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EVALUATION OF HEAT-TREATED TOMATO SEEDLINGS AS AN ECO-FRIENDLY INSECTICIDE OF THE RICE WEEVIL, *SITOPHILUS ORYZAE*

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ABSTRACT

Solanum lycopersicum L., or tomatoes, are cultivated and consumed all over the year and are regarded as the second-most significant crop in the world. *S. lycopersicum* is vulnerable to insect pests, and to extreme climatic fluctuations including heat-stress. In the current study, chemical composition and insecticidal effect was investigated in the leaves of heat-shocked *S. lycopersicum* seedlings. Tomato seedlings were subjected to heat shock at 40℃; (HS) in comparison to control at 22℃; (Con). Under the two heat regimens, leaves were extracted by ethanol. The levels of secondary metabolites (SMs) using gas chromatography-mass spectrometry (GC-MS), and insecticidal activity against the rice weevil, *Sitophilus oryzae* using fumigation assay were measured. Data were statistically manipulated. For Con and HS, GC-MS tests identified 11 and 18 molecules, respectively. Additionally, responses of tomato plant to heat shock (HS) resulted in more SMs compounds than in the case of control plants (Con). Out of 18 compounds, 13 were detected in HS but not in Con extracts. HS extract showed more insecticidal activity (100% mortality at 5000 ppm at day 1) than Con extract (60% mortality at 5000 ppm at day 1) against *S. oryzae* using fumigation assay. Strong positive correlation between mortality and concentration (R^2= *0.95*) was demonstrated. LC_{50}'s were estimated at 1-, 2- and 3-days post-exposure (DPE). Based on LC_{50} values, HS extract exhibited more activity (39.52 and 26.85 ppm) than Con extract (1687.82 and 208.61 ppm) at 1 and 2 DPE, respectively. However, the difference was insignificant at 3 DPE (10.79 for HS and 11.08 for Con). The insecticidal effect was attributed to the bioactive compounds identified by GC-MS (methyl ketones and phthalic acid esters). Both Con and HS extracts could be regarded safer substitution of the conventional hazardous insecticides against *S. oryzae*, and candidates for IPM programs, too. Additional research is recommended to reveal issues related to the potential environmental impacts, human and animal health, method of application, mode of action and the pathway of tomato leaf extracts, as well.

Keywords: *Solanum lycopersicum*, tomatoes, climatic changes, secondary metabolites, heat shock, insecticidal activity.

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INTRODUCTION

Insect infestation of stored agricultural products causes considerable quantitative damage (Gabarty and El Nour, 2016; Bharathi *et al.*, 2017; Taddese *et al.*, 2020). Food and Agriculture Organization (FAO) documented that 17% of food production is lost within the store-life period. Ten percent is devastated by insects, and 7% by rodents, mites, and plant diseases (Bouchelos, 2018). The postharvest damage caused by insects is estimated up to 20% and 9% in developing and developed countries, respectively (Shankar and Abrol, 2012). Besides the quantitative and qualitative damages (protein, amino-acid, carbohydrates and fats changes), human health risks caused by aflatoxin-producing fungi are major challenges (Kumar *et al.*, 2020; Stathers *et al.*, 2020; Liu *et al.*, 2021).

The lesser rice weevil, *Sitophilus oryzae* is a major pest of stored grains and cereal-based products within the store-life period (Saroukolai *et al.*, 2010). Both adult and larvae of *S. oryzae* can infest a wide variety of grains, including rice (*Oryza sativa*), wheat (*Triticum aestivum*), maize (*Zea mays*), barley (*Hordeum vulgare*), and sorghum (*Sorghum bicolor*), both in the field and during storage (Roy *et al.*, 2024; Syanizam *et al.*, 2024). Larvae preferentially feed on the grain germ, consuming vital proteins and vitamins, while adults primarily consume the endosperm, reducing carbohydrate content (Akbar *et al.*, 2021) and may cause up to 80% damage under prolonged storage (Roy *et al.*, 2024).

Traditional insect control in stored products includes plant-derived materials and widespread chemical insecticides (Shankar and Abrol, 2012; Kedia *et al.*, 2015). However, the overuse of chemical insecticides poses significant risks, including accumulation in ecosystems, resistance development, and carcinogenic

effects (Shankar and Abrol, 2012; Mahanta *et al.*, 2021; Rani *et al.*, 2021). Consequently, there is an urgent global need for eco-friendly, natural, and non-persistent insecticidal alternatives for stored grains (Ebadollahi *et al.*, 2021; Narayanankutty *et al.*, 2021; Garrido-Miranda *et al.*, 2022).

