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Original Article

Repellent Effect of Garlic against Stored Product Pests

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Intact garlic clove, grated garlic and its volatile extract applied on brown rice showed a repellent effect but no insecticidal activity against two stored product pests, *i.e.* the maize weevil and the red flour beetle. Neither repellency nor insecticidal activity was observed with garlic or its extract against two agricultural pests, diamondback moth larvae and green peach aphids. In comparative tests, hot pepper and "wasabi" mustard demonstrated only weak repellency, if any, although volatile components of "wasabi" mustard showed insecticidal activity against these insects. Volatile components of garlic were trapped and subjected to GC-MS analysis. Four major peaks resolved were sulfide compounds produced by the rapid degradation of allicin and a cyclic compound produced by dehydration. It remains to be determined whether allicin itself, the degradation products, or the mixture of these are responsible for the repellent effect.

Key words: garlic, stored-product pests, repellency, volatile compounds.

INTRODUCTION

Garlic, *Allium sativum* L., is generally used as a spice in cooking or pickling, and its other functions such as anti-protozoal, anti-fungal, anti-carcinogenic, hypo- and hyper-glycemic and insecticidal properties are also well recognized.[1-5] Garlic is one of many so-called botanical pesticides used to control insect pests on crops as well as nematodes.[6]

Although it is often known that Japanese farmers, as a traditional practice, place garlic in grain "storage containers" to protect commodities from stored product pests, few studies have evaluated actual effects of garlic against these pests.[7] Furthermore, recently, a few commercial products utilizing certain spices are on the market in Japan as grain protectants, *e.g.* "Komebitsu Sensei" (Aramic Co., Ltd.) and "Wasabichan" (Konishi Co., Ltd.). However, their efficacy has not been evaluated under laboratory conditions.

The present study was undertaken to examine repellent effect as well as insecticidal effect of garlic against stored product pests. A few other spices were also tested for comparison.

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MATERIALS AND METHODS

1. *Spices and Rice*

A basket of garlic, *Allium sativum* L., hot pepper, *Capsicum frutescens* L., "wasabi" mustard, *Eutrema wasabi* Maxim., were purchased from a local store in Japan. Brown rice, *Oryza sativa* L., variety koshihikari, grown in Chiba prefecture, Japan, was obtained from a farm.

2. *Insects*

The maize weevil, *Sitophilus zeamais* M., colony collected in Matsudo and maintained in the laboratory on brown rice and an insecticide susceptible strain of the red flour beetle, *Tribolium castaneum* H., obtained from the Phillipines and maintained in the laboratory on rice bran were used throughout the study. The green peach aphid, *Myzus persicae* Sulz., and the diamondback moth (DBM), *Plutella xylostella* L., were collected from Kagoshima and maintained in the laboratory on Chinese cabbage and cabbage, respectively.

3. *Repellency Test against Stored Product Pests*

To evaluate repellent effect of garlic, five combinations of treatment were tested *viz* 1) rice *vs.* rice (general control to check the natural distribution of insects), 2) rice *vs.* rice supplemented with intact garlic clove, 3) none *vs.* rice supplemented with intact garlic clove, 4) rice *vs*. rice supplemented with grated garlic and 5) none *vs*. rice supplemented with grated garlic. At one end of a plastic container (25×20×10 cm, length×width×height) a lump of 5 g brown rice was placed and supplemented with a peeled garlic clove (2–3 g), grated garlic (1 g), or nothing, while brown rice alone or nothing was placed at the other end of the plastic container. A group of 10 adult individuals of maize weevil or red flour beetle picked up at random from each stock culture was released in the middle of the plastic container. The container was capped with a nylon mesh, and then covered with a cardboard box (35×30×15 cm, length×width×height) to eliminate the effect of light. The container was kept at room temperature and the distribution of insects was recorded at 30 min and 24 hr. Each experiment was replicated thrice with freshly prepared materials.

4. Repellency Test against Aphids and DBM Larvae

Fifty g of peeled garlic was grated, wrapped with two layers of cheesecloth, and squeezed to extract concentrated garlic juice. Three ml of the extract was obtained and diluted 50 and 100 fold. A piece of cabbage leaflet (5×5 cm) dipped in diluted garlic extract and dried at room temperature was placed at one end of a plastic container (15×10×5 cm, length×width×height), while untreated cabbage leaflet was placed at the other end of the container. For control, untreated cabbage leaflets were placed at both ends of the container. A group of 10 DBM larvae (3–4 days old) were released in the middle of the plastic container. The container was capped with a nylon mesh, covered with a cardboard box, kept at room temperature, and the distribution of insects was recorded at 12 and 24 hr.

