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# Fumigant and contact toxicity of *Allium sativum* (Alliaceae) essential oil against *Sitophilus oryzae* L. (Coleoptera: Dryophthoridae)

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**ABSTRACT**

*In this study, Allium sativum (Alliaceae) essential oil was extracted and evaluated for repellent, insecticidal, feeding inhibitory, oviposition inhibitory and acetylcholinesterase enzyme inhibitory activities against rice weevil, Sitophilus oryzae. In the repellency assay, A. sativum oil repelled S. oryzae adults significantly at 0.2% concentration. A. sativum oil caused fumigant and contact toxicity in S. oryzae adults. In fumigation toxicity assay, median lethal concentrations ($LC_{50}$) were found to 0.30 and 0.24 µl/cm$^3$ of A. sativum oil after 24 and 48 h exposure of S. oryzae adults respectively. In contact toxicity assay, median lethal concentrations ($LC_{50}$) were found to 0.17 and 0.13 µl/cm$^2$ of A. sativum oil after 24 and 48 h exposure of S. oryzae adults respectively. A. sativum oil showed significant feeding deterrent activity in S. oryzae adults. Exposure of S. oryzae adults to sub-lethal concentrations of A. sativum oil significantly inhibited oviposition and acetylcholinesterase enzyme (AchE) activity in a concentration dependent manner.*

**Keywords:** *Allium sativum*, essential oil, *Sitophilus oryzae*, insecticides

## INTRODUCTION

With the beginning of agricultural practices, storage of food grains started as a safeguard against poor harvests and famine. Since then, insects also started damaging stored grains both qualitatively and quantitatively. To protect stored grains from insect infestation, several synthetic pesticides have been used. But, these synthetic pesticides have increased the risk of ozone depletion, neurotoxicity, carcinogenicity, teratogenicity and mutagenic effects among non-target species and cross-resistance and multi resistance in insects [1]. This has led to increased public awareness on human safety and possible environmental damage diverting attention towards plant products especially volatile chemicals in stored-grain insect pest management. Essential oils are highly volatile and non-persistent. Some of these exhibit adulticidal, larvicidal and antifeedant activity, capacity to delay development, adult emergence and fertility, and have deterrent effects on oviposition [2-8]. There are 17,500 aromatic species among higher plants belonging to families Alliaceae, Apiaceae, Asteraceae, Cupressaceae, Lamiaceae, Lauraceae, Myrtaceae, Piperaceae, Poaceae, Rutaceae and Zingiberaceae. Approximately 3,000 essential oils are known; 10% of them have commercial importance in cosmetic, food and pharmaceutical industries. They are generally recognized as safe (GRAS) by the U.S. Food and Drug Administration. Essential oils are natural, complex, secondary metabolites characterized by a strong odour and low density. Biological activities of essential oils depend on their chemical composition, which, in turn, varies with plant parts used for extraction, extraction method, plant phenological stage, harvesting season, plant age, soil nature and environmental conditions [9]. There are very complex natural mixtures that can contain 20 to 60 compounds at different concentrations, characterized by two or three major components at fairly high concentrations (20 to 70%) compared with other components present in trace amounts. The components include two groups of distinct biosynthetical origin. The main group is composed of terpenes and terpenoids and the other of aromatic and aliphatic constituents, all characterized by low molecular

weight [9]. *Allium sativum* (Garlic) belonging to the family Alliaceae is one of the most important constituent of human food and ingradients of Ayurvedic medicines since ancient time. It is used as diuretic, gastrointestinal, antihypertension, antioxidant, heart stimulant, antirhumetic, and hypolipidemic agent. Garlic bulbs contain a number of active compounds, especially sulphur containing compounds responsible for the pharmacological activities. Steam distillation of garlic bulb produces essential oils containing mainly of diallyl, allyl methyl and dimethyl mono to hexa sulfide [10]. These components contribute to acaricidal, antibacterial, fungicidal, insecticidal, molluscicidal, nematicidal and antiparasitic properties of garlic. In the present study, essential oil was extracted from the bulbs of *A. sativum* and evaluated for their biological activities against rice weevil, *S. oryzae*.

## MATERIALS AND METHODS

### Extraction of oil

*A. sativum* bulbs were purchased from Gorakhpur, U.P., India. Ground bulbs were hydrodistilled in Clevenger apparatus continuously for 4 h at $100^0$C to yield essential oil. The oil extracted was collected and kept in Eppendorf tubes at $4^0$C until use.

