Evaluation of freshly prepared juice from garlic (*Allium sativum* L.) as a biopesticide against the maize weevil, *Sitophilus zeamais* (Motsch.) (Coleoptera: Curculionidae)

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Abstract: Freshly prepared garlic (*Allium sativum* L.) juice, containing the antimicrobial allicin, was evaluated as a possible grain protectant against the maize weevil, *Sitophilus zeamais* (Motsch.). Each experiment was set out in Completely Randomized Design (CRD) with four replications, and there was a control treatment. Adult mortality and weight loss percentage were investigated. There was an observed increase in adult mortality following days of exposure in all treatments. Statistically significant ($p < 0.05$) reduced grain loss was also observed in all the treatments when compared with the control. The juice samples were freshly prepared from an indigenous Nigerian garlic cultivar (GUN) and a cultivar purchased from a supermarket in Germany (GAG). These garlic juice samples exhibited lethal effects causing at least 90% adult mortality in contact toxicity tests. The amount of allicin in GUN was 1.88 mg/ml according to High Pressure Liquids Chromatography (HPLC) analysis, while the amount of allicin in GAG was 3.50 mg/ml. This study highlights the potential of *A. sativum* containing allicin for biorational control of maize grains against *S. zeamais* infestation and damage.

Key words: allicin, *Allium sativum*, biopesticide, *Sitophilus zeamais*, stored product

Introduction

Maize (*Zea mays* L.) or corn is a major source of dietary carbohydrate as well as the most important cereal in Sub-Saharan Africa (IITA 2009). The maize weevil, *Sitophilus zeamais* (Motsch.) (Coleoptera: Curculionidae), is a major pest of stored maize grain in many regions of the world including Nigeria (Adedire 2001). Although synthetic insecticides have long been widely used in the control of insect pests, the indiscriminate application of synthetic products has led to various problems. Toxic residues in the treated products, environmental pollution, and growing resistance against insecticides by insects and pests are due to indiscriminate application of synthetic insecticides (Huang *et al*. 1997). There is an urgent need to continue the search for eco-friendly, cheap, sustainable, and safe plant protection agents that will not contaminate food products in their use as grain protectants in storage systems for small holder farmers. Moreover, because they are often viewed as "mild" on the environment, compounds of biogenic origin are generally more positively regarded compared to substances partially or completely chemically synthesised in laboratories (Slusarenko *et al*. 2008). For these reasons, biogenic origin compounds are more likely to gain wider acceptance among farmers in the long run. In addition, crop protection agents of natural origin have the advantage of possessing novel modes of action against insects. They also have the potential to reduce the risk of cross-resistance while offering new leads for the design of target-specific molecules (Zhou *et al*. 2012).

In the last few decades, there has been a heightened interest in plants like garlic. These plants have been equipped by evolution to defend themselves against invading pathogens and pests. The interest in these plants is not only because of environmental concerns trailing the use of chemically synthesised plant protection products, but also because of farmer and consumer preference for organic farming strategies and produce, respectively (Slusarenko *et al*. 2008; Nwachukwu *et al*. 2012). For many such plants, protection against pathogens and pests often comes in the form of sulphur-containing secondary metabolites synthesised following external attacks on them (Nwachukwu *et al*. 2012). Allicin (diallylthiosulfinate), the major antimicrobial substance in garlic, has attracted the attention of investigators because of its widely acclaimed potency. Garlic is known for its positive effect on health, particularly the prevention of cardiovascular diseases and certain digestive cancers (Lalla *et al*. 2013). Previous studies have shown that garlic also possesses some insecticidal, fungicidal, acaricidal, nematicidal, and bactericidal properties (Lalla *et al*. 2013). With

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a widespread antimicrobial activity comparable to those of common antibiotics like ampicillin (Curtis *et al*. 2004) and penicillin (Cavallito *et al*. 1944), it is hardly surprising that this compound has shown activity against some of the world's most notable plant pathogens including *Phytophthora infestans* and *Pseudoperonospora cubensis* (Portz *et al*. 2008).

