__Original Research Article__

Exploring the Insecticidal and Repellent activity of Garlic Essential Oil against *Sitophilus oryzae* Infestation in Stored Maize

ABSTRACT

The potential of garlic essential oil for its insecticidal and repellent properties against the rice weevil, *Sitophilus oryzae* was investigated through various bioassays. The results of the contact toxicity assay showed that the mortality rates were dependent on the concentration of the garlic essential oil, and its effectiveness increased with time. This was evident from the decreasing median lethal doses (LC_{50}) over the course of the study, ranging from 7.13 µl/ 20g of seeds (1 DAT) to 3.40 µl / 20g of seeds (5 DAT). The highest repellency rate of 88.89 per cent was achieved with a concentration of 6µl over a 4-hour period. This finding was further supported by the negative preference indices, which ranged from -1.0 to -0.1. With regard to fumigant toxicity assay, the LC_{50} values decreased as the study progressed, ranging from 4.72 µl /96cm^3 (1 DAT) to 1.70 µl/96cm^3 (5 DAT). Collectively, these results underscore the potential of garlic essential oil as a promising compound for the management of *S. oryzae.*

Keywords: Garlic essential oil, rice weevil, Sitophilus oryzae, toxicity, repellency, median lethal concentration

1. INTRODUCTION

Several factors contribute to post-harvest losses of food grains, yet insect damage presents the most serious threat. Nearly 100 species of stored product pests cause economic losses in storage and it has been reported that about 10-20 per cent of the world's total grain production is lost through pest infestation at storage levels [1]. In India, about 10-30 per cent of agricultural products are lost due to pests every year, with insect pests accounting for 26 per cent of the total [2]. In the case of stored maize, insects have been identified as the primary cause of grain losses, accounting for up to 20-30 per cent of total losses. Around 37 species of arthropod pests have been reported to be associated with stored maize and their by-products [3]. Among them, *Sitophilus oryzae* (Coleoptera: Curculionidae) is the most predominant one, causing up to 85 per cent weight loss in stored maize in

India and other countries [4]. It feeds voraciously on all cereals like rice, wheat, maize, oats and barley, making them unfit for human consumption [5]. They start their infestation in the field itself and subsequently reach the storage grains. They destroy the seeds by consuming them from the inside making them chaffy, and eventually, the viability of the seed is affected [6]. Further, they result in the development of carcinogenic fungus *Aspergillus* sp and other mold causing fungi.

In past years, control of rice weevils, and other stored-grain insect pests was mainly achieved with conventional insecticides. But indiscriminate use of these synthetic insecticides resulted in the development of resistance, residues, undesirable effects on non-target organisms, and other environmental hazards [7,8,9]. Furthermore, these pesticides have lately been proven to degrade seed viability and germination, as well as to be ozone-depleting substances, necessitating more limited usage of these insecticides [10]. This awareness has created a worldwide interest in the development of alternative strategies that are more eco-friendly. So, green insecticides, especially essential oils, show promise for tackling stored grain pests [11,12,13]. Essential oils are non-phytotoxic, less harmful to non-target organisms, and easily biodegradable leaving no toxic residues or by-products posing little threat to the environment or human health than synthetic pesticides [14].

In this context, garlic essential oil has shown greater promise as a natural and eco-friendly option for controlling stored pests. Garlic essential oil contains various compounds that possess insecticidal, repellent, and antifeedant properties [15,16] making it effective against a wide range of stored pests. And when used appropriately, it leaves no harmful residues on the treated grains or food products highly complying with food safety and quality standards. Though these essential oils have been tested for their toxicity properties, comprehensive studies must be conducted before they could be recommended for use as pesticides to inhibit post-harvest biodeterioration of food commodities, hence extending their shelf life [10].

2. MATERIAL AND METHODS

2.1 Insect rearing

Insects were initially obtained from the Department of Seed Technology, TNAU, Coimbatore and the mass culturing of rice weevil (*Sitophilus oryzae*) was carried out from January to July (2023) at Central Instrumentation Laboratory, TNAU, Coimbatore. Fresh rice grains were used to raise the rice weevil adults. The grains were initially sun-dried to kill any other existing insect stages. About 50 pairs of freshly emerged adult weevils were released into the plastic containers containing 500g of rice grains. The containers were tightly covered with muslin cloth and the culture was kept at a temperature of 27 ± 2^0 C, a relative humidity of 65 ± 5% and a photoperiod of 12:12 hours of light to dark. The F_1 generation adults which emerged after 35-40 days after inoculation were utilized for the toxicity studies. For a constant supply of insects for the studies, concurrent subcultures were maintained [17].