### Insects

Rice weevil, *S. oryzae* was used to determine the insecticide nature of *A. sativum* essential oil. The insects were reared on whole wheat grain in the laboratory at 28±$4^0$C, 75±5% RH, and photoperiod of 10:14 (L:D) h.

### Repellent activity

Repellency assay was performed in glass petri dishes (diameter 8.5 cm, height 1.2 cm). Test solutions of different dilutions (0.2, 0.4, 0.8 and 1.6% vol:vol) of *A. sativum* essential oil were prepared in acetone. Whatman filter papers were cut into two halves and each test solution was applied to filter paper half as uniform as possible using micropipette. The other half of the filter paper was treated with acetone only. Essential oil treated and acetone treated halves were dried to evaporate the acetone completely. Both treated and untreated halves were then attached with cellophane tape in a manner so that seepage of the test samples from one half to other half can be avoided and placed at the bottom in each petri dish. Forty *S. oryzae* adults were released at the centre of the filter paper disc and the petri dish was covered and kept in dark. Six replicates were set for each concentration of essential oil. After 4 h of treatment, number of adults in treated and untreated halves was counted. Percent repellency (PR) was calculated using formula:

$$PR = (C-T)/(C+T) \times 100$$

C = number of insects in the untreated halves and T = number of insect in treated halves
Preference index (PI) was calculated using formula:
PI = (percentage of insects in treated halves - percentage of insects in untreated halves)/ (percentage of insects in treated halves + percentage of insects in untreated halves)

PI values between - 1.0 and - 0.1 indicate repellant essential oil, - 0.1 to + 0.1 neutral essential oil and + 0.1 to + 1.0 attractant essential oil.

### Fumigant toxicity

Insecticidal effect of *A. sativum* essential oil was tested against *S. oryzae* adults by fumigation. Formulations of different dilution (8, 12, 16 and 20 µl/ml solvent) of essential oil were made in acetone. Ten adults taken from the laboratory culture were placed with 2 g of wheat grains in glass petri dish (diameter 8.5 cm, height 1.2 cm). Filter paper strip (2 cm diameter) was treated with *A. sativum* essential oil formulations and left for two minutes for evaporation of acetone. Treated filter paper was pasted on the undercover of petri dish, closed, air tightened with parafilm and kept in dark in conditions applied for rearing of insect. Six replicates were set for each concentration of essential oil and control. After 24 and 48 h of fumigation, mortality in adults was recorded. Median lethal concentration ($LC_{50}$) was calculated using POLO programme [11]. Analysis of variance (ANOVA) and correlation and linear regression analysis were conducted to define concentration-response relationship [12].

**Contact toxicity**
Contact toxicity of *A. sativum* essential oil was determined against *S. oryzae* adults. Formulations of different dilution (8, 12, 16 and 20 µl/ml solvent) of essential oil were made in acetone, applied on bottom surface of glass petri dish (diameter 8.5 cm, height 1.2 cm) and left for two minutes for evaporation of acetone. Ten adults taken from the laboratory culture were released at the centre of petri dish, covered and kept in dark in conditions applied for rearing of insect. After 24 and 48 h of fumigation, mortality in adults was recorded. Median lethal concentration ($LC_{50}$) was calculated using POLO programme [11]. The analysis of variance (ANOVA) and correlation and linear regression analysis were conducted to define concentration-response relationship [12].

**Antifeedant activity (AFA)**
Antifeedant activity of *A. sativum* essential oil was studied using flour disks. Flour disks were prepared by mixing 10 g wheat flour with 50 ml water until completely suspended. Wheat flour suspension was pipetted (200 µl) onto a plastic sheet, held for 24 h at room temperature and dried in oven at $60^0$C for 1 h. Flour disks were weighed between 70-76 mg each. Flour disk was treated with 3, 6, 9 and 12µl of *A. sativum* essential oil, weighed, placed in glass petri dish and released twenty-five adults in each petri dish. Insects were allowed to feed and flour disks were reweighed after 4 days. Six replicates were set for each concentration of essential oil and control. Antifeedant activity (AFA) was calculated using formula:

$$AFA = [C-T/C]\times 100$$

C = consumption of flour disk in control group, and T = consumption of flour disc in treated group. The analysis of variance (ANOVA) was performed to test the significant antifeedant activity of *A. sativum* essential oil in insect [12].