Each experiment was replicated thrice with freshly prepared materials. In case of repellency test against the green peach aphid, the same procedure was employed except that cabbage was replaced by Chinese cabbage.

5. Mortality Test by Vapor Action against Stored Product Pests

Either 1 g of grated garlic, 1 g of grated "wasabi" mustard, or a 5.3 cm long hot pepper minced and grated was put in a petri dish (9×2 cm, diameter×height) and placed at the bottom of a glass cylinder (12×18 cm, diameter×height). A group of 10 stored product pests along with a pinch of brown rice placed in a petri dish was kept on a 10 cm high platform. The cylinder was covered with a glass plate and sealed with grease. The cylinder was kept at room temperature and mortality was recorded at 24 and 48 hr. Each treatment was replicated thrice.

6. Mortality Test against Aphids and DBM Larvae

An appropriate amount of diluted solutions of garlic extract was sprayed using a chromatographic sprayer against a cabbage leaflet inoculated with 10 DBM larvae (3–4 days old) or against a Chinese cabbage leaflet inoculated with 10 green peach aphids. The treated leaflets and insects were placed in a plastic container, which was capped with a nylon mesh and kept at room temperature. Mortality was recorded at 12 and 24 hr. A leaflet and insects without spraying served as control. Each treatment was replicated thrice.

7. Separation and Identification of Volatile Components

One hundred g of grated garlic was put in a 500 ml Erlenmeyer flask that was connected with two pieces of silica Sep-Pak® cartridge (Waters Corporation, Milford, Massachusetts, USA) through a rubber stopper. One cartridge was used to clean the incoming air, while the other was used for trapping the volatile components of grated garlic using a mini pump (MP-603T, SIBATA) at the flow rate of 1 *l*/min for 10 hr at room temperature (20–25°C). The trapped materials were eluted by 2×1 ml of acetonitrile and 3 μl of the elute was injected for gas chromatography and mass spectrometry (GC-MS) analysis. The GC-MS analysis was carried out using a Shimadzu Gas Chromatograph-Mass Spectrometer QP 2000 GF (Shimadzu Corporation) under the following conditions-column: Shimadzu CBI-S30-025 (30 m×i.d. 0.32 mm), oven temperature: 50 to 200°C with hold time of 1 min and ramp rate of 8°C/min, injection temperature: 70 to 220°C with ramp rate of 10°C/min, carrier gas: He 0.8 kg/cm^2, ionization: electron impact (EI) at 220°C.

Repellent effect of the volatile components was examined employing the same procedure as described above. One ml of the acetonitrile solution was poured over 5 g of brown rice and subjected to the bioassay after evaporation of acetonitrile.

RESULTS AND DISCUSSION

1. Repellent Effect

Tables 1 and 2 show the results of repellency experiment of garlic against the maize weevil and the red flour beetle, respectively. When the insects were released in the container and allowed to choose between the two untreated brown rice heaps, which served as control, there was no significant difference in their distribution between the heaps at both 30 min and 24 hr with either insect. In clear contrast, more insects chose the untreated rice when the other was supplemented with an intact garlic clove or a pinch of grated garlic demonstrating the repellent effect of garlic against both stored product pest species. The effect appeared more pronounced with grated garlic than intact garlic clove.

It is interesting to note that the repellent effect was not strong enough to keep the insects off the rice when only the rice supplemented with an intact garlic clove was given (Table 1 and at 24 hr in Table 2), although the rice supplemented with grated garlic still exerted strong repellency even when given alone without untreated control rice (Tables 1 and 2). These results suggest that garlic has repellent effect against the maize weevil and the red flour beetle, and that the effect is exerted mildly with an intact garlic clove but strongly when the garlic is grated.

Table 3 shows the result of similar experiment against two agricultural pests, *i.e.* the green peach aphid and the DBM larvae. In contrast to the results with the stored product pests, the garlic extract did not show any repellent effect against these insects, indicating the repellent effect of garlic does not work for all insects.