Plant-derived extracts, secondary metabolites (SMs), and microbial enzymes offer eco-friendly insecticidal alternatives to synthetic pesticides, proving selective, potent, and less likely to cause resistance or harm non-target organisms (Tozlu *et al.*, 2011; Batiha *et al.*, 2021). Confirmed examples include chinaberry (*Melia azedarach*), tobacco (*Nicotiana tabacum*), aztec tobacco (*N. rustica*) (Shah and Qadir, 2014; Androutsopoulou *et al.*, 2021; Ghoneim *et al.*, 2021), English lavender (*Lavandula angustifolia*), Aleppo Pine (*Pinus halpensis*), French marigold (*Tagetes patula*) and garlic (*Allium sativum*) (Mobki *et al.*, 2014). Additionally, botanical essential oils (EOs) are recognized as biodegradable, low-toxicity compounds with diverse insecticidal actions including fumigant, contact, repellent, antioxidant, and antifeedant properties (Hashem *et al.*, 2018; Ju *et al.*, 2019; El-Refai *et al.*, 2020; Mehta and Kumar, 2020; Azeem *et al.*, 2022; Visakh *et al.*, 2022).

Global tomato production and consumption significantly increased between 1978-2008 due to its nutritional value (FAO, 2008; Razifard *et al.*, 2020), leading to substantial agricultural by-products like leaves and seedlings (Colvine, 2008; FAOSTAT, 2022). While some studies show insecticidal effects of tomato extracts against various pests (Ghada *et al.*, 2017; Spring *et al.*, 2022; Setyaningrum *et al.*, 2023), research on heat-treated tomato seedling extracts for stored-product insects is lacking, despite our prior work showing increased phenolics, flavonoids, and antioxidant activity in heat-stressed tomato seedlings (Alhaithloul *et al.*, 2021).

Therefore, this study aimed to determine if heat stress enhances tomato seedlings to secrete volatile secondary metabolites (SMs) with insecticidal activity against adult *S. oryzae*. All evaluations were conducted on tomato seedlings, *S. lycopersicum* because the heat-stress might have a maximum impact on the young plants, leading to induction of potent volatile bioactive secretions with insecticidal properties. Herein, tomato seedlings were heat-treated, their phytochemicals identified via GC-MS, and the insecticidal potency of their ethanol extracts was evaluated against *S. oryzae* adults under laboratory conditions.

MATERIALS AND METHODS

Colonization of *Sitophilus oryzae*: A colony of *S. oryzae* was originally collected from infested grains of wheat, and established under laboratory conditions at the Biology Department, College of Science, Jouf University, Saudi Arabia. Rearing incubator was adjusted to temperature of 28±2 °C and relative humidity of 65±5%. Fifty pairs of one to two-day-old adult insects were placed in a jar with one kilogram of their food grains. To encourage oviposition and mating, the jars were kept within the incubator for a maximum of seven days. To re-infest fresh wheat seeds, the parental adults were removed, and the remaining diet with eggs were placed in new jars with fresh seeds. Each new jar was covered with a piece of mesh and secured with rubber bands to keep emerging insects within the jars. For all experiments, the new emerging beetles were utilized (Rani, 2012).

Plant materials: Twenty pots of 35 days-old seedlings of the tomato, *S. lycopersicum* (cultivar GS12 F1), were thermally treated as will be explained in experimental design. Tomato seeds were purchased from Nur-Sultan company (AstanaAgroA, Kazakhstan). Germination and acclimatization conditions of the seeds were done following Alhaithloul *et al.* (2021).

Experimental design: The experiment layout was designed as: 3 (replicates)* 10 (pots/ replicate)* 3 (times repetition). Each ten of the twenty tomato seedling pots (one seedling each) were exposed to a different heat treatment: ten pots were heat-shocked for one hour each day for seven days at 40 °C (HS), while the other ten were grown at 22 °C as a control group (Con). Heat regimens (Con and HS) were applied by keeping seedlings within digital incubators (Thomas Scientific Inc., USA), which were adjusted to the requested time intervals and temperatures. Before and after thermal exposure, seedlings were kept under controlled growing chamber. Each experiment was repeated thrice with three biological replicates (Alhaithloul *et al.*, 2021).