2.2 Collection and preparation of garlic sample

The garlic cloves were obtained from the local markets of Coimbatore, Tamil Nadu (11°1′6″N 76°58′21″E). The garlic was peeled manually and oven dried at 60^0 C for three days. After complete drying of the sample, these cloves were pounded into powder by using a mortar and pestle. The garlic powder was then sieved and stored in an airtight plastic container for further use [18].

2.3 Extraction of garlic essential oil using Soxhlet apparatus

The extraction process of garlic essential oil was carried out using the Soxhlet apparatus using hexane as the solvent. 30g of powdered garlic sample was weighed, packed in a piece of Whatman No.1 filter paper, and sealed tightly. This filter paper containing the sample was loaded into the thimble of the Soxhlet apparatus for the extraction process. The sample solvent ratio for the extraction process was 1:10. Hence 300ml of the solvent hexane was filled in the round bottom flask of the apparatus. This solvent was made to boil at 50-60^0 C for about 12 hrs until the completion of the extraction process. The solvent-dissolved oils were separated through a rotary evaporator after refluxing the solvent under reduced temperature and pressure which removes the excess solvent and results in oil yield. This extracted garlic essential oil was stored in a refrigerator at 4^0 C for subsequent analysis [18].

2.4 Bioassays

2.4.1 Contact toxicity

The different concentrations of the extracted garlic essential oil (3,5,7,9,11 and 13µl/ml of solvent) were made in acetone. These concentrations for the contact toxicity bioassay were initially fixed by carrying out the preliminary range-finding test. Each of these doses was treated with 20g of maize seeds placed over Petri dishes, using a micropipette and stirred continuously for one minute to ensure the uniform spread of essential oil over the seed surface. The treated samples were left for a few minutes to ensure complete evaporation of the solvent. Freshly emerged adult weevils of 30 numbers were introduced into each Petri plate. Two controls were used in the experiment i.e., seeds treated with acetone and the seeds treated without acetone or essential oil. All the treatments were replicated thrice and maintained in the laboratory conditions of 27 ± 2 ºC, 65 ± 5% RH. Insect mortality was observed for five days on a daily basis and LC_{50} worked out and expressed as µl/20g of seeds [19].

2.4.2 Fumigant toxicity

Different formulations of garlic essential oil (1,2,3,4,5 and 6µl/ml of solvent) were made in acetone. A preliminary range-finding test was initially conducted to fix the concentrations for the fumigant toxicity test. These concentrations were impregnated into Whatman No.1 filter paper discs and accommodated into Petri dishes of 9 cm diameter having a volume of $96cm^3$. Two filter paper discs, one treated with acetone and the other without essential oil or acetone were taken as controls. The treated acetone was made to evaporate completely from the filter paper discs before introducing the test insects. In each Petri plate, about 30 freshly emerged adults were introduced and kept in the dark in laboratory conditions for 24 hours. Each treatment was replicated thrice while being kept in laboratory conditions of 27 ± 2 ºC, 65 ± 5% RH. Insect mortality was recorded every day for up to five days and LC_{50} worked out and expressed as $µl/96cm^3$ [20].

2.4.3 Repellency

The repellency assay was performed using glass Petri dishes. A preliminary range-finding test was initially conducted to fix the concentrations for the repellent bioassays. These fixed concentrations of garlic essential oil (1,2,3,4,5 and 6µl/ml of solvent) were prepared in acetone. The Whatman filter papers were cut in half, and each test solution was applied to one side of the filter paper as evenly as possible with a micropipette. The other half of the filter paper was maintained as control which was treated with acetone only. The essential oil-treated and acetone-treated halves were dried to completely evaporate the solvent. Both the treated and untreated halves were then fastened using cellophane tape and fixed to the bottom of each petri dish in such a way that no seepage of test solution happen between the two halves of the filter papers. Thirty *S. oryzae* adults were released at the centre of the filter paper disc, covered and then kept in the dark. Three replications were maintained for each treatment and control. The number of adults in the treated and untreated half was counted at 1, 2, 3, and 4hrs after treatment [20].
Percentage repellency (PR) was calculated using the following formula,

