Bioefficacy of organic extracts of *A. sativum* against *S. zeamais* (Coleoptera; Dryophthoridae)

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Abstract

Laboratory based experiments were carried out to determine the fumigant toxicities, repellent and progeny emergence inhibition activities of Methanolic, Hexane and Methanolic: Hexane blend extracts of *Allium sativum* on *Sítophilus zeamais* (Coleoptera: Dryophthoridae). Four different concentrations (25%, 50%, 75% and 100%) of the extracts were tested. The fumigant toxicities of the *A. sativum* extracts were found to be dependent on both concentration and exposure time. The maximum toxicities (96.15%) was recorded for higher concentration of blend extracts and minimum (6.41%) with hexane extracts of *A. sativum* at lowest concentration of (25%). The three different *A. sativum* extracts recorded significantly good level of repellent activities and reduction in progeny emergence.

Keywords: *Allium sativum*; *Sítophilus zeamais*; Fumigant toxicity; Repellency

Introduction

Maize (*Zea mays* L.) is one of the major world’s leading cereal crops together with wheat and rice. Maize provides carbohydrates, iron, vitamins, proteins and minerals to human body [1]. It is also used as livestock feed and raw material for several processing plants [1,2]. Maize is largely infested with insect pests in the fields and stores. The major pests that cause post-harvest infestation and loss in corn include, the maize weevil (*Sítophilus zeamais*), larger grain borer (*Prostephanus truncatus*) and granary weevil (*Sitophilus granamensis*) [3]. Their damage to stored grains and grain products accounts for 5–10% of the total loss in the temperate zone and approximately 20–30% in the tropical zone [4]. Such damage may reach up to 40%, in countries that have not embraced modern storage technologies [5]. There is increasing interest in utilization of botanical products as a cheaper, sustainable and environmentally friendly means of protecting stored grains from infestation by insects [6]. The objectives of this study were to evaluate the fumigant toxicities, repellent activities and new adult emergence inhibition activities of three different extracts of *A. sativum*. These activities were all carried out with the aim of establishing an alternative plant derived pesticide.

Materials and Methods

Insect culture

Adults of *S. zeamais* were obtained from infested maize grains at Rodi-market in Homa-bay town, Kenya. Whole grains used for culture were first cleaned and disinfected by placing them in a deep freezer at -40°C for 3 days and then air dried for 2 days to prevent moldiness and transferred into two plastic jars. A hundred pairs of *S. zeamais* were introduced into each jar, covered with muslin cloth for seven days to allow oviposition. After seven days, all adults were removed and the set up was then kept for forty nine days at moderate temperature of 26 ± 30°C, relative humidity of 70% and 12:12 h (Light:Darkness) to allow emergence of progenies.

Collection and preparation of plant materials

*Allium sativum* (Garlic) were obtained from Githurai, a local market in Nairobi, Kenya and transported to the Biochemistry and Biotechnology laboratories at Kenyatta University for studies. Garlic cloves were cut into small pieces to increase their surface area for quick drying. The chopped clove pieces were then spread in dark room for two weeks to dry. The dry pieces were subsequently milled using a blender to obtain powder that was used in extraction.

Extraction

Five hundred grams of *A. sativum* powder was put in conical flasks and Seven hundred and fifty millilitres of methanol were added to the conical flask and corked. The mixture was allowed to stand for three days. After soaking, the extracts were filtered into separate labeled containers using Whatmann’s No. 1 filter papers and funnels under vacuum pump pressure. A similar procedure was repeated for hexane extractions. For blend extraction, methanol and hexane were mixed in the ratio 1:1 to make one litre of blend solvent. The extracts were then concentrated at 40°C using rotary evaporator for 8 h.

Preparation of extract concentration

Prior to application, the concentrate were diluted with hexane, methanol and a blend of the two solvents at a concentration of 1 g/ml and this was termed as stock solution (100%) as suggested by Deshmukh and Borie [7] with limited modification. Four different concentrations of the extracts were used in this experiment (25%, 50%, 75% and 100%). The different concentrations were made as follows; 25% extract concentration was prepared by diluting 1 ml stock solution by 3 ml of solvent to make up to 4 ml. The 50% extract concentration was prepared by diluting 2 ml of stock solution by 2 ml of the solvent.
to make up to 4 ml while 75% concentration was prepared by 1 ml of the solvent 3 ml of stock solution added to make up to 4 ml.