Allicin is a phytoanticipin, which means that its synthesis, from preformed precursors already present in garlic, occurs prior to any external attack or irritation, and so there is no expenditure of energy involved (van Etten 1994; Nwachukwu *et al*. 2012). Allicin is formed as a volatile organosulfur compound following the disruption of garlic tissues either by crushing, piercing, or wounding. The substrate alliin (S-allyl-L-cysteine sulfoxide) held in the cytosol prior to tissue disruption, reacts with the now liberated vacuolar enzyme, alliinase, to give allicin with pyruvate and ammonia as by-products.

Over the years, various studies investigating the possible use of agents of biogenic origin as crop protectants have highlighted the potency of natural products, such as biopesticides (Qi and Burkholder 1981; Isman 2000; Lee *et al*. 2004; Gonzalez-Coloma *et al*. 2010). For instance, studies examining the fumigant and contact insecticidal effects of 22 plant essential oils against the bean weevil, *Acantho scelidesobtectus* (Regnault-Roger *et al*. 1993), and of 28 plant essential oils against four adult members of the order *Coleoptera* including the rice weevil *Sitophilus oryzae,* a close relative of *S. zeamais* (Shaaya *et al*. 1991) have added to the impressive body of evidence in the literature clearly demonstrating the efficacy of using natural pesticides as biocontrol agents in the open field and also have acted as stored product protectants against pests related to the maize weevil. While none of the two works just cited examined the use of agents from garlic but instead studied the use of other natural compounds including those from lavender, coriander, lemon, and celery, such as α-terpineol, α-terpinene, β-caryophyllen, and carvacrol among others, they provide materials for fascinating comparative analyses of chemical compounds in plants as crop protectants, especially since allicin; the major biologically active agent in fresh garlic juice, is only found in garlic (Cavallito *et al*. 1944; Cavallito and Bailey 1944; Jain and Apitz-Castro 1987).

Research on the potential applications of biologically active compounds from garlic abound in literature. For instance, steam-distilled garlic oil has been tested for toxicity against the eggs, larvae, and adults of *Tribolium castaneum*, and against the adults of *S. zeamais* (Ho *et al*. 1996). Extracts from garlic (and other plants) have been vapourised and used in fumigation tests involving *T. castaneum* and *S. zeamais* (Huang *et al*. 2000). There have also been tests investigating the insecticidal action of garlic compounds on *Sitotroga cerealella* (Yang *et al.* 2012). In addition to garlic, the potential for the use of other alliums in preserving stored plant products has also been demonstrated (Ofuya *et al*. 2010). Other investigations include those of Hamed *et al.* (2012), Adedire and Ajayi (1996), and Arannilewa *et al.* (2006). Each of these works used garlic essential oils obtained by steam distillation or solvent extraction.

Although the search for and use of plant materials with grain protectant ability is not new (Lale 1992; Odeyemi 1993; Udo 2005; Asawalam and Emosairue 2006), given the growing role of allicin from garlic in crop protection, we decided to evaluate the efficacy of freshly prepared *Allium sativum* L. juice as a protectant of maize grain against infestation by *S. zeamais*.

Materials and Methods

S. zeamais culture

Adult *S. zeamais* was bred in the laboratory at 27±2°C, 60–65% relative humidity (RH) and 12 h : 12 h light : dark regime. *S. zeamais* was obtained from stocks maintained at the Crop Science Laboratory, Michael Okpara University of Agriculture, Umudike, Nigeria. The feed medium used was whole maize grains, purchased from the Umuahia main market, Abia State, Nigeria. Fifty pairs of *S. zeamais* were introduced into one litre glass jars containing 400 g of weevil-susceptible maize grains. The jars were then covered with nylon mesh held in place with rubber bands. Freshly emerged adults of *S. zeamais* were subsequently used for the experiments.