Extract preparation: Seedling leaves of the two experimental groups have been, immediately, processed at the end of the stress (42 days-old). Leaves were frozen using liquid nitrogen and ground into powder using laboratory mill. For each treatment, ≈100 g powder from each biological replicate was extracted with 200 mL of 80% ethanol (Sigma–Aldrich, Saint Louis, MO, USA). This mixture was then stirred at room temperature for three days. Each separate mixture was filtered using Whatman (No. 1) filter paper. The filtrates from all replicates (6 replicates) were then concentrated using a speed vacuum (Modulspin 31, Biotron, Seoul, Korea). The resulting crude ethanol extracts were stored in sealed, dark vials at 4°C until used.

Phytochemical analysis using GC-MS: As previously noted, the GC-MS methodology was utilized to analyze phytochemical components of *S. lycopersicum* seedling leaves (Visakh *et al.*, 2023). In brief, TSQ 8000 Evo system (Thermo Fisher Scientific, Waltham, MA, USA) was employed, including a TG-1MS capillary column and an autosampler, utilizing helium gas as a carrier. The

sample was introduced using 1: 200 as the split ratio. The obtained mass spectra were fully analyzed with the Xcalibur 1.1 software. After that, each component was identified by cross-referencing it with the NIST database to allow for a thorough understanding of the phytochemical composition. Mass spectra were scanned between 35 and 500 m/z as the temperature was gradually increased. Subsequently, the relative percentages were determined by measuring the peak area of every component.

Fumigant insecticidal toxicity: Fumigation assay was employed because the GC-MS results confirmed the presence of many volatile (VOCs) and semi-volatile (SVOCs) compounds in tomato extracts. Consequently, it is logically to link mortality results to the presence of those VOCs and SVOCs detected by GC-MS. The fumigant toxicity assay against adult *S. oryzae* was performed following Germinara *et al.* (2012). Briefly, 250, 500, 1000, 1500, 2500, and 5000 ppm concentrations were prepared and tested for each extract. Untreated paper disc was used as control. A 2.0-cm filter-paper disc, treated with the required concentration, was hanged in the center of the fumigation chamber (tightly-capped 500 mL glass jar) by an iron wire. Before capping, fifteen adult insects were put inside the chamber and 50 g of intact-kernel wheat were placed at chamber's base. Bioassays were performed in the fumigation chambers at 26±2 °C and 60±5% relative humidity. Adult mortality statistics and percentages were noted at 1-, 2-, and 3-days post-exposure (DPE). Insect was calculated dead if it exhibited no movement after exposed to fresh air for 12 hours. Each experiment was replicated five times.

Data analyses: LC_{10}, LC_{50} and LC_{90} values of the fumigation bioassays were determined by probit analysis. One-Way Analysis of Variance (ANOVA) and Duncan's post-hoc pairwise analyses were employed for testing the results of fumigation phytotoxicity assays. *S-student* test was performed to compare between the number and area of compounds in the case of Con and HS extracts. All analyses were performed by using SPSS version 26 software.

RESULTS

Phytochemical analysis using GC-MS: All resolved signals from the GC-MS chromatograms of the extracts of *S. lycopersicum* seedlings are shown in Table 1. The main chemicals found in Con extract were 1,3-Propanediol, 2-(hydroxymethyl)-2-nitro (44.16%), Diethyl Phthalate (26.70%), Oxirane, 2,3-dimethyl (14.10%), Acetic anhydride (4.30%), and 2-Propanone, 1,1-dichloro (3.44%). In the meantime, the main compounds found in HS extract were as follows: 2-Propyl-tetrahydropyran-3-ol (19.11%), 1,3-Propanediol, 2-(hydroxymethyl)-2-nitro (17.80%), cis-Vaccenic acid (8.20%), 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl)-ester (6.10%), Dibutyl phthalate (6.03%), Diethyl Phthalate (5.90%), Acetic anhydride (5.02%), and 2-Furancarboxaldehyde, 5-(hydroxymethyl) (3.10%). Eight compounds with previous reported insecticidal activities were resolved from the Con and HS extracts. These compounds are chemically classified as methyl ketones or phthalic acid esters (Table 1).