**Determination of Fumigant Toxicity**

Fumigant toxicity test was done as follows; twenty grams of maize grains were put into each of the four plastic vials. One milliliters of each of the extracts were then added and the mixture shaken for five minutes to ensure uniform coating of grains. The set up was left for two hours to allow the traces of the solvent to evaporate. Twenty adult *S. zeamais* (2-3 days old) were introduced into each vial and then covered with lid. Several tiny openings were made on the sides of plastic vials to ensure ventilation. In solvent control, twenty grams of maize was treated with pure solvent only and twenty adult insects introduced. For positive control, the same weight of maize grains was treated with a conventional pesticide (*Actellic*) and twenty adult *S. zeamais* introduced. Four replicates were made for each treatment.

The weevil mortality was assessed 6, 24, 48, 60 and 72 h after the insects were introduced. The insects were confirmed dead when there was no response to probing with a sharp pin at the abdomen [8]. Corrected mortality percentages were obtained using Abbott formula [9].

Where $Pr$ represent corrected mortality, $Pt$ represent percentage mortality in various extracts treatments and $Pc$ represent percentage mortality in solvent control.

**Determination of progeny emergence inhibition**

After 96 h duration , both dead and alive insects were removed from the vials and the set up maintained at 26°C ± 3 for 42 more days to check for the emergence of F1 generation. The number of adults that emerged from each vial was counted and recorded. The Percentage Inhibition Rate (% IR) in progeny emergence was calculated using the formula described by Tapandjoo et al. [10].

Where;

$Cn=$number of emerged insects in the control

$Nt=$the number of insects recorded in the treated half

$Nc=$number of insects recorded in the control half

**Determination of repellent activity**

Repellent activity was assessed using the area preference method [11]. Test areas consisted of 10 cm Whatman's No. 1 filter papers cut in half. Each extract solution (1 ml) was applied to a half-filter-paper disc uniformly with a pipette. The other half of the filter paper was either treated with methanol or hexane alone. Extract-treated and control half-discs were then air-dried to evaporate the solvent completely. Full discs were re-made by attaching treated halves to untreated halves of the same dimensions with cello-tape. Each filter paper was then placed in a Petri dish and ten adult weevils released at the center of each filter paper disc and then covered. Four replicate were made for each treatment and number of insects present on treated (Nt) and control (Nc) areas was recorded after one hour for five hours. These numbers were then used to calculate percent repellency of each extract by using the formula described by Thien et al. [12].

Where;

$Cn=$number of emerged insects in the control half

$Nt=$the number of insects recorded in the treated half

**Statistical analysis**

The numerical data on mortality, repellency and new adult emergence were tabulated in Microsoft excel spread sheets then exported to Minvib version 17.0 software and subjected to descriptive statistics. The results obtained were expressed as mean ± standard deviation (SEM). One way analysis of variance (ANOVA) was carried out to obtain statistical significance difference between means of different treatments followed by Tukey’s post hoc test to compare and separate the means. The value $P ≤ 0.05$ was considered significant.

**Results**

**Fumigant toxicity of methanolic extract of *A. sativum* against *S. zeamais***

Methanolic extract of *A. sativum* showed fumigant toxicity on *S. zeamais* as indicated by percentage of corrected mortality (Table 1). The 100% extract concentration showed toxicity of 76.92% within 96 h of insects’ exposure to the extract, while 75% extract concentration killed 65.38% of *S. zeamais* within the same duration of 96 h (Table 1).

The lowest concentration of the extract used in this study (25%) induced no mortality within 6 h after *S. zeamais* were exposed to the extract. This percentage mortality gradually increased to 35.9% after 96 h. When the extract concentration was increased to 50%, mortality increased to 46.2% within 96 h of fumigant toxicity study (Table 1).

The percentage mortality recorded by 25% and 50% extract concentrations were not significantly different for the duration between 6 h and 72 h following *S. zeamais* introduction into the vials (p>0.05; Table 1). Besides, the mortality rate recorded at the 6th hour in all the test concentration (25-100%) demonstrated no significant difference and were comparable to the control at that same hour (p>0.05; Table 1).