Some spices such as garlic, hot pepper and "wasabi" mustard have been traditionally used as cultural methods to protect stored grains in Japan. However, when minced hot pepper and grated "wasabi" mustard were examined for repellent effects in the present study employing the same method as that used for garlic, little repellency was observed in most combinations tested against the maize weevil and the red flour beetle (Tables 4–7). When these insects were given only rice with

Table 1 Repellent effect of garlic against the maize weevil.

	Insect distribution (%)[1]	
Combinations tested	30 min	24 hr
Rice	33.3±5.8[a]	56.7±5.8[b]
Rice	43.3±5.8[a]	43.3±5.8[b]
Elsewhere	23.3±11.5	0
Rice	60.0±17.3*	70.0±10.0*
Rice with intact garlic	16.7±5.8*	13.3±5.8*
Elsewhere	23.3±23.1	16.6±5.8
Rice	86.7±11.5**	93.3±11.5**
Rice with grated garlic	3.3±5.8**	0**
Elsewhere	10.0±10.0	6.7±11.5
Rice with intact garlic	70.0±17.3*	80.0±10.0*
Elsewhere	30.0±17.3*	20.0±10.0*
Rice with grated garlic	0**	3.3±5.8**
Elsewhere	100.0±0**	99.7±5.8**

[1] Mean±S.D. of three replications. [a–b] The difference between the same alphabet is not significant at $P=0.05$. *,** The difference is significant at $P=0.05$ and $P=0.01$, respectively, in the same column.

Table 2 Repellent effect of garlic against the red flour beetle.

	Insect distribution (%)[1]	
Combinations tested	30 min	24 hr
Rice	36.7±5.8[a]	43.3±5.8[b]
Rice	40.0±10.0[a]	46.7±15.3[b]
Elsewhere	23.3±15.3	10.0±10.0
Rice	90.0±10.0**	90.0±10.0**
Rice with intact garlic	3.3±5.8**	3.3±5.8**
Elsewhere	6.7±11.5	6.7±5.8
Rice	96.7±5.8**	90.0±10.0**
Rice with grated garlic	0**	0**
Elsewhere	3.3±5.8	10.0±10.0
Rice with intact garlic	10.0±10.0**	46.7±15.3[c]
Elsewhere	90.0±10.0**	53.3±15.3[c]
Rice with grated garlic	0**	0**
Elsewhere	100.0±0**	100.0±0**

[1] Mean±S.D. of three replications. [a–c] The difference between the same alphabet is not significant at $P=0.05$. **The difference is significant at $P=0.01$ in the same column.

Table 3 Repellent effect of garlic against the diamondback moth larvae and the green peach aphid.

Insects and stages tested	Combinations tested	Insect distribution (%)[1]	
		12 hr	24 hr
Myzus persicae	Leaflet[2]	50.0±10.0[a]	46.7±5.8[b]
Apterous adults	Leaflet	36.7±5.8[a]	46.7±5.8[b]
	Elsewhere	13.3±5.8	6.7±5.8
Myzus persicae	Leaflet[2]	43.3±5.8[c]	40.0±10.0[d]
Apterous adults	Leaflet with garlic extract	43.3±5.8[c]	46.7±5.8[d]
	Elsewhere	13.3±5.8	13.3±5.8
Plutella xylostella	Leaflet[3]	46.7±15.3[e]	46.7±15.3[f]
3–4 days old larvae	Leaflet	53.3±15.3[e]	46.7±15.3[f]
	Elsewhere	0	0
Plutella xylostella	Leaflet[3]	46.7±11.5[g]	50.0±10.0[h]
3–4 days larvae	Leaflet with garlic extract	36.7±5.8[a]	50.0±10.0[h]
	Elsewhere	0	0

[1] Mean±S.D. of three replications. [2] Chinese cabbage. [3] Cabbage. [a–h] The difference between the same alphabet is not significant at $P=0.05$.

Table 4 Repellent effect of hot pepper against the maize weevil.

