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Fumigant toxicity of garlic essential oil with the combined effect of ozone gas and determination of phytochemical residues from the treated rice weevil *Sitophilus oryzae* and wheat grains

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Abstract

Ozone gas (OEG) is a strong oxidizing agent and it has been recognised as potential fumigant to manage stored product insect pests. The present study was carried out to understand the ozonation effect on garlic essential oil (GEO) fumigation and residual characteristics on the fumigated food grain and insect pests. Fumigant toxicity of GEO and OEG against *Sitophilus oryzae* adults were evaluated using 1 kg of wheat grain at 30 to 120 µL/kg and 0.2 to 2.4 g/h concentrations, respectively. In order to saturate GEO in stored wheat grain, OEG was passed at 1 g/h flow rate along with GEO at 225 µL/kg concentration. Mortality of *S. oryzae* adults was observed and phytochemical residues were collected from the treated grains and beetles at different exposure durations. After 72 h of exposure, 64.10 µL/kg of LC_{50} value was obtained for GEO fumigation against *S. oryzae* adults. Fumigant toxicity of GEO was increased with the combined treatment of OEG at specific flow rate of 1 g/h and 100% mortality was achieved ($P \leq 0.05$) within 72 h exposure. In gas chromatography analysis, diallyl sulfide, diallyl disulfide and diallyl trisulfide compounds were detected as major fumigant residues from the treated *S. oryzae* adults and wheat grain. The residual level of phytochemical fumigants was remarkably reduced in GEO+OEG treatment as compared to GEO alone treatment. The study results suggests that GEO fumigation with the combination of OEG at mild dose could be a promising method in order to prevent *S. oryzae* infestation in stored wheat grain.

Keywords: Ozone, Garlic essential oil, *Sitophilus oryzae*, Phytochemical residue, Fumigation, Eco-friendly insecticide

Introduction

Plant essential oils and phytochemical volatiles have been widely recognized as promising alternative natural sources to conventional gaseous fumigants against stored product insects (SPI). However, the vapour pressure and diffusion properties of plant volatile molecules are less ($\leq$1 mmHg at 20°C) as compared to gaseous fumigants with respect to the practical applications (Dambolena et al., 2016). Garlic (*Allium sativa*) essential oil (GEO) recorded as a one of the potential bio-fumigant against several SPI (Demeter et al., 2021). In-order to enhance the fumigant action, GEO was investigated in combination of carbon dioxide (CO_2) and 4.9-fold increased fumigant toxicity was recorded against *Tribolium confusum* (Herbst) as compared to GEO alone treatment (Işikber, 2010). Ozone (O_3) gas (OEG) is also a potential fumigant and its insecticidal activities have been reported against a number of SPI species (Kells et al., 2001; Iskiber and Oztekin, 2009; Mishra et al., 2019). According to Graham (1997), OEG had been recognized as safe for application in food processing sector under GRAS status. Rajendran and Sriranjini (2008) suggested that phytochemical fumigant studies with carrier gases are needed to validate the potentiality of essential oil based bio-fumigants for SPI control. The *Sitophilus oryzae* (Linnaeus), commonly known as rice weevil is a major SPI that causes severe damages in a wide range of cereals including rice, wheat, maize. During the past few decades, a wide range of plant essential oils and phytochemical volatiles have been extensively studied for the control of *S. oryzae*. In our recent study, we have investigated the fumigant toxicity of selected essential oils against *S. oryzae* adults and analyzed the phytochemical residue profiles on the fumigated rice grain (Vendan et al., 2017). In another study, we have evaluated the persistence and ingestion characteristics of five different phytochemical volatiles in *S. oryzae* adults (Sahu et al., 2021). The present study was aimed to investigate the fumigant toxicity of combination of GEO with OEG and to determine the phytochemical residues on the treated wheat grain and *S. oryzae* adults.

Materials and methods

Essential oil and chemicals

The garlic essential oil, HPLC-grade n-hexane solvent and other chemicals were procured from Sigma-Aldrich Chemicals Pvt. Ltd., India.