In the case of the Con and HS extracts, respectively, 11 and 18 compounds were identified out of the total detectable peaks (27 and 41) of the GC-MS chromatograms. Out of the 18 compounds of HS extract, 13 were specific to HS but not detected in Con extract. Meanwhile, out of the 11 compounds of Con extract, 6 were specific to Con but not detected in HS extract. Five compounds were extracted from both HS and Con extracts. Out of these 5 compounds, concentration of 3 (Acetic anhydride, Tridecanoic acid, methyl ester and n-Hexadecanoic acid) was higher, and concentration of 2 (1,3-Propanediol, 2-(hydroxymethyl)-2-nitro and Diethyl Phthalate) was lower in HS than in Con. Statistically, there was a substantial variation ($P<0.001$) in both the number and the total estimated area of the detected compounds. In the meantime, there was a marginally positive correlation found in both situations with respect to heat change ($R^2= 0.08$ for number, $R^2= 0.04$ for area). For the Con and HS extracts, the separated signals' retention times ranged from 7.9 to 16.5 and 8.8 to 23.3 minutes, respectively (Table 1).

Fumigant insecticidal toxicity: Mortality of adult *S. oryzae* observed by fumigation bioassay using seven concentrations of Con extract at 3 successive DPE is summarized in Table 2. In general, higher concentrations and longer exposure times were associated with higher death rates. When compared to control, there was a significant increase ($P<0.00$) in the percentage mortalities of adult *S. oryzae* at 1, 2 and 3 DPE for all used concentrations of the Con extract. Pairwise comparisons between differences in mortalities resulted by the different concentrations of Con extract at 1, 2 and 3 DPE are indicated by the superscript letters in Table 2. At 1 DPE, concentrations 1000, 1500, 2500 and 5000 ppm resulted in significantly higher mortalities ($P<0.001$) than 250 and 500 ppm. Meanwhile, the concentration 5000 ppm resulted in significantly higher mortalities ($P<0.001$) than 1000 and 1500 ppm. At 5000 ppm of Con extract, the highest percentage of mortalities were observed, displaying 60, 84.33, and 93.67% mortality at 1, 2 and 3 DPE, respectively (Table 2).

The adult *S. oryzae* mortality caused by fumigation bioassay using seven concentrations of HS extract at 3 successive DPE is summarized in Table 3. In general, higher concentrations and longer exposure times were associated with higher death rates. Using the seven

HS extract concentrations, the same significant pattern ($P< 0.001$) of mortality in adult *S. oryzae* was reiterated at 1, 2 and 3 DPE when compared to control. Pairwise comparisons between differences in mortality resulted by the different concentrations of HS extract at 1, 2 and 3 DPE are indicated by the superscript letters in Table 3. By application of the concentration of 250 ppm of HS extract, 80, 84 and 88% mortalities were recorded at 1, 2 and 3 DPE, respectively. However, 100% mortality was achieved by applying 5000 ppm of HS extract at 1, 2 and 3 DPE (Table 3).

Additionally, LC_{50} and LC_{90} values of both Con and HS extracts were estimated against *S. oryzae* at 1, 2 and 3 DPE (Table 4). Based on the 95% confidence limits interference, significant differences of both LC_{50} and LC_{90} values between Con and HS extracts against *S. oryzae* were recorded at both 1 and 2 DPE (Table 4). However, insignificant differences of both LC_{50} and LC_{90} values between Con and HS extracts against *S. oryzae* were recorded at 3 DPE (Table 4).

Table 1. Total volatile compounds identified by GC-MS analyses of the extracts of heat treated tomato seedlings.