Insect distribution (%)[1]		
Combinations tested	30 min	24 hr
Rice	36.7±11.5[a]	50.0±10.0[b]
Rice	33.3±5.8[a]	50.0±10.0[b]
Elsewhere	30.0±10.0	0
Rice	40.0±10.0[c]	53.3±11.5[d]
Rice with intact hot pepper	26.7±5.8[c]	40.0±10.0[d]
Elsewhere	33.3±15.3	6.7±5.8
Rice	53.3±11.5[e]	63.3±11.5[f]
Rice with grated hot pepper	23.3±5.8[e]	36.7±5.8[f]
Elsewhere	23.3±5.8	0
Rice with intact hot pepper	46.7±5.8[g]	100.0±0.0**
Elsewhere	53.3±5.8[g]	0**
Rice with minced hot pepper	33.3±5.8[h]	43.3±15.3[i]
Elsewhere	66.7±20.8[h]	56.7±15.3[i]

[1] Mean±S.D. of three replications. [a–i] The difference between the same alphabet is not significant at $P=0.05$. **The difference is significant at $P=0.01$ in the same column.

Table 5 Repellent effect of hot pepper against the red flour beetle.

Insect distribution (%)[1]		
Combinations tested	30 min	24 hr
Rice	43.3±15.3[a]	46.7±11.5[b]
Rice	40.0±10.0[a]	50.0±10.0[b]
Elsewhere	16.7±5.8	3.3±5.8
Rice	56.7±15.3[c]	56.7±15.3[d]
Rice with intact hot pepper	40.0±5.8[c]	40.0±10.0[d]
Elsewhere	3.3±5.8	3.3±5.8
Rice	56.7±15.3[e]	50.0±10.0[f]
Rice with grated hot pepper	33.3±5.8[e]	36.7±5.8[f]
Elsewhere	10.0±5.8	13.3±5.8
Rice with intact hot pepper	60.0±10.0[g]	73.3±5.8**
Elsewhere	40.0±10.0[g]	26.7±5.8**
Rice with minced hot pepper	60.0±10.0[h]	33.3±5.8*
Elsewhere	40.0±10.0[h]	66.7±15.3*

[1] Mean±S.D. of three replications. [a–h] The difference between the same alphabet is not significant at $P=0.05$. *The difference is significant at $P=0.05$ in the same column. ** The difference is signifact at $P=0.01$ in the same column.

Table 6 Repellent effect of "wasabi" mustard against the maize weevil.

Insect distribution (%)[1]		
Combinations tested	30 min	24 hr
Rice	43.3±15.3[a]	43.3±5.8[b]
Rice	46.7±5.8[a]	43.3±5.8[b]
Elsewhere	10.0±10.0	3.3±5.8
Rice	46.7±15.3[c]	50.0±10.0[d]
Rice with intact "wasabi"	43.3±5.8[c]	46.7±11.5[d]
Elsewhere	10.0±10.0	3.3±5.8
Rice	63.3±15.3*	66.7±11.5*
Rice with grated "wasabi"	33.3±5.8*	36.7±5.8*
Elsewhere	3.3±5.8	6.7±5.8
Rice with intact "wasabi"	70.0±10.0*	63.3±5.8*
Elsewhere	30.0±10.0*	36.7±5.8*
Rice with grated "wasabi"	70.0±10.0*	36.3±5.8*
Elsewhere	30.0±10.0*	63.7±15.3*

[1] Mean±S.D. of three replications. [a–d] The difference between the same alphabet is not significant at $P=0.05$. *The difference is significant at $P=0.05$ in the same column. ** The difference is significant at $P=0.01$ in the same column.

Table 7 Repellent effect of "wasabi" mustard against the red flour beetle.

Insect distribution (%)[1]		
Combinations tested	30 min	24 hr
Rice	46.7±15.3[a]	43.3±11.5[b]
Rice	36.7±5.8[a]	50.0±10.0[b]
Elsewhere	16.7±11.5	6.7±5.8
Rice	53.3±20.8[c]	40.0±10.0[d]
Rice with intact "wasabi"	36.7±5.8[c]	56.7±11.5[d]
Elsewhere	10.0±10.0	3.3±5.8
Rice	53.3±15.3[e]	66.7±11.5[f]
Rice with grated "wasabi"	43.3±5.8[e]	36.7±5.8[f]
Elsewhere	3.3±5.8	6.7±5.8
Rice with intact "wasabi"	70.0±10.0*	63.3±15.3*
Elsewhere	30.0±10.0*	36.7±11.5*
Rice with grated "wasabi"	66.7±11.5*	40.0±10.0[g]
Elsewhere	33.3±5.8*	60.0±10.0[g]

[1] Mean±S.D. of three replications. [a–g] The difference between the same alphabet is not significant at $P=0.05$. *The difference is significant at $P=0.05$ in the same column.

minced hot pepper (Table 5) or grated "wasabi" mustard (Table 6) significantly ($P=0.05$) more insects were found elsewhere at 24 hr indicating weak repellent effect. The effect, however, appeared extremely low as compared to that observed with grated garlic (Tables 1 and 2).