Insect culture

Different species of SPI were regularly maintained in the insectary with controlled temperature (30 ± 2°C), relative humidity (75 ± 5%) and photoperiod conditions (13:11; light: dark). From the stock culture, a sub-culture of *S. oryzae* was prepared and maintained with wheat grain. Newly emerged (one-week-old) *S. oryzae* adults in the sub-culture were used for fumigation bioassays.

Fumigation bioassays

Fumigant toxicity of GEO was evaluated against *S. oryzae* adults at 30, 60 and 120 µL/kg concentrations with 1 kg of wheat grain in 1.5 L container under airtight condition. Thirty individuals of *S. oryzae* adults were released into each container filled with wheat grain. Garlic essential oil was loaded on the Whatman No 1 filter paper strips (15 x 1 cm) and inserted at the center of wheat grains inside the container. Control sample was maintained without GEO treatment. Three replicates were maintained for each concentration treatment. Mortality of *S. oryzae* adult was recorded at 24, 48 and 72 h of treatment durations.

Fumigant toxicity of OEG was evaluated against *S. oryzae* adults at 0.2, 1.0 and 2.4 g/h flow rates with 1 kg of wheat grain in 1.5 L container under airtight condition. OEG was generated using atmospheric oxygen with the help of an ozone generator (Model A5G, Faraday Ozone Products Private Limited, Coimbatore, India) and passed into the fumigation container containing wheat grains and inoculated 30 individuals of *S. oryzae* adults. The OEG was passed into experimental containers through the inlet (bottom of the container) and the remaining OEG was liberated through the outlet of the container (top of the container). Ozone gas fumigation was carried out for 1.0 h and the fumigated grain was stored up to 72 h for assessing the beetle mortalities. Control sample was maintained without OEG treatment. For each treatment condition, three replicates were maintained with control samples. After 24, 48 and 72 h of treatment, *S. oryzae* adults were separated from the wheat grain using sieves (Test Sieves, Jayant Scientific India) and the beetle mortalities were recorded.

Fumigant toxicity of GEO+OEG combination (225 µL/kg of GEO and 1.0 g/h flow rate of OEG) was evaluated against *S. oryzae* adults at 0.25, 0.50 and 1.00 h exposures with 1 kg of wheat grain in 1.5 L container. Concentration of GEO and flow rate of OEG were determined based on the obtained LC_{90} values and flow rate option in the OEG generator. Similar to the above method (OEG fumigation bioassay), GEO+OEG fumigation experimental set up was prepared and GEO was loaded on the Whatman No 1 filter paper strip (15 x 1 cm) and placed at the end of the OEG inlet connecting to the 1.5 L container containing wheat grains and inoculated 30 *S. oryzae* adults. Garlic essential oil exposure without OEG treatment vice versa was maintained for comparative analysis and control sample was maintained without any fumigant treatment. Three replicates were maintained for each concentration and treatment. Treated beetles were separated and mortality was recorded at 24, 48 and 72 h of treatment duration, similar to the above bioassays.

Fumigant residue analysis

Fumigation experiments were carried out similar to the above method (GEO+OEG fumigation bioassay) and the fumigated grain and beetles were used for fumigant residues analysis. After 72 h of GEO and GEO+OEG fumigation treatments, three replicates of each treatment were pooled into single samples. Randomly, 50 g of wheat grain and 30 individuals of *S. oryzae* adults were separated from the pooled samples. Both wheat grain and beetle samples were soaked for 1 h in 100 and 5 ml of HPLC-grade hexane, respectively. Then the hexane extract was collected by filtration using Whatman No 1 filter paper and all the extracts were concentrated up to 1 mL by air-drying. Final concentrates were filtered using PTFE syringe filter (0.2 µm pore size) and all the hexane extract samples were stored at -20°C till further use.