**Oviposition inhibition**
Ten 3-4 days old *S. oryzae* adults of mixed sex were fumigated with two sublethal concentrations viz. 40 % and 80 % of 24-h $LC_{50}$ of *A. sativum* essential oil for 24 h and reared on wheat grain in a 250 ml plastic box for 10 days. After 45 days, adults were discarded and number of $F_1$ progeny was counted. Six replicates were set for each concentration of essential oil and control. Analysis of variance (ANOVA) was performed to test the significant oviposition changes in insect [12].

**Acetylcholinesterase enzyme (AChE) activity determination**
*S. oryzae* adult insects were fumigated with two sublethal concentrations viz. 40% and 80% of 24-h $LC_{50}$ of *A. sativum* essential oil as in toxicity assay. After 24 h of fumigation, adults were utilized for determination of acetylcholinesterase enzyme activity [13]. Fumigated insects were homogenized in phosphate buffer saline (50 mM, pH 8) and centrifuged. Supernatant was used as the acetylcholinesterase source. To 0.1 ml of enzyme source, added 0.1 ml substrate acetylthiocholine iodide (ATChI) (0.5 mM), 0.05 ml chromogenic reagent 5,5-dithiobis 2-nitrobenzoic acid (DTNB) (0.33mM) and 1.45 ml phosphate buffer (50 mM, pH 8). Acetylcholinesterase enzyme activity was determined by measuring changes in the optical density at 412 nm by incubating the reaction mixture for 3 min at $25^0$C. Enzyme activity was expressed as mmol of 'SH' hydrolysed $min^{-1}$ $mg^{-1}$ protein. Each enzymatic assay was replicated six times. Analysis of variance (ANOVA) was performed to test the significant changes in enzyme activity [12].

## RESULTS

**Repellent activity**
Repellency was found 33.33, 54.16, 75.0 and 95.83% at 0.2, 0.4, 0.8 and 1.6% concentrations of *A. sativum* essential oil respectively (Table 1). Preference Index (PI) was found -0.33, -0.54, - 0.75 and -0.95 at 0.2, 0.4, 0.8 and 1.6% concentrations of *A. sativum* essential oil respectively (Table 1). This essential oil showed significant ($F = 112.01$, $P<0.01$) concentration dependent repellant activity against *S. oryzae* adults evidenced from the negative values of Preference Index (Table 1).

**Fumigant toxicity**
Fumigation of *S. oryzae* adults with *A. sativum* essential oil caused toxicity by vapour action. Median lethal concentrations ($LC_{50}$) were 0.30 and 0.24 µl/$cm^3$ air after 24 and 48 h of exposure respectively (Table 2). Regression

analysis showed concentration-dependent mortality in *S. oryzae* adults against *A. sativum* essential oil (F = 164.84 for 24h and 133.75 for 48h; P<0.01, Table 3).

**Contact toxicity**

*A. sativum* essential oil caused contact toxicity in *S. oryzae* adults. Median lethal concentration ($LC_{50}$) of *A. sativum* essential oil was 0.17, and 0.13 µl/cm$^2$ against *S. oryzae* adults after 24 and 48 h of exposure respectively (Table 2). Regression analysis showed concentration-dependent mortality in *S. oryzae* adults against *A. sativum* essential oil (F = 113.70 for 24h and 131.55 for 48h; P<0.01, Table 3).

**Antifeedant activity (AFA)**

*A. sativum* essential oil significantly decreased consumption of flour disk by *S. oryzae* adults. Consumption of flour disk was reduced to 81.72, 50.91, 33.16 and 13.73 % of control when treated with 3, 6, 9 and 12 µl/disk of *A. sativum* essential oil (F = 111.12, P<0.01; Table 4).

**Oviposition inhibition**

Fumigation of *S. oryzae* adults with *A. sativum* essential oil significantly reduced oviposition potential. Reduction in oviposition was 49.45 and 15.78% of the control when *S. oryzae* adults were fumigated with 40 % and 80 % of 24-h $LC_{50}$ of *A. sativum* essential oil respectively (F = 175.32, P<0.01; Table 5).

**Acetylcholinesterase enzyme (AChE) activity**

Fumigation of *S. oryzae* adults with *A. sativum* essential oil significantly reduced AChE activity. AChE activity was reduced to 77.51 and 52.20% of control when *S. oryzae* adults were fumigated with 40 % and 80 % of 24-h $LC_{50}$ of *A. sativum* essential oil respectively (F = 205.78, P<0.01; Table 6).