Sr. No.	Compounds	RT in min		Area (mAU*s)		Concentration (%)	
		Con	HS	Con	HS	Con	HS
1	2-Propanone, 1,1-dichloro-*	7.999	ND	39670	ND	3.44	ND
2	Oxirane, 2,3-dimethyl-	8.780	ND	162585	ND	14.10	ND
3	2-Propyl-tetrahydropyran-3-ol	ND	8.783	ND	546605	ND	19.11
4	2-Furancarboxaldehyde, 5-(hydroxymethyl)-*	ND	9.615	ND	88859	ND	3.10
5	Acetic anhydride	9.778	9.777	49616	145212	4.30	5.02
6	Iberin nitrile	ND	11.157	ND	38990	ND	1.40
7	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	11.740	11.783	509140	513032	44.16	17.80
8	Diethyl Phthalate**	ND	12.149	ND	439131	ND	15.20
9	1,5-Diazocine, octahydro-1,5-dinitroso-	ND	12.386	ND	11305	ND	0.40
10	Diethyl Phthalate**	12.934	12.929	307872	169751	26.70	5.90
11	α-D-Mannopyranoside, methyl 3,6-anhydro-	13.032	ND	17284	ND	1.50	ND
12	Isoamyl nitrite	13.223	ND	16342	ND	1.42	ND
13	Tridecanoic acid, methyl ester	15.228	15.229	11880	32510	1.03	1.13
14	n-Hexadecanoic acid	15.476	15.479	5793	82273	0.50	2.90
15	Dibutyl phthalate**	ND	15.615	ND	174251	ND	6.03
16	Phthalic acid, cyclobutyl isobutyl ester**	15.632	ND	25753	ND	2.23	ND
17	Oxacycloheptadec-8-en-2-one*	ND	16.544	ND	28016	ND	1.00
18	9,15-Octadecadienoic acid, methyl ester, (Z,Z)-	16.548	ND	6972	ND	0.61	ND
19	Methyl (Z)-5,11,14,17-eicosatetraenoate	ND	16.620	ND	19481	ND	0.70
20	Phytol	ND	16.706	ND	34210	ND	1.20
21	cis-Vaccenic acid	ND	16.944	ND	236314	ND	8.20
22	3-Heptadecen-5-yne, (Z)-	ND	17.002	ND	68438	ND	2.40
23	Octadecanoic acid	ND	17.112	ND	40353	ND	1.42
24	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester**	ND	23.295	ND	174998	ND	6.10

RT: Retention time; ND: Not detected by the used column; *: Methyl ketone; **: Phthalic acid ester.

Table 2. Percentage mortality of *S. oryzae* adults following fumigation bioassay of ethanol extract of *S. lycopersicum* seedlings treated with 22 °C

Conc ppm/L air	% Mortality of *S. oryzae*		
	Day 1	Day 2	Day 3
Control	$0.00 \pm 0.00^{a*}$	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
250	30.00 ± 3.00^{b}	53.33 ± 3.06^{b}	78.00 ± 3.00^{b}
500	30.00 ± 5.00^{b}	58.33 ± 2.08^{b}	83.67 ± 2.52^{c}
1000	50.00 ± 5.00^{c}	71.33 ± 2.08^{c}	85.67 ± 2.52^{cd}
1500	50.00 ± 3.00^{c}	75.00 ± 5.00^{cd}	87.67 ± 2.52^{cd}
2500	55.00 ± 5.00^{cd}	78.00 ± 2.00^{d}	90.33 ± 3.06^{de}
5000	60.00 ± 3.00^{d}	84.33 ± 4.04^{e}	93.67 ± 3.22^{e}

Conc: extract concentration; *: Difference between means within the column with the same letter are not significant at $P\leq 0.05$ (Duncan's post-hoc tests).

Table 3. Percentage mortality of *S. oryzae* adults following fumigation bioassay of ethanol extract of *S. lycopersicum* seedlings treated with 40 °C.

Conc	% Mortality of *S. oryzae*		
ppm/L air	Day 1	Day 2	Day 3
Control	0.00 ± 0.00[a*]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
250	80.00 ± 5.00[b]	84.00 ± 4.00[b]	88.00 ± 2.00[b]
500	80.00 ± 8.00[b]	85.00 ± 3.00[b]	91.00 ± 4.00[b]
1000	90.00 ± 6.00[c]	93.00 ± 3.00[c]	94.00 ± 4.00[bc]
1500	90.00 ± 5.00[c]	93.67± 6.66[cd]	96.67 ± 2.52[bc]
2500	95.67 ± 4.93[cd]	97.00 ± 3.00[cd]	99.00 ± 1.00[c]
5000	100.00 ± 0.00[d]	100.00 ± 0.00[d]	100.00 ± 0.00[c]

Conc: extract concentration; [*]: Difference between means within the column with the same letter are not significant at $P \leq 0.05$ (Duncan's post-hoc tests).

Table 4. LC values of ethanol extracts of heat-treated *S. lycopersicum* seedlings against *S. oryzae* adults following fumigation bioassay.