Gas Chromatography analysis

Phytochemical fumigant residues were analyzed using a gas chromatograph coupled flame photometric detector (GC-FPD) (Agilent 7890B, version 2019) system. HP-5 capillary column (30 m x 0.32 mm x 0.25 µm film thickness) was used and split less mode of injection was followed for analyses of all the samples. Nitrogen was used as a carrier gas in a constant flow mode (25 mL/min). About 4 µL of hexane extract samples were injected into the column and the following specifications were employed in the GC-FPD system for analysis; initial temperature at 150°C for 1 min, ramped to 210°C at the rate of 20°C/min, and ramped again at the rate of 4°C/min to 300°C for the last 10 min. The compounds detected in the tested samples were identified by comparing the respective retention times of the standard and control samples.

Data analysis

Percentage corrected mortality of *S. oryzae* adults was calculated using Abbott's correction formula (Abbott, 1925). The significance of results was analysed by one-way ANOVA and the effective treatments were separated by Tukey's multiple range test. Differences between means were considered significant when $P \leq 0.05$. The mortality data were further used for Probit analysis to estimate the LC_{50}, LC_{90}, LT_{50} and LT_{90} values of treatments (Finney, 1971). ANOVA and Probit analysis were performed using the SPSS (16.0 version) software program.

Results and discussion

In the present study, combined treatment of GEO with OEG was investigated for evaluating synergistic fumigant toxicities against *S. oryzae* adults. In the first phase of the study, fumigant toxicity of GEO and OEG was evaluated against *S. oryzae* adults with 1 kg of wheat grain at three different concentrations (30, 60 and 120 µL/kg of GEO and 0.2, 1.0 and 2.4 g/h of OEG). After 72 h of treatment, 24.3 and 41.8% of mortality were recorded for 30 and 60 µL/kg of GEO concentrations, respectively, whereas at 24 and 48 h treatments mortality was nil (Fig. 1a). Remarkably, 76.5% of mortality was observed for 120 µL/kg of GEO concentration with 1.0 kg of wheat grain. About 64.10 and 224.24 µL/kg of LC_{50} and LC_{90} values were recorded respectively, at 72 h of fumigation treatment in the present study. Previously, Chaubey (2016) recorded 0.24 $\mu L/cm^3$ of LC_{50} value for GEO against *S. oryzae* adults for 48 h of fumigation exposure without food. Most recently, Demeter et al. (2021) studied the fumigant toxicity of twenty-five different essential oils with 8 g of wheat grain against *S. granarius* (Linnaeus), and they highlighted that GEO was most toxic with 0.64% for LC_{50} value at 24 h exposure. In the present study in OEG fumigation, no mortality was observed for 0.2 g/h exposure during 24 to 72 h of treatment durations (Fig. 1b). Remarkably, 100% mortality of *S. oryzae* adult was observed for 1.0 and 2.4 g/h of OEG exposures at 72 h treatment duration. The Probit analysis results revealed 0.45 g/h for LC_{50} and 0.60 g/h for LC_{90} of OEG against *S. oryzae* adults at 72 h of treatment duration (Fig. 1b). Previously, Kells et al. (2001) reported 100% mortality of *S. zeamais* Motschulsky adult for 50 ppm of OEG fumigation on 8.9 tonnes of maize at 72 h of treatment.

In the second phase of the study, fumigant toxicity of combination of GEO+OEG (225 µL/kg of GEO and 1.0 g/h flow rate of OEG) was evaluated against *S. oryzae* adults with 1 kg of wheat grain at 0.25, 0.50 and 1.00 h of fumigation exposures. In GEO and OEG alone treatments, mortalities were not observed for 0.25 and 0.50 h fumigation exposures up to 72 h of treatment duration (Fig. 1c). For 1 h fumigation exposure, 30 and 100% mortality rates were recorded for GEO and OEG alone treatments at 72 h treatment duration. Fumigant toxicity of combination of GEO+OEG was directly proportional to the fumigation exposures and treatment durations (Fig. 1c). Remarkably, 100% mortality of *S. oryzae* adult was recorded for 1 h of fumigation of GEO+OEG combination and OEG alone at 72 h of treatment. Interestingly, fumigant toxicity of GEO (225 µL/kg) was increased with the combination of OEG (1.0 g/h flow rate) treatment and 96.66% of mortality of *S. oryzae* adult was recorded within 48 h of treatment period compared to GEO and OEG alone treatments with the significance of $P \leq 0.05$. About 0.28 and 0.74 h of least LT_{50} & LT_{90} values were recorded for GEO+OEG combination at 72 h of treatment duration, respectively (Fig. 1c).