$$PR = \frac{C - T}{C + T} \times 100$$

Where C is the number of insects in the untreated halves and T is the number of insects in the treated halves
Preference Index (PI) was calculated using the following formula,

$$PI = \frac{(\%\text{ of insects present in treated halves} - \%\text{ of insects present in untreated halves})}{(\%\text{ of insects present in treated halves} + \%\text{ of insects present in untreated halves})}$$

PI values ranging between - 1.0 and - 0.1 - repellent essential oil,
- 0.1 to + 0.1 neutral essential oil
+ 0.1 to + 1.0 attractant essential oil.

3. RESULTS AND DISCUSSION

3.1 Contact toxicity

The median lethal doses (LC_{50}) for ascertaining the contact toxicity at different days of exposure is summarized in Table 1. In the contact toxicity assay, the median lethal doses (LC_{50}) were found to be

7.13 5.04, 3.88, 3.62, and 3.40 µl/20 g of seeds after 1, 2, 3, 4 and 5 days after treatment (DAT). It was noted that the median lethal doses (LC_{50}) in contact toxicity decreased with an increasing exposure period. These LC_{50} values ranging from 7.31µl (1 DAT) to 3.40 µl (5 DAT) per 20g of seeds, showed that the mortality rate of the adult weevils increased with increasing period of time. Regression study revealed a concentration-dependent relationship between garlic essential oil and mortality in *Sitophilus oryzae* adults. Similar results have been obtained by Chaubey (2014) where the median lethal concentrations (0.52 µl/cm^2, 0.44 µl/cm^2, 0.37 µl/cm^2, 0.27 µl/cm^2) decreased with increasing period of time (24hrs, 48hrs, 72hrs and 96hrs) when applied on vials against bruchid adults [21].

3.2 Fumigant toxicity

The median lethal doses (LC_{50}) of rice weevil adults exposed to various concentrations of garlic essential oil for ascertaining the fumigant toxicity are summarized in Table 2. In fumigant toxicity assay, the median lethal concentrations (LC_{50}) were found to be 4.72 3.78, 2.54, 2.41, and 1.71 µl/96cm^3 after 1, 2, 3, 4 and 5 DAT. Regression study revealed a concentration-dependent relationship between the garlic essential oil and the mortality of *Sitophilus oryzae* adults. These results were in accordance with Mobki *et al*.(2014) who tested the toxicity of garlic extract against larvae and adults of *Tribolium castaneum*. According to the results of fumigant bioassays, the garlic extract tested was highly toxic when applied to *T. castaneum,* with insecticidal activity dependent on oil concentration and exposure period [22].

3.3 Repellency

The garlic essential oil was found to repel the adults of *Sitophilus oryzae* oil at all concentrations in the repellency tests (Table 3). The results clearly indicated that the repellent activity of garlic essential oil is concentration-dependent and the per cent repellency (PR) increased with increasing concentration and duration of exposure. At the concentration of 6µl/96cm^3, maximum repellency (88.8 per cent) was observed at 4 hrs of exposure. At all concentrations and exposure period, the preference index was in the range of -1.0 and -0.1, proving that the tested oil is repellent in nature. As a result, this finding supports the fact garlic essential oil could be an effective deterrent of *Sitophilus oryzae*. In a similar study, Chaubey (2013) recorded the per cent repellency of garlic essential oil against *S. oryzae* as 33.33, 54.16, 75.0 and 95.83 per cent for 0.2, 0.4, 0.8 and 1.6 per cent concentrations of garlic essential oil, respectively. The respective preference index was observed as -0.33, -0.54, - 0.75 and - 0.95 at 0.2, 0.4, 0.8 and 1.6 per cent concentrations of garlic essential oil respectively [23]. Chaubey (2014) noted that garlic essential oil inhibited oviposition in pulse beetle and this reduction was noted in terms of per cent repellency as 31.84, 42.5, 52.23 and 70.74 at 0.056, 0.085, 0.113 and 0.169µl/cm^3 concentrations of garlic essential oil respectively [21]. In another study, it was revealed that the repellent activity of the garlic extract was significantly influenced by the applied concentration and exposure duration. An increase in activity was also noted on exposing insects for a longer period of time. The PR values for the concentrations ranging from 0.62 to 2.13 µl/cm^2 were found to be 46.66 to 96.66 per cent, respectively [22].