Temperature	DPE[*]	LC_{50}	95% confidence limits		LC_{90}	95% confidence limits	
			Lower	Upper		Lower	Upper
22 °C	1	1687.82[a]	556.56	5118.44	140773.37[a]	46420.299	426906.80
40 °C		39.52[b]	12.85	121.49	1087.23[b]	353.66	3342.41
22 °C	2	208.61[a]	71.82	605.96	11622.22[a]	4001.15	33759.28
40 °C		26.85[b]	8.009	89.97	664.95[b]	198.4	2228.63
22 °C	3	11.08[a]	1.88	54.05	2056.79[a]	383.61	11027.75
40 °C		10.79[a]	2.78	50.005	380.26[a]	89.66	1612.76

[*]DPE: days post-exposure; LC_{50} and LC_{90}: the concentrations causing 50 and 90% mortality of *S. oryzae*. LC_{50} and LC_{90} with the same letter are insignificant in the same DPE (based on the interference of 95% confidence limits).

DISCUSSION

It has been demonstrated that a wide variety of chemicals derived from plants have insecticidal effects which makes them useful in integrated pest management (IPM) programs of stored-product insects (Abdelgaleil *et al.*, 2016; Abdelgaleil *et al.*, 2022). These compounds could be beneficial to their pest selectivity and negligible impact on the ecosystem and non-target organisms (Saroukolai *et al.*, 2010). Moreover, a large number of these compounds have little mammalian toxicity and may be biodegraded by soil microbes (Krzyzowski *et al.*, 2020). Plant-derived extracts and their main components complement each other as effective tools for managing pests, especially stored product ones (Zapata and Smagghe, 2010). Herein, seedlings of heat-treated *S. lycopersicum* were extracted in ethanol, characterized using GC-MS, and tested against *S. oryzae* through fumigation bioassay under controlled laboratory settings. The primary shared components identified in the two extracts were 1,3-Propanediol,2-(hydroxymethyl)-2-nitro (44.16 and 17.80%), Diethyl Phthalate (26.70 and 5.90%) and Acetic anhydride (4.30 and 5.02%) in the case of Con and HS extracts, respectively. Additionally, some treatment-specific compounds were identified (Oxirane, 2,3-dimethyl (14.10%) and 2-Propanone, 1,1-dichloro (3.44%) in the case of Con extract, and 2-Propyl-tetrahydropyran-3-ol (19.11%), cis-Vaccenic acid (8.20%), 1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl)-ester (6.10%), Dibutyl phthalate (6.03%), and 2-Furancarboxaldehyde, 5-(hydroxymethyl) (3.10%) in the case of HS extract. The chemical composition of *S. lycopersicum* extracts, partly, resembled those previously documented for *S. lycopersicum* grown worldwide. Nonetheless, there were some variations in the constituent compounds and percentages. Numerous factors, including location, timing of the collection, climate, environment, plant nutrition, solvent, and extraction technique, could be responsible for this variation (Talano *et al.*, 2003; Camejo *et al.*, 2005; Oller *et al.*, 2005; Camejo *et al.*, 2006; Chang *et al.*, 2006; Camejo *et al.*, 2007; Carvalho *et al.*, 2011; Singh *et al.*, 2014; Santoso *et al.*, 2018; Alhaithloul *et al.*, 2021; Setyaningrum *et al.*, 2023; Shubham *et al.*, 2023). Both number and area of SM tend to increase in respect to temperature increase.

Our study demonstrated that both Con and HS extracts were toxic to *S. oryzae* using fumigation assay. HS extract was more efficacious against *S. oryzae* (LC_{50}= 39.52, 26.85 and 10.79 at 1, 2 and 3 DPE) than Con extract (LC_{50}= 1687.82, 208.61 and 11.08 at 1, 2 and 3 DPE). This higher insecticidal activity could be attributed to the higher content of both methyl ketones (4.1%) and