### Table 1: Mean % of corrected mortality ± SE with exposure period (hours, h)

<table>
<thead>
<tr>
<th>Concentration extract (%)</th>
<th>Mean % of corrected mortality ± SE with exposure period (hours, h)</th>
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<tbody>
<tr>
<td></td>
<td>6 h</td>
</tr>
<tr>
<td>Control</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>25</td>
<td>0.00 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>50</td>
<td>1.25 ± 1.25&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>75</td>
<td>2.50 ± 2.50&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>100</td>
<td>2.50 ± 1.44&lt;sup&gt;b&lt;/sup&gt;</td>
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</tbody>
</table>
Table 1: Fumigant toxicity of hexane extract of A. sativum on adult S. zeamais, Values followed by the same superscript within the same column are not significantly different by one-way ANOVA (P ≤ 0.05) followed by Tukey’s test.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Mean % corrected mortality ± S.E with exposure period (hours, h)</th>
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<tbody>
<tr>
<td></td>
<td>6 h</td>
</tr>
<tr>
<td>Control</td>
<td>0.00 ± 0.00b</td>
</tr>
<tr>
<td>25% extract</td>
<td>0.00 ± 0.00b</td>
</tr>
<tr>
<td>50% extract</td>
<td>2.50 ± 1.44b</td>
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<tr>
<td>75% extract</td>
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</tr>
<tr>
<td>100% extract</td>
<td>3.75 ± 2.39b</td>
</tr>
<tr>
<td>Actellic</td>
<td>91.25 ± 5.15a</td>
</tr>
</tbody>
</table>

Table 2: Fumigant toxicity of hexane extract of A. sativum on adult S. zeamais, Values followed by the same superscript within the same column are not significantly different by one-way ANOVA (P ≤ 0.05) followed by Tukey’s test.

<table>
<thead>
<tr>
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<th>Mean % corrected mortality ± S.E with exposure period (hours, h)</th>
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<tr>
<td></td>
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<tr>
<td>Control</td>
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<tr>
<td>100% extract</td>
<td>67.50 ± 3.23h</td>
</tr>
<tr>
<td>Actellic</td>
<td>91.25 ± 5.15a</td>
</tr>
</tbody>
</table>

Table 3: Fumigant toxicity of hexane: methanolic blend extract of A. sativum on adult S. zeamais, Values followed by the same superscript within the same column are not significantly different by one-way ANOVA (P ≤ 0.05) followed by Tukey’s test.

Fumigant toxicity of hexane extract of A. sativum against S. zeamais

The hexane extract of A. sativum demonstrated low fumigant toxicity on S. zeamais (Table 2). The results clearly revealed that 25% hexane extract concentration induced only 6.41% mortality rate after 96 h exposure period of S. zeamais to extract components (Table 2). Similarly, low mortality results were also observed with 50% extract concentration within a similar period of 96 h (Table 2). Meanwhile, 19.23% and 24.36% mortality was recorded for 75% and 100% extract concentrations (Table 2). Moreover, there was gradual slight increase in mortality rate with time for every extract concentration that was studied and the results noted for all the extract treatments were significantly lower than actellic (p<0.05; Table 2).

The hexane: methanolic blend extracts of A. sativum generally showed more fumigant toxicity effect on S. zeamais than individual hexane and methanolic extracts of the same plant particularly at high concentrations of the extract (Figures 1 and 2).
Repellent activity of methanolic extract of *A. sativum* on *S. zeamais*

The methanolic extract of *A. sativum* showed repellent activities on adults *S. zeamais* in a dose independent fashion (Table 4). During the first hour, all tested dosages recorded repellence rate above 65% and the results recorded for 50, 75 and 100% extract concentrations were not significantly different following paired T test (*P* ≤ 0.05). During the second hour, all tested concentrations recorded repellence rate above 75% and the results were not significantly different (*P* > 0.05). The extract concentration of 75% and 100% demonstrated increased repellent activity in the third hour but slightly dropped in the fourth hour (Table 4). In the fourth hour, 100% repellent activity was achieved by 100% extract concentration (Table 4). The repellence rates recorded by the other extract concentrations were equally higher. The extract concentration of 25% demonstrated increased repellent activity effects in the second hour but slightly dropped in the third hour (Table 4). In the fifth hour, all tested concentrations recorded repellence rate above 75% and the results were not significantly different following paired T test (*P* ≤ 0.05). The mean percentage repellence activities of the extract for the five hour period ranged between 77.5 and 90 (Table 4).