**Table 1. Percent repellency of *A. sativum* oil against *S. oryzae***

| % Conc. (vol:vol) | Percent Repellency (PR) * Mean±SE | Preference Index** (PI) | F-value*** |
|---|---|---|---|
| 0.2 | 33.33±0.53 | - 0.33 | 112.01 |
| 0.4 | 54.16±0.64 | - 0.54 | |
| 0.8 | 75.0±0.76 | - 0.75 | |
| 1.6 | 95.83±0.93 | - 0.96 | |

**Percent repellency (PR) was calculated as: PR = (C-T)/(C+T) ×100*
*Where C = number of insects in the untreated halves and T = number of insect in treated halves*
***Preference index (PI) was calculated as:*
*PI = (percentage of insects in treated halves - percentage of insects in untreated halves)/ (percentage of insects in treated halves + percentage of insects in untreated halves).*
*PI value between -1.0 to -0.1 indicates repellant essential oil, -0.1 to +0.1 neutral essential oil and +0.1 to +1.0 attractant essential oil.*
****F-values significant (P<0.01)*

**Table 2. Fumigant and contact toxicity of *A. sativum* oil against *S. oryzae* adults**

| Essential oil | Exposure period | $LC_{50}$ | LCL | UCL | g-value | Heterogeneity | t-ratio |
|---|---|---|---|---|---|---|---|
| Fumigant toxicity | 24 h | 0.30µl/cm$^{-3}$ | 0.026µl/cm$^{-3}$ | 0.034µl/cm$^{-3}$ | 0.16 | 0.32 | 3.96 |
| | 48 h | 0.24µl/cm$^{-3}$ | 0.021µl/cm$^{-3}$ | 0.027µl/cm$^{-3}$ | 0.15 | 0.34 | 4.06 |
| Contact toxicity | 24 h | 0.17µl/cm$^{-2}$ | 0.014µl/cm$^{-2}$ | 0.020µl/cm$^{-2}$ | 0.17 | 0.31 | 4.21 |
| | 48 h | 0.13µl/cm$^{-2}$ | 0.012µl/cm$^{-2}$ | 0.015µl/cm$^{-2}$ | 0.14 | 0.29 | 4.33 |

*$LC_{50}$ represents lethal concentration that cause 50% mortality*
*LCL and UCL represent lower confidence limit and upper confidence limit respectively*

**Table 3. Regression analysis of fumigant and contact toxicity of *A. sativum* oil against *S. oryzae* adults**

| Essential oil | Exposure period | Intercept | Slope | Regression Equation | Correlation coefficient | F-value* |
|---|---|---|---|---|---|---|
| Fumigant toxicity | 24 h | - 7.16 | 3.46 | Y = - 7.16+3.46X | 0.987 | 164.84 |
| | 48 h | - 7.56 | 4.42 | Y = - 7.56+4.42X | 0.990 | 133.75 |
| Contact toxicity | 24 h | - 6.31 | 5.14 | Y = - 6.31+5.14X | 0.978 | 113.70 |
| | 48 h | - 3.92 | 6.97 | Y = 3.92+6.97X | 0.975 | 131.55 |

*Regression analysis was performed between different concentration of essential oil and response of adults/larvae*
**F-values significant (P<0.01)*

**Table 4. Feeding inhibitory activities of *A. sativum* in *S. oryzae* adults**

| Conc (µl/disk) | Consumption of flour disk in mg (Mean±SE) | AFA* | F-Value** |
|---|---|---|---|
| 0 | 19.15±1.24(100) | 0.0 | 111.12 |
| 3 | 15.65±1.16(81.72) | 14.87 | |
| 6 | 9.75±1.15(50.91) | 44.80 | |
| 9 | 6.35±0.96(33.16) | 73.30 | |
| 12 | 2.63±0.64(13.73) | 86.56 | |

*Values in parentheses indicate per cent change with respect to control taken as 100%*
**Antifeedant Activity (AFA) was calculated using formula: AFA = [C-T/C]×100*
*where, C = consumption of flour disk in control group, and T = consumption of flour disc in treated group.*
***F-values significant (P<0.01)*