Preparation and application of *A. sativum*

The *A. sativum* (garlic) bulbs used for the study were purchased from the Umuahia main market, Nigeria (GUN) and from a supermarket in Aachen, Germany (GAG). The garlic juice was prepared by blending axillary buds from composite garlic bulbs using a NAKAI Japan Model 1706 Extractor. Prior to High Pressure Liquids Chromatography (HPLC) determination of allicin, the juice was introduced into a sterile 50-ml Falcon tube and centrifuged (Megafuge 1.0R; Heraeus Instruments, Osterode, Germany) at 5,000 rpm (3,000 g) for 10 min to separate the majority of the pulp from the liquid. Remnants of the pulp were then carefully removed from the top of the liquid with a clean spatula. A diaphragm vacuum pump (Vacuubrand GmbH, Wertheim, Germany) was used to separate the remaining pulp from the pure liquid juice, under pressure. The pure filtrate was then transferred to a second sterile Falcon tube and sealed preparatory to HPLC analysis.

The weight of 50 g of clean and un-infested Bende white maize variety, which had not been pre-treated with weevil repellents, was taken using an MP Citizen Electronic weighing balance. Subsequently, this maize was introduced into four sterilised plastic vials. To each plastic vial, 1 ml of each garlic juice type was added. The contents were mixed thoroughly by manual agitation of the vials. A control experiment containing no garlic juice was also set up. Five pairs of adult *S. zeamais* were introduced into the treated and untreated maize grains. The lids of the plastic vials were perforated in order to maintain aerobic conditions in the vials. Muslin textile material was used to secure the top of the plastic vials and served to ensure aeration while preventing entry or exit of insects. The contents of the plastic vials were then shaken gently for proper and uniform mixing. Each treatment was replicated four times. The samples were arranged in a completely randomised design on a laboratory table.

Mortality and damage assessment assays

The number of dead insects in each vial was counted at 7, 14, 21, 28, and 35 days after treatment (DAT) to estimate mortality. Maize weevil mortality was assessed as:

Number of dead insects/Total number of insects × 100.

Data on the adult weevil mortality percentage were corrected using Abbott's formula (Abbott 1925) thus:

$$P_t = \frac{P_o - P_c}{100 - P_c},$$

where:
P_t – corrected mortality (%); P_o – observed mortality (%); P_c – the control mortality (%).

Weight loss was assessed by re-weighing the grains to determine the weight loss percentage. The weight loss percentage was calculated following the method of the FAO (FAO 1985) as follows:

$$\text{The weight loss percentage} = \frac{[U_a N - (U+D)]}{U_a N} \times 100,$$

where:
U – weight of undamaged fraction in the sample, N – total number of grains in the sample, U_a – average weight of undamaged grains, D – weight of damaged fraction in the sample.

Contact toxicity test by topical application

One ml fresh dosage garlic-juice sample were applied uniformly to the bottom of the plastic vials. A control was set up in which there was no garlic juice. Five male and five female adult weevils of about 5 days old were introduced separately into each vial. Each treatment was replicated four times and weevil mortality was recorded after 12, 24, 36, and 48 h of exposure. Insects were presumed dead if they remained immobile and did not respond to five jabs with a blunt dissecting probe after an arbitrary 5-min recovery period.

HPLC determination of allicin in garlic juice

Determination of the amount of allicin in the garlic-juice preparations was performed using a JASCO HPLC. The method used was taken from Krest and Keusgen (2002). To dilute freshly prepared garlic juice, HPLC-grade water was used at a ratio of 1 : 10. Thereafter, 1 ml of the diluted sample was introduced into a sterile vial with the injection volume set at 20 μl. In order to protect the column, the diluted garlic juice was passed through a polyethersulfon membrane (0.2 μm pore size, Steriflip, Millipore), before introduction into the vial and subsequent injection into the HPLC (JASCO Chromatography Data System, with Intelligent UV detector, Jasco Labor-u. Datentechnik GmbH, Groß-Umstadt, Germany). As an internal standard, 1.5 ml of a 0.05 mg/ml solution (in methanol) of butyl-4-hydroxybenzoate was used. Using the HPLC software ChromPass (version 1.8.6.1), a mixed gradient elution [solvent A, 30% (v/v) HPLC grade methanol adjusted to pH 2.0 with 85% (v/v) orthophosphoric acid; solvent B, 100% HPLC grade methanol] was performed. Elution spectra were recorded between 200–600 nm with detection at 254 nm for the chromatogram.