Previously, Işikber (2010) studied the combined effect of GEO with CO_2 treatment and 98.3% of mortality of *Tribolium confusum* adult with 0.38 µL/L of LC_{50} value was recorded within 24 h of exposure period without food. The present study results suggested that GEO+OEG combination treatment was better than GEO and OEG alone treatments against *S. oryzae* adults in with food condition.

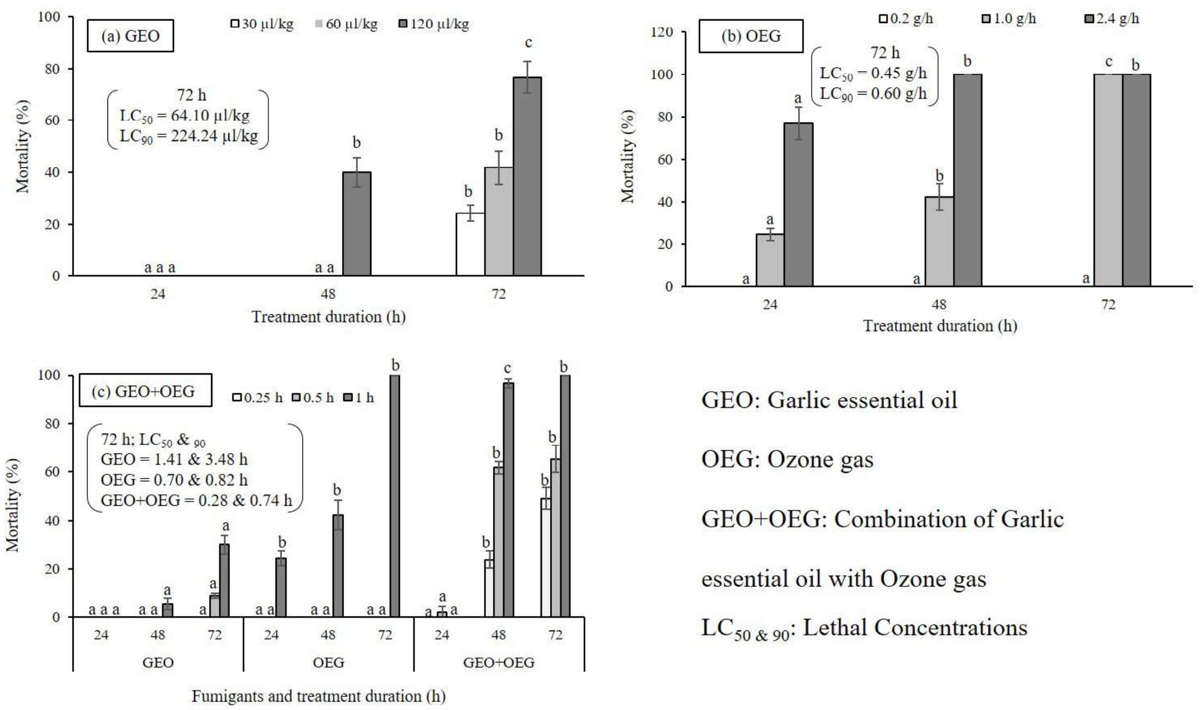

Fig. 1. Mortality of *Sitophilus oryzae* adults due to the fumigation effect of garlic essential oil, ozone gas and combination of garlic essential oil with ozone gas.

Each vertical bar is a mean of three replicates with standard error (% Mean ± SE). (a & b). Means within a concentration (60, 120 and 240 µL/kg) and between treatment duration (24, 48 and 72 h), different letters are significantly ($P \leq 0.05$) different from each other as determined by Tukey's test. (c). Means within a fumigant exposure (0.25, 0.50 and 1.00 h) and between different treatment duration (24, 48 and 72 h), different letters are significantly ($P \leq 0.05$) different from each other as determined by Tukey's test.