Table 1. Contact toxicity of garlic essential oil against rice weevil (*Sitophilus oryzae*)

Exposure period	LC_{50} (µl/20g of seeds)	95% Fiducial limit		Regression equation	Chi square
		LL	UL		
1 DAT	7.131	6.158	8.258	y=3.5428+1.7119x	3.557
2 DAT	5.043	4.142	6.140	y=3.8937+1.5763x	3.916
3 DAT	3.875	3.090	4.859	y=3.9256+1.8107x	3.975
4 DAT	3.622	2.906	4.515	y=3.8792+1.9849x	3.406
5DAT	3.401	2.785	4.153	y=3.7179+2.3751x	3.691

(DAT- Days after treatment ; LC_{50}- Median Lethal Concentration)

Table 2. Fumigant toxicity of garlic essential oil against rice weevil (*Sitophilus oryzae*)

Exposure period	LC_{50} (µl)	95% Fiducial limit		Regression equation	Chi square
		LL	UL		
1 DAT	4.720	4.217	5.282	y=0.9291+6.3119	2.233
2 DAT	3.787	3.383	4.240	y =3.6335+2.361x	3.353
3 DAT	2.548	2.279	2.849	y=3.9848+2.5426x	4.770
4 DAT	2.413	2.091	2.786	y=4.2664+1.9512x	8.122
5DAT	1.708	1.453	2.008	y=4.4556+2.3203x	7.992

(DAT- Days after treatment ; LC_{50} - Median Lethal Concentration)

Table 3. Repellency of garlic essential oil against rice weevil (*Sitophilus oryzae*)

Treatments (Concentration)	Per cent Repellency (PR) (%)				Preference Index (PI)			
	1 hr	2hrs	3hrs	4hrs	1 hr	2hrs	3 hrs	4 hrs
1 µl	26.67	35.56	48.89	62.22	-0.27	-0.36	-0.49	-0.62
2 µl	40.00	46.67	57.78	71.11	-0.40	-0.47	-0.58	-0.71
3 µl	46.67	60.47	68.89	68.89	-0.47	-0.60	-0.69	-0.69
4 µl	51.11	60.00	62.22	75.56	-0.51	-0.60	-0.62	-0.76
5 µl	62.22	71.11	71.74	84.44	-0.62	-0.71	-0.72	-0.84
6 µl	75.56	80.00	86.67	88.89	-0.76	-0.80	-0.87	-0.89

4. CONCLUSION

This study demonstrates that garlic essential oil is an effective natural insecticide. Its multiple insecticidal properties, including contact toxicity, fumigant activity, and repellency, showcase its potential for effective pest control [22]. The concentration-dependent effects highlight the importance of precise application. As we seek sustainable alternatives to traditional pesticides, garlic essential oil emerges as a viable option. Further research could refine its application methods and explore synergistic effects with other natural compounds. This progress is key to environmentally friendly pest control solutions that tap into nature's capabilities. In this context, garlic essential oil represents a promising avenue for advancing natural insecticides in modern agriculture.

