to control stored-grain insect pests for decades, and only methyl bromide and phosphine remain in use today (Rajendran., 2016) [18]. In developing countries, the traditional use of plants and plant derivatives for protection of stored products is long established. During storage, stored grain products are usually destroyed by stored grain insect pests. These insect pests of stored grain have been recorded causing severe weight loss and the quality of stored grain products. In this sense, the insecticidal qualities of numerous plant products against stored grain pest have been assessed.

For the control of stored-grain insect pests and to reduce the economic damage, chemical pesticides have been used from a long time. But these synthetic insecticides pose great ecological disturbances by entering through the food chain and ultimately causing human illness. In this regard, insecticidal nature of many plant originated chemicals has been established. Since, these plant based insecticides are biodegradable, less toxic to mammals, more selective in action and may retard the development of insect resistance, cause no ecological disturbances and they are eco-friendly.

Given that insect growth regulators depend on juvenile hormones, the characteristics of plant extracts that regulate the insect growth are highly intriguing and unusual. The enzyme ecdysone plays a major role in shedding of old skin and the phenomenon is called ecdysis or moulting. Ecdysone activity is reduced when active plant components enter the larvae's body, preventing them from moulting and dying while still in the larval stage (Isman et al., 1990) [9].

In the present study, the phytochemical characters of the *P.longum* was investigated, phytosterols is present in ethanol extract and alkaloids and phytosterols were present in the methanol extract. The result of phytochemical analysis of *P.longum* was supported by Khushbu et al., (2011) [10] where they confirm presence of these compounds in *P.longum* along with protein, carbohydrates etc.

Insecticidal activity of *P.longum* ethanol and methanol extract was studied at different concentrations (100, 200, 300, 400 and 500mg/L). Insecticidal activity of solvent extracts was calculated based on mortality of Sitophilus after treatment. High mortality normally indicates potential insecticidal activity of *P.longum* extracts. Data pertaining to the insecticidal activity clearly revealed that 100% insecticidal activity was recorded at 500mg/L concentration of methanol extract of *P.longum* was more effective which took 24 hours as compared to ethanol extract which took 48 hours. The obtained results were supported by the results obtained by Thambi and Cherian (2015) [22] where they obtained 100% mortality at 500ppm concentration of ethyl acetate extracts of *Manihot esculenta* at 72 hours.

In the insecticidal activity of *P.longum* extracts mixed with rice grains, among 5 concentrations 5g/100ml concentration

looks more effective. It took 48 hours to attain complete mortality. The *P.longum* ethanol extract mixed with rice grains took 48 hours to attain complete mortality. The solvent ethanol used as control shows 10% mortality, methanol shows 10% and ethanol mixed with rice grains shows 10% mortality. The mortal rate recorded in control clearly shows that 100% mortality is not achieved by solvents, but by *P.longum* extracts.

The results of repellence percentage were supported to the observation of Chaubey (2007) [6] who studied the repellent activity of three essential oils against stored product beetle *Tribolium castaneum* and Chaubey (2012) [7] who studied *Zingiber officinale* (Zingiberaceae) and *Piper cubeba* (Piperaceae) essential oils against *Sitophilus oryzae*. The repellency of 500mg/L concentration of *P.longum* extract was reflected by reduced numbers of insects on treated side of filter paper. It can be presumed that this reduction was caused by chemosensory effects, either through olfactory or gustatory. The antenna of Sitophilus is highly sensitive against *P.longum* extracts therefore; it shows maximum repellency against Sitophilus species.

Varying activity by different concentration of plant extract indicated that the pest controlling and repellent factors were not uniformly present. This is clearly seen in this investigation that percent mortality and repellency was different in different concentration of *P.longum* extracts. In the present study, the evaluation of *P.longum* as grain protectant was done which will definitely make a landmark in the field of stored-grain insect pest management. The *P.longum* which was studied under different parameters were proved very useful in controlling the insect pest population.

Conclusion

The experimental studies demonstrated that the grain protection potential of *P.longum* extract against Sitophilus species proved by insecticidal, and repellent activity. The *P.longum* is the natural insecticide and it does not have any toxic hazard from the treated grain. As compared to ethanol and methanol extracts, *P.longum* methanol extract has better activity than ethanol extract. The potential insecticidal and repellent activity of *P.longum* extracts might be present due to the presence of bioactive compounds. Therefore, *P.longum* could be considered an ideal grain protectant from the point of view of safety and seed viability.

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Author's contributions

Sowmya G contributed to bioefficacy experiments and literature review. Sanjay M S and Meghashree A M were involved in phyto-chemical analysis and statistical analysis, Ramyashree M was involved in experiment supervision, key inputs in project and reviewed and revised the manuscript. All authors have read and approved the final manuscript.

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