In this present study, phytochemical fumigant residues were examined and the diallyl sulfide (DAS), diallyl disulfide (DADS) and diallyl trisulfide (DATS) were detected as major residues in the GEO alone and GEO+OEG combination treated wheat grain and *S. oryzae* adults (Fig. 2 and Table 1). Remarkably, residual level of phytochemicals was decreased (99.97 and 99.98% of DADS and

DATS residues, respectively) in GEO+OEG treated wheat grains compared to GEO alone treated wheat grain. According to Law and Kiss (1991), OEG may not sediment as fumigant residue on the surface of fumigated samples and hence fumigant residues were not examined for OEG treatment in the present study.

The residual property of phytochemical fumigants may be linked to the polar surface area of fumigant molecules (Vendan et al., 2017). Interestingly, in the present study phytochemical residual level was decreased (99.98 and 99.97% of DADS and DATS residues, respectively) in GEO+OEG treated wheat grains when compared to GEO alone treated wheat grains. In *S. oryzae* adults, DADS level was increased from 39.98 peak area (GEO treatment) to 585.27 peak area (GEO+OEG treatment), which might be attributed to the enhanced mortality of *S. oryzae* adults. More recently, it was reported that phytochemical fumigant persistence on the body surface of *S. oryzae* adults was positively correlated with the fumigant toxicity and fumigation exposure (Sahu et al., 2021).

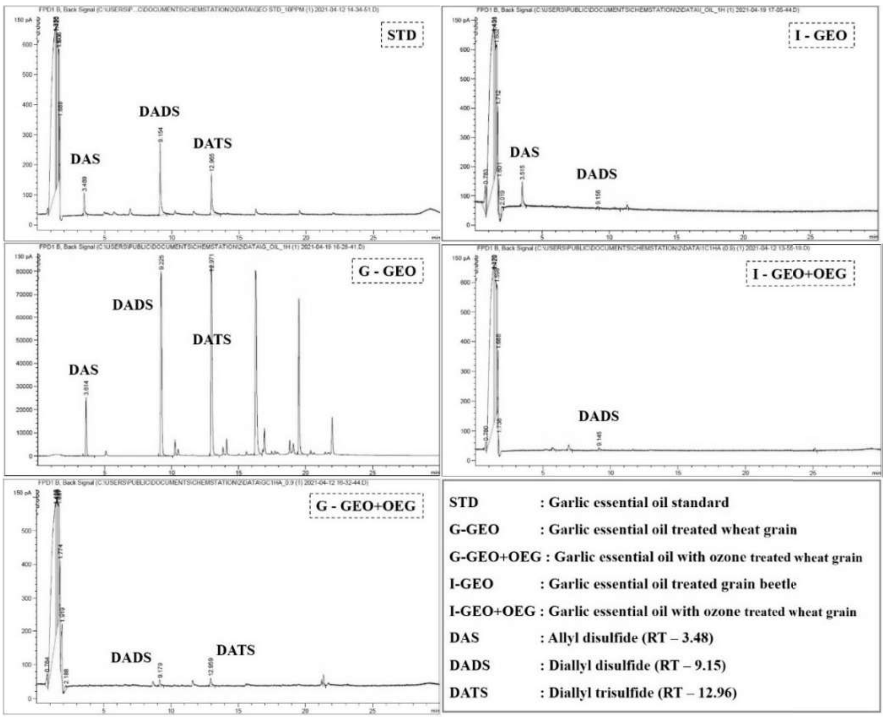

Fig. 2. GC-FPD chromatograms of phytochemical residues detected from the treated wheat grain and *Sitophilus oryzae* adults.

Table 1. Phytochemical residual level on treated wheat and *Sitophilus oryzae* adults.