2. *Insecticidal Activity*

Insecticidal activity (killing effect) of the three spices by vapor action was examined (Table 8). While "wasabi" mustard resulted in high mortality against the two stored product pests, garlic and hot pepper showed little toxicity, if any. The potential insecticidal activity of garlic was also examined by directly spraying 100-fold

Table 8 Mortality by vapor action of some spices against the stored product pests.

Insects tested	Spices tested	Mortality (%)[1]	
		24 hr	48 hr
Sitophilus zeamais	Garlic grated	0	0
	Hot pepper minced	0	9.4±11.6
	"Wasabi" mustard grated	70.0±20.0	100.0±0
Tribolium castaneum	Garlic grated	0	0
	Hot pepper minced	7.0±6.1	7.0±6.1
	"Wasabi" mustard grated	66.7±15.3	96.7±5.8

[1] Mean±S.D. of three replications.

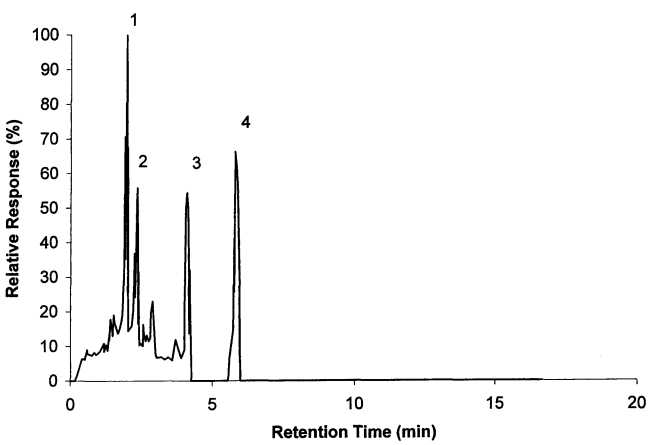

Fig. 1 GC chromatogram of volatile components of garlic.

and 50-fold diluted solutions of the extract against DBM larvae and green peach aphids. At these concentrations, no significant mortality was observed after treatment for these insects.

3. *Separation of Volatile Components of Garlic*

Fig. 1 shows GC chromatogram of the vapor collected from grated garlic. The vapor consisted of four major peaks in addition to numerous small peaks.

Fig. 2 shows the mass-spectra of the four major peaks. By comparison of the fragment ion patterns obtained with reference values given in literature,[8] these peaks were identified as diallyl disulfide (peak no. 1), $C_6H_{10}S_2$ (peak no. 2), a cyclic compound, 3-vinyl-[4H]-1,2-dithiin (peak no. 3) and diallyl sulfide (peak no. 4).

In order to confirm that the volatile components collected from garlic were repellent, the mixture was applied to a heap of brown rice and tested against the maize weevil and the red flour beetle (Table 9). Although the difference in insect distribution between the treated and untreated rice was not significant at 30 min with either species, significantly more insects were found on untreated rice at 24 hr suggesting the repellent effect of the volatile components of garlic.

The present study confirmed that garlic demonstrates repellent effect against the two stored product pests, *i.e.*

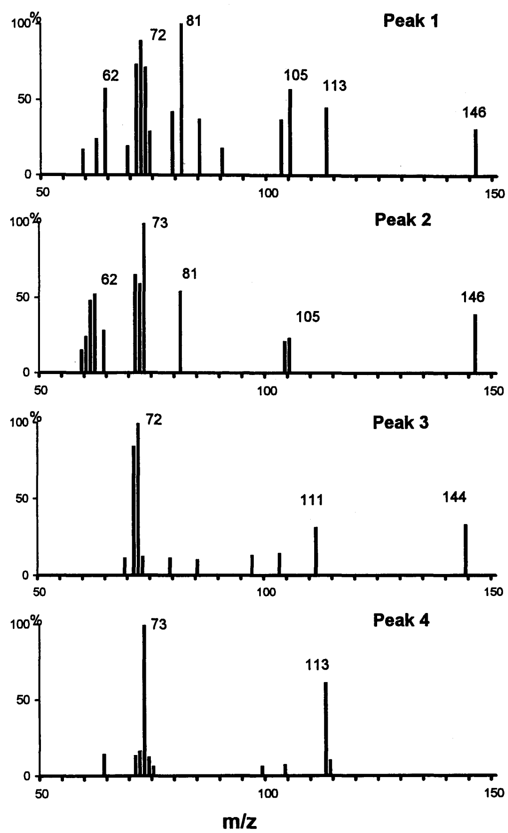

Fig. 2 Mass spectra of the four GC peaks.
Peak 1: Diallyl Disulfide; Peak 2: $C_6H_{10}S_2$; Peak 3: 3-vinyle-[4H]-1,2-dithiin; Peak 4: Diallyl Sulfide.