REFERENCES

1. Phillips, T. W & Throne, T. E. (2010). Biorational approaches to managing stored-product insects. *Annual review of entomology*, *55*, 375-397.
2. Tyagi, S. K., Guru, P. N., Nimesh, A., Bashir, A. A., Patgiri, P., Mohod, V., & Khatkar, A. B. (2019). Post-harvest stored product insects and their management.
3. Girma Demissie, Tadele Tefera, &Abraham Tadesse. (2023). Management of the maize weevil, *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae) using botanical insecticides on three maize genotypes. *Pest managment journal of Ethiopia*, *12*, 49–58.
4. Alam, M. J., Ahmed, K. S., Hossen, B., Mozammel, H., &Hoque, A. B. M. Z. (2019). Storage pests of maize and their status in Bangladesh. *Journal of bioscience and agriculture research*, *20*(02), 1724-1730.
5. Kim, S. I., &Lee, D. W. (2014). Toxicity of basil and orange essential oils and their components against two coleopteran stored products insect pests. *Journal of Asia-Pacific entomology*, *17*(1), 13-17.
6. Sallam, M. N., & Bothe, C. (1999). Chapter ii Insect damage: damage on post-harvest. *Entomol. Res*, *53*, 301-310.
7. Cherry, R., &Nagata,R. (2005). Development of resistance in southern chinch bugs (Hemiptera: Lygaeidae) to the insecticide bifenthrin. *Florida entomologist*, *88*(2), 219-221.
8. Prasad, P. S., Amarananjundeswara, H., Shetty., Sandhya, G. C., Basavaraj, V. N., & Ramegowda, G. K. (2020). Management of potato tuber moth during storage by using biological agents in Hassan district of Karnataka. *Int. J. Curr. Microbiol. App. Sci*, *9*(9), 465-470.
9. He, Y., Du, J., luo, J., Chen, S., &Yuan, R. (2020). Coreactant-free electrochemiluminescence biosensor for the determination of organophosphorus pesticides. *Biosensors and bioelectronics*, *150*, 111898.
10. Kedia, A., Prakash, B., Mishra, P. K., Singh, P., & Dubey, N. K. (2015). Botanicals as eco-friendly biorational alternatives of synthetic pesticides against *Callosobruchus* spp.(Coleoptera: Bruchidae)—a review. *Journal of food science and technology*, *52*, 1239-1257.
11. Saxena, S., & Pandey, A. K. (2001). Microbial metabolites as eco-friendly agrochemicals for the next millennium. *Applied microbiology and biotechnology*, *55*, 395-403.
12. Singh, H. P., Batish, D. R., & Kohli, R. K. (2001). Allelopathy in agroecosystems: an overview. *Journal of crop production*, *4*(2), 1-41.
13. Rajapakse, R. H. S. (2006). The potential of plants and plant products in stored insect pest management.
14. Mishra, A. K., & Dubey, N. (1994). Evaluation of some essential oils for their toxicity against fungi causing deterioration of stored food commodities. *Applied and environmental microbiology*, *60*(4), 1101-1105.
15. Grainge, M., &Ahmed, S. (1988). *Handbook of plants with pest-control properties*. John wiley & sons limited.
16. Ali, A. M., Mohamed, D. S., Shaurub, E. H., & Elsayed, A. M. (2017). Antifeedant activity and some biochemical effects of garlic and lemon essential oils *on Spodoptera littoralis* (boisduval) (Lepidoptera: Noctuidae). *J entomolzool stud*, *5*(3), 1476-1482.
17. Devi, M. A., Nameirakpam, b., devi, T. B., Mayanglambam, S., Singh, K. D., Sougrakpam, S., ... &Rajashekar, Y. (2020). Chemical compositions and insecticidal efficacies of four aromatic essential oils on rice weevil *Sitophilus oryzae* l. *International journal of tropical insect science*, *40*, 549-559.
18. Gafar, M. K., Itodo, A. U., Warra, A. A., & Abdullahi, I. (2012). Extraction and physicochemical determination of garlic (*Allium sativum* l) oil. *International journal of food and nutrition science*, *1*(2), 4-7.
19. Nenaah, G. E., & Ibrahim, S. I. (2011). Chemical composition and the insecticidal activity of certain plants applied as powders and essential oils against two stored-products coleopteran beetles. *Journal of pest science*, *84*, 393-402.
20. Chaubey, M. K. Insecticidal activities of *Anethum graveolens* l. and *Illicium verum*.

21. Chaubey, M. K. (2014). Biological activities of essential oil against pulse beetle (Coleoptera: Bruchidae). *Herba polonica*, *60*(2), 41-55.
22. Mobki, M., Safavi, S. A., Safaralizadeh, M. H., &Panahi, O. (2014). Toxicity and repellency of garlic (*Allium sativum* l.) Extract grown in Iran against *Tribolium castaneum* (herbst) larvae and adults. *Archives of phytopathology and plant protection*, *47*(1), 59-68.
23. Chaubey, M. K. (2013). Insecticidal effect of *Allium sativum* (Alliaceae) essential oil. *Journal of biologically active products from nature*, *3*(4), 248-25