**Table 5. Oviposition inhibitory activities of *A. sativum* oil in *S. oryzae* adults**

| Conc | No. of progeny emerged (Mean±SE) | F-Value* |
|---|---|---|
| Control | 152.0±3.56(100%) | 175.32 |
| 40% of 48h-$LC_{50}$ | 75.16±2.67(49.45) | |
| 80% of 48h-$LC_{50}$ | 24.0±1.06(15.78) | |

*Values in parentheses indicate per cent change with respect to control taken as 100%*
**F-values significant (P<0.01)*

**Table 6. Effect of 40 and 80% of 24h-$LC_{50}$ of *A. sativum* oil on Acetylcholinesterase enzyme (AChE) activity in *S. oryzae* adults**

| Conc. | Enzyme activity* | F- value** |
|---|---|---|
| Control | 0.0886±0.0019(100) | 205.78 |
| 40% of 24h-$LC_{50}$ | 0.0686±0.0015(77.51) | |
| 80% of 24h-$LC_{50}$ | 0.0462±0.0009(52.20) | |

**Enzyme activity was expressed as mol of 'SH' hydrolysed $min^{-1}mg^{-1}$ protein*
*Values in parentheses indicate per cent change with respect to control taken as 100%*
***F-values significant (P<0.01)*

## DISCUSSION

Among plant-based insecticides, plant volatiles have received much attention in the scientific community in insect pest management programme [2-8]. *Acorus calamus*, *Syzygium aromaticum*, *Hyptis spicigera*, *Ocimum canum* and *Vepris heterophyla* essential oils exhibited repellent activity, insecticidal effect and inhibition of progeny in *S. oryzae* [14,15]. Essential oil components also have been evaluated for their role in insect pest management programme. Linalool and linalyl acetate exhibited significant fumigant toxicity to rice weevils [16]. Menthol, methonene, limonene, β-pipene, α-pipene, and linalool exhibited toxicity in *S. oryzae* and inhibited AChE activity [17]. In this study, repellant, insecticidal, feeding, oviposition and AChE inhibitory activities of *A. sativum* essential oil against *S. oryzae* was studied. This essential oil showed significant repellant activity against *S. oryzae* adults as evidenced by the negative values of the PI. *A. sativum* essential oil induced high mortality in *S. oryzae* adults when treated by fumigation or contact methods. The index of significancy of potency estimation, g-value indicates that the mean value is within the limits of all probabilities (P<0.1, 0.5 and 0.01) as it is less than 0.5. Values of t-ratio greater than 1.6 indicate that the regression is significant. Values of heterogeneity factor less than 1.0 denotes that model fits the data adequate. *A. sativum* essential oil reduced progeny production in *S. oryzae* fumigated. This ultimately reduced damage caused by the insect. *A. sativum* essential oil decreased consumption of flour disk by *S. oryzae* adults. Similar results have been shown by *Schinus molle*, *Alpinia conchigera*, *Zingiber zerumbet* and *Curcuma zedoaria* essential oils in *T. castaneum* and *S. oryzae* [3,18-20]. Little is known about the mode of action of essential oils and their constituents in insects, but studies suggested their neurotoxicity [21-22]. In this study, fumigation of *S. oryzae* adults with *A. sativum* significantly reduced AChE activity. Recent research has demonstrated the interference of monoterpenes with acetylcholinesterase activity in insects [19-20]. Essential oils are lipophilic in nature and can be inhaled or ingested. The rapid action against insect pests is indicative of a neurotoxic mode of action and interference with the neuromodulator octopamine [23] or GABA-gated chloride channels [24]. Several essential oil components act on the octopaminergic system of insects. Octopamine is a neurotransmitter, neurohormone, and circulating neurohormone–neuromodulator, and its disruption results in total breakdown of the nervous system [25]. Thus, the octopaminergic system of insects represents a target for insect control. Low molecular weight terpenoids are too lipophilic to be soluble in the haemolymph after crossing the cuticle, and the proposed route of entry is tracheae [26]. Most insecticides bind to receptor proteins in the insect and interrupt

normal neurotransmission leading to paralysis and death. Recent evidence suggests that low molecular weight terpenoids with different structures may also bind to target sites on receptors that modulate nervous activity [25].

## CONCLUSION

In conclusion, present study indicates that *A. sativum* essential oil causes repellency, contact toxicity, fumigant toxicity, feeding inhibition and oviposition inhibition, and also acts on AChE enzyme activity in *S. oryzae*. Since *Allium* is safe for human as it is an integral part of our food, *A. sativum* can be considered as a natural alternative in the eco-friendly management of stored-grain insects.

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