the maize weevil and the red flour beetle. The effect was stronger with grated garlic than with intact garlic clove. The result is consistent with the fact that alliin, a colorless, odorless and water-soluble flavor precursor, present in the intact cells of garlic comes in contact with an enzyme allinase upon injury of the cells and is converted to the sulfur-containing product allicin.[9,10]

It is reasonable to assume that allicin is produced to a lesser extent by peeling the intact garlic clove and to a greater extent by grating the garlic. The volatile compound allicin is known to be very unstable and breaks down rapidly into strong smelling different compounds.

Table 9 Repellent effect of a mixture of volatile components of garlic against the stored product pests.

Insects tested	Combinations tested	Insect distribution (%)[1]	
		30 min	24 hr
Sitophilus zeamais	rice	23.3±15.3[a]	50.0±17.3[b]
	rice	26.7±11.5[a]	43.3±15.3[b]
	elsewhere	50.0±17.3	6.7±15.4
	rice	56.7±15.3[c]	73.3±15.2*
	rice with volatile compound mixture	13.3±15.4[c]	13.3±5.8*
	elsewhere	30.0±10.0	13.3±15.3
Tribolium castaneum	rice	30.0±10.0[d]	33.3±5.8[e]
	rice	33.3±15.3[d]	40.0±10.0[e]
	elsewhere	36.7±15.3	26.7±11.5
	rice	30.0±10.0[f]	70.0±10.0*
	rice with volatile compound mixture	13.3±5.8[f]	13.3±5.8*
	elsewhere	56.7±15.3	16.7±15.4

[1] Mean±S.E. of the three replications. [a–f] The difference between the same alphabet is not significant at $P=0.05$. * The difference is significant at $P=0.05$ in the same column.

Brodnitz et al.[3)] reported that allicin undergoes complete decomposition at 20°C after 20 hr giving diallyl disulfide (66%), diallyl trisulfide (9%), diallyl sulfide (14%) and sulfur dioxide. Block[2)] mentioned that allicin self-decomposes to yield cyclic compounds 2-vinyl-[4H]-1,3-dithiin or 3-vnyl-[4H]-1,2-dithiin; while Brodnitz et al.[3)] postulated the cyclic compounds were dehydration products of allicin formed during gas chromatography. In the present study, neither alliin nor allicin was found from volatile components of grated garlic. It is conjectured allicin was decomposed to diallyl disulfide; diallyl sulfide, 3-vinyl-[4H]-1,2-dithiin and other sulfide compounds during collection of the vapor that lasted for 10 hr or during gas-chromatography analysis.

It is not clear whether allicin itself or the decomposition products or both are responsible for the repellent effect of garlic.

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要　　約

ニンニクの貯穀害虫に対する忌避効果

G. K. M. M. ラハマン，本山直樹

玄米にニンニクの小片，オロシ，および揮発性成分抽出物を処理すると，貯蔵害虫のコクゾウムシとコクヌストモドキに対して忌避効果を示したが，殺虫活性はなかった．ニンニクおよびその抽出物は供試した農業害虫のコナガ幼虫とモモアカアブラムシに対しては忌避効果も殺虫活性も示さなかった．一方比較試験に供試したトウガラシとワサビについては，いずれも貯蔵害虫に対して忌避効果がないか，あるいは著しく弱い効果しか示さなかったが，ワサビの揮発性成分は貯蔵害虫両種に対して殺虫活性を示した．ニンニクの揮発性成分を捕集しGC-MSで分析したところ，4つの主要なピークが分離され，アリシンの急速な分解によって生成したスルフィド化合物と，その脱水素反応によって生成した環状化合物と推定された．忌避効果がアリシン自体，その分解物，あるいはそれらの混合物に由来するかについては不明である．