RT (min)	Compound	Residue Level (Peak area#)					
		GEO		OEG		GEO + OEG	
		Grain	Insect	Grain	Insect	Grain	Insect
3.48	Diallyl sulfide	532.44	46.90	nt	nt	-	-
9.15	Diallyl disulfide	2572.54	39.98	nt	nt	0.61	585.27
12.96	Diallyl trisulfide	2812.55	-	nt	nt	0.78	-

RT= Retention Time, GEO= Garlic essential oil, OEG= Ozone gas, GEO+OEG= Combination of Garlic essential oil with Ozone gas. #Peak area (pA*s) per gram of wheat grain and beetle, nt= not tested.

Conclusion

The current study revealed that the fumigant toxicity of GEO against *S. oryzae* adults had significantly increased during the combination treatment of GEO+OEG. Additionally, the residual level of phytochemical fumigants was reduced in GEO+OEG treatment as compared to GEO alone treatment. It was also noted that the trace levels of DAS, DADS and DATS residues detected in the treated wheat grain were safe for consumption with reference to GRAS status. This study results could be used for the further studies for the development of environment-friendly bio-fumigant process for the safe storage of food grains from insect pests.

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References

Abbott WS (1925) A method of computing the effectiveness of an insecticide. J Econ Entomol **18**(2): 265-267.

Chaubey MK (2016) Fumigant and contact toxicity of *Allium sativum* (Alliaceae) essential oil against *Sitophilus oryzae* L. (Coleoptera: Dryophthoridae). Entomol Appl Sci Letters **3**(2): 43-48.

Cullen PJ, Tiwari BK, O'Donnell CP, Muthukumarappan K (2009) Modelling approaches to ozone processing of liquid foods. Trends Food Sci Technol **20**(3-4): 125-136.

Dambolena JS, Zunino MP, Herrera JM, Pizzolitto RP, Areco VA, Zygadlo JA (2016) Terpenes: natural products for controlling insects of importance to human health—a structure-activity relationship study. Psyche Pp.1-17.

Demeter S, Lebbe O, Hecq F, Nicolis SC, Kemene TK, Martin H, Fauconnier M-L, Hance T (2021) Insecticidal activity of 25 essential oils on the stored product pest, *Sitophilus granarius*. Foods **10**(2): 200.

Finney DJ (1971) Probit analysis. a statistical treatment of the sigmoid response curve. Cambridge University Press: London, UK. 333 p.

Graham DM (1997) Use of ozone for food processing. Food Technol **51**(6): 72-75.

Işikber AA (2010) Fumigant toxicity of garlic essential oil in combination with carbon dioxide (CO_2) against stored-product insects. Proc 10th Intern Working Conf on Stored Prod Prot, Julius-Kühn-Archive **425**: 371-376.

Işkiber AA, Öztekin S (2009) Comparison of susceptibility of two stored-product insects, *Ephestia kuehniella* Zeller and *Tribolium confusum* du Val to gaseous ozone. J Stored Prod Res **45**(3): 159-164.

Kells SA, Mason LJ, Maier DE, Woloshuk CP (2001) Efficacy and fumigation characteristics of ozone in stored maize. J Stored Prod Res **37**(4): 371-382.

Law SE, Kiss EG (1991) Instrumentation for ozone-based insect control in agriculture. Automated Agriculture for the 21st Century: Proc of the 1991 Symposium. Chicago, IL, USA.

Mishra G, Palle AA, Srivastava S, Mishra HN (2019) Disinfestation of stored wheat grain infested with *Rhyzopertha dominica* by ozone treatment: process optimization and impact on grain properties. J Sci Food Agric **99**(11): 5008-5018.

Rajendran S, Sriranjini V (2008) Plant products as fumigants for stored-product insect control. J Stored Prod Res **44**: 126-135.

Sahu U, Ibrahim SS, Vendan SE (2021). Persistence and ingestion characteristics of phytochemical volatiles as bio-fumigants in *Sitophilus oryzae* adults. Ecotoxicol Environ Saf **210**: 111877.

Vendan SE, Manivannan S, Sunny AM, Murugesan P (2017) Phytochemical residue profiles in rice grains fumigated with essential oils for the control of rice weevil. PLoS ONE **12**(10): 1-17 e0186020.