<table>
<thead>
<tr>
<th>Concentration (% extract)</th>
<th>PR (mean% ± S.E)</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
<th>5 h</th>
<th>PR (mean%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>65.00 ± 2.89</td>
<td>77.50 ± 2.50</td>
<td>85.00 ± 2.89</td>
<td>72.50 ± 4.79</td>
<td>87.50 ± 4.79</td>
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<td></td>
</tr>
<tr>
<td>50</td>
<td>75.00 ± 2.89</td>
<td>97.50 ± 2.50</td>
<td>80.00 ± 9.13</td>
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<td>80.00 ± 5.77</td>
<td>100.00 ± 5.57</td>
<td>97.50 ± 2.50</td>
<td>90.5</td>
<td></td>
</tr>
<tr>
<td>Actellic (control)</td>
<td>87.50 ± 4.79</td>
<td>87.50 ± 4.79</td>
<td>85.00 ± 6.45</td>
<td>97.50 ± 2.50</td>
<td>95.00 ± 5.00</td>
<td>90.5</td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Repellent activity of methanolic extract of *A. sativum* on *S. zeamais*. Values followed by the same superscript within the same column are not significantly different by one-way ANOVA (*P* ≤ 0.05) followed by Tukey’s test. Superscript n values were means obtained over the 5 h duration.

Repellent activity of hexane extract of *A. sativum* on *S. zeamais*

The hexane extract of *A. sativum* had significant repellent activity against *S. zeamais* (Table 5). The greatest repellent activities were recorded by 100% extract concentrations which showed 90% repellent activity during fourth and fifth hour of exposure (Table 5). The lowest repellent activity was noted for 25% extract concentration, which caused 27.5% repellence activity on the second hour and a maximum of 67.5% on the fifth hour of study (Table 5). Other extract concentrations used in the study also caused high repellence effects.

The extract concentration of 50% caused a low repellent effect of 35% in the first hour and a higher repellent effect of 85% in the fifth hour. In addition, all the extract concentrations tested demonstrated no significant difference in repellent activities in the third and fifth hours (Table 5). The mean percentage repellence activities of the extract for the five hour period were between 41 and 80% (Table 5). The repellence activities of actellic were significantly higher than low concentration of the extract but comparable to higher concentration of the extract (Table 5).

**Figure 1**: Comparison of fumigant toxicities of methanolic and methanolic: hexane blend extracts of *A. sativum* against *S. zeamais*. Bar graphs followed by the same superscript within the same hour are not significantly different following paired T test (*P* ≤ 0.05).

**Figure 2**: Comparison of fumigant toxicities of hexane and methanolic: hexane blend extracts of *A. sativum* against *S. zeamais*. Bar graphs followed by the same superscript within the same hour are not significantly different following paired T test (*P* ≤ 0.05).
Discussion

In this study, methanolic extract of A. sativum demonstrated toxicity against maize weevils with over 76% mortality at higher dosage treatment. These results are in agreement with the findings of Lalla et al. [13] that showed similar toxicity rates of garlic oils on C. maculatus which are closely related to S. zeamais. Ibrahim and Garba, [14] work on pest management also found garlic powder to be effective against maize weevil.

Other studies carried out by Mobki et al. [15] documented greater fumigant toxicities of garlic extracts against red flour beetle. This difference may be due to reasons based on solvents of extractions, extract concentration used and susceptibility of the two beetles, T. castaneum and S. zeamais to garlic components.

In addition, the level of activity of the crude extract tested was dependent on the concentration used, the least concentration had 35.9% mortality while the highest applied concentration achieved up to 76% mortality rate. This is similar to the findings of Cheubey [16] that showed a positive concentration dependent correlation of A. sativum oils versus mortality in pulse beetle.

Methanolic extract of A. sativum did not have significant acute toxicity on S. zeamais, as very low mortality rates were observed by 6th and 24th hour periods. This is in agreement with the results of Park and Shin [17] that recorded less than 30% mortality of Japanese termite when subjected to garlic treatment up to high doses of 7.6 (ul/L). Organosulphur compounds such as diallyl trisulfide and diallyl disulfide are the most active constituents of garlic extracts that have insecticidal effects on a range of insects. These major components of

Table 5: Repellent activity of hexane extract of A. sativum on S. zeamais, Values followed by the same superscript within the same column are not significantly different (P ≤ 0.05) following Tukey’s test. Superscript m values were based on four extract concentration, five replicates of 20 insects in each replication. Superscript n values were means obtained over the 5 h duration.