phthalic acid esters (33.23%) in heat-shocked tomato leaves than in control (3.44 and 28.93%, for methyl ketones and phthalic acid esters, respectively). Additionally, this toxicity was concentration and exposure-time dependent in both extracts. We did not compare Con and HS extracts to conventional insecticides because we believed that the comparison with such hazardous insecticides is not useful due to: (i) stored food products which will be consumed by humans and animals, (ii) 100% mortality was obtained at 1 DPE by the concentration of 5000 ppm of HS extract. Similar results were presented by Ghada *et al.* (2017) who demonstrated that tomato leaf extract exhibited a direct relationship between its concentration and mortality against *Aphis gossypii*, with estimated LC_{50} and LC_{90} values of 439.89 ppm and 1483.36 ppm, respectively. Different fractions of methanolic extract of tomato leaves were used against the brine shrimp (*Artemia* sp.), and demonstrated strong positive correlation between mortality and concentration (R^2= *0.95*). LC_{50} were estimated between 0.69 and 205.79 µg/ml for dichloromethane and aqueous partitions, respectively (Afreen *et al.*, 2016). Additionally, ethanolic tomato leaves extract was reported to possess repellent action against the mosquito, *Aedes aegypti* with a conclusion of using this extract in mosquito repellent lotion (Setyaningrum *et al.*, 2023). Setyaningrum *et al.* (2023) explained this behavior by pointing to the tomato leaf extract's alkaloids and saponins. Alkaloids were reported as insect nerve toxins, metamorphosis inhibitor, and possessing an insect-repellent odor (Aseptianova *et al.*, 2017; Santoso *et al.*, 2018). Meanwhile, saponins are insect stomach toxins causing troubles in the insect digestion and absorption (Santoso *et al.*, 2018). Furthermore, a plant extract with a high flavonoid and triterpene content demonstrated antifeeding effect against the fifth larval instar of *Ctenopsteustis obliquana* (Thoison *et al.*, 2004). At 6 DPE, a biologically active phenolic extract of tomato hairy roots (100 µl/g) demonstrated mortality of 40.0% against *S. litura* and 53.34% against *H. armigera* (Singh *et al.*, 2014). Furthermore, aqueous extract of tomato leaves was found very efficacious against the 4 larval instars of *A. aegypti* mosquitoes at 1, 2 and 3 DPE (Spring *et al.*, 2022). This toxicity was attributed to the presence of bioactive compounds like phenolics (Spring *et al.*, 2022). Many publications reported that the potential insecticidal activity of tomato leaves extracts is due to their bioactive compounds (Trease and Evans 1996; Kishore *et al.*, 2011; Raiola *et al.*, 2014). In the present study several compounds with already reported insecticidal effect have been identified in the tomato extracts such as: methyl ketones (2-Propanone, 1,1-dichloro, 2-Furancarboxaldehyde, 5-(hydroxymethyl), and Oxacycloheptadec-8-en-2-one) and phthalic acid esters (Diethyl Phthalate, Dibutyl phthalate, Phthalic acid, cyclobutyl isobutyl ester, and 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester).

By extrapolating the results of the current research and the previous research, it is clear that the insecticidal efficacy of tomato leaf extracts is possibly, due to the presence of a variety of bioactive substances (Jiang *et al.*, 2012), rather than their repellent smell (Aseptianova *et al.*, 2017; Santoso *et al.*, 2018). These key compounds include: phenolics (Singh *et al.*, 2014; Spring *et al.*, 2022), alkaloids (Aseptianova *et al.*, 2017; Setyaningrum *et al.*, 2023), saponins (Santoso *et al.*, 2018; Setyaningrum *et al.*, 2023), flavonoids and triterpenes (Thoison *et al.*, 2004), methyl ketones (Antonious, 2016), and phthalic acid esters (Roy, 2020; Huang *et al.*, 2021). These compounds harm insects in several ways: Phthalic acid esters disrupt the acetylcholinesterase enzyme (Xu and He, 2010; Roy, 2020; Huang *et al.*, 2021), methyl ketones interfere with both the cholinergic system (*e.g.* acetylcholinesterase and nicotinic acetylcholine receptors) and the octopaminergic system (by affecting octopaminergic and tyramine receptors) (Isman, 2006; Rattan, 2010), and flavonoids and triterpenes act as antifeedants, stopping insects from eating (Thoison *et al.*, 2004). Additionally, alkaloids are nerve toxins, metamorphosis inhibitors, and insect repellents, as well (Aseptianova *et al.*, 2017; Santoso *et al.*, 2018). Moreover, saponins are well-known insect stomach toxins (Santoso *et al.*, 2018).

Conclusion: Herein, we reported that heat-shocked (HS) extract of *S. lycopersicum* was more efficacious than Con (22 °C) extract against *S. oryzae*, as fumigant agents at 1, 2 and 3 DPE. This toxicity was concentration and exposure-time dependent. Toxicity of these extracts were explained by the bioactive substances identified by GC-MS in the extracts (*e.g.* methyl ketones and phthalic acid esters).

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