<table>
<thead>
<tr>
<th>Concentration (% extract)</th>
<th>PR (mean% ± S.E)</th>
<th>PR (mean%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h</td>
<td>2 h</td>
</tr>
<tr>
<td>25</td>
<td>35.00 ± 5.00</td>
<td>27.50 ± 4.79</td>
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<tr>
<td>Actellic (control)</td>
<td>87.50 ± 4.79</td>
<td>87.50 ± 4.79</td>
</tr>
</tbody>
</table>

Table 6: Repellency activity of Hexane: Methanolic blend extracts of A. sativum on S. zeamais, Values followed by the same superscript within the same column are not significantly different (P ≤ 0.05) determined by one way ANOVA followed by Tukey’s test. Superscript m values were based on four concentrations, four replicates. Superscript n values were means obtained over the 5 h duration.
garlic have been associated with arrest of normal respiratory events within the insects [18]. In addition, alcohol extraction results in products of more complex mixture of compounds such as allicin, diallyl methyl trisulphide, ajoene and vinyldithiin which might have contributed to the extract's insecticidal properties [19].

The results of this study showed that hexane extracts of A. sativum had comparatively low toxic activity against S. zeamais since only 24% mortality rate was recorded by the highest applied concentration at the end of the study period. Although there is limited knowledge on activity of hexane extract of garlic against beetle, Rao et al. [20] also found that hexane extracts of custard apple had low activity against Khapra beetle compared to ethyl acetate extract of the same plant. A closely similar experiment by Viglianco et al. [21] showed that hexane extracts of three plants, Aloysia polystachia, Solanum argentinum and Tillandsia recurvata had low potency against S. oryzae than chloroform and ethanol extracts of the same plant. The low performance of this extract used could have been due to low concentrations of active compounds in the extract since it has been found that active principles in garlic readily dissolve in solvents of higher polarity than those of less polarity [22]. Grieve [23] also stated that alkyl compounds found in Alliacea family are readily obtained using solvents of greater polarities such as water as compared to other solvents.

The hexane extracts of garlic had no significant acute toxicity against maize weevils with only 3.85% mortality being recorded after 24 h at highest applied concentration. This could be attributed to presence of potent compounds in low concentration. It is also noteworthy, that as much as hexane extract of garlic showed presence for phenolic, flavonoids and saponins, their levels could have been low to induce an effective mortality on the tested pest.

The hexane: methanolic blend extracts of A. sativum showed greater activity against S. zeamais (Table 4). The extract was able to induce acute toxicity 88% within 24 h. By the end of experiment, 96% mortality was recorded which was comparatively higher than that noted with the actellic treatment (Table 4). Although there is limited information about the activity of hexane; methanolic blend extract of garlic, the results obtained were in agreement with findings of Hamed et al. [24], who recorded 78-100% mortality rate of S. oryzae within 3-14 days following exposure to garlic essential oils. Other similar works done by Lalla et al. [13] on C. maculatus, which are closely related to S. zeamais revealed strong toxicity of garlic essential oils against the bruchid, indicating up to 100% mortality within 3 days of exposure to high concentrations of the oils.

Since the solvent blend used for extraction had both polar and non-polar components, the manifested activity of extract can therefore be attributed to fact that more active components were captured within the extract especially allicin and alkyl compounds in garlic. A wide range of insect species are sensitive to organosulphur compounds derived from Alliacea compounds [13]. These compounds affect the normal respiratory events in the insects [25]. In addition, an allicin and diallyl sulhide induces pain and inflammation by activating Transient Receptor potential Ankyrin-1 (TRPA-1) ion channel in neural cells of the insect. Induction of such pain cause considerable stress to weevils leading to more mortality [19].

Garlic extracts produces pungent smell which has been attributed to presence of allicicous compounds majorly organosulphur compounds such as allicin and diallyl sulphide. Such odor from these compounds repels feeding insects [26]. Yang et al. [18] also attributed to high behavior repellent response of adult grain moths to two active constituents, diallyl trisulphide and diallyl disulphide present in garlic extracts. From the results of this study, different garlic extracts had immense efficacy against the development of F1 progeny of S. zeamais. Previous studies on effect of A. sativum components on adult emergence of C. chinensis (coleoptera; bruchidae) also recorded high inhibition rates [16]. Garlic components such as diallyl sulphide and diallyl trisulphide have behavioral deterrence and hinder oviposition of store-grain pests [18]. Such attributes could be the reason for the observed low adult emergence upon exposure to the three different garlic extracts used in this study. Moreover, these active constituents are ovicidal and larvicidal to eggs and larvae of beetles that have been evaluated [27].

Conclusion

Based on the findings of the present study, we conclude that methanolic and methanolic:hexane blend extracts of A. sativum have great potential of grain protection against S. zeamais infestation to maize in stores. Further studies to isolate pure compounds of A. sativum and determine the mode of action is suggested.

References