Statistical analysis

Data obtained were subjected to analysis of variance (ANOVA) and the significant mean differences (p > 0.05) were separated by using the Student-Newman-Keuls (SNK) test.

Results

Mortality

The effect of fresh garlic juice on the mortality of *S. zeamais* is presented in figure 1. The results obtained show that fresh GUN juice with a cumulative mortality rate of 73% and GAG at 87% mortality rate, were significantly more effective in causing *S. zeamais* mortality at 28 DAT compared to the control.

Contact toxicity test by topical application

Upon exposure to adult *S. zeamais*, fresh GUN juice caused 100% mortality, while GAG juice caused 90% mortality (Fig. 2) 48 h after topical application. In the control there was zero mortality.

Effect on grain weight

There was significant weight loss of the control (Fig. 3) when compared with the maize grains treated with gar-

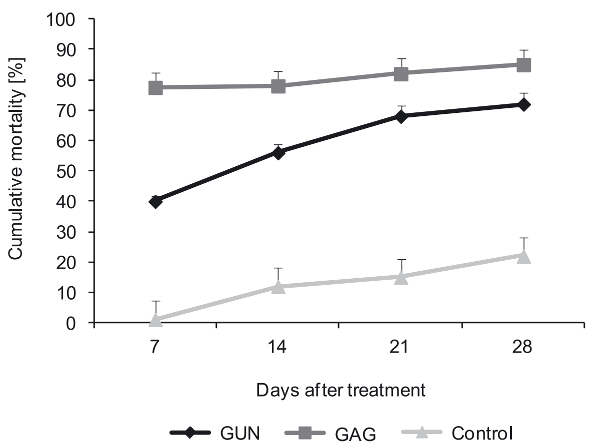


Fig. 1. Cumulative mortality of freshly emerged adults of *S. zeamais* after introduction onto maize grains treated with freshly prepared *A. sativum* juice. GUN – Nigerian garlic cultivar; GAG – German garlic cultivar

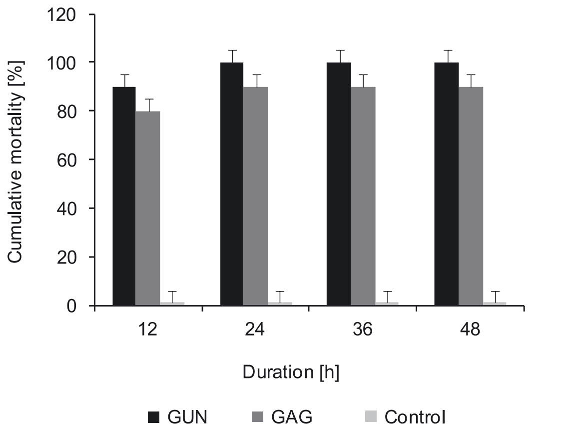


Fig. 2. Contact toxicity of fresh garlic juice against *S. zeamais* adult at 48 h after treatment. Explanations – see fig. 1

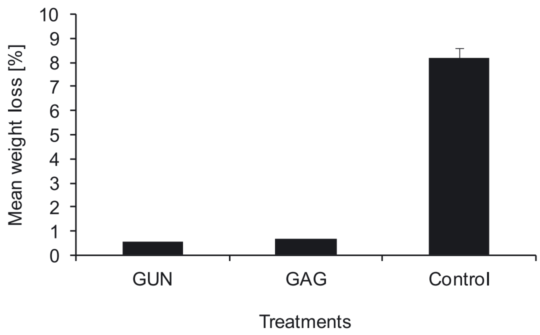


Fig. 3. Effect of *A. sativum* juice on weight loss of maize grains. Explanations – see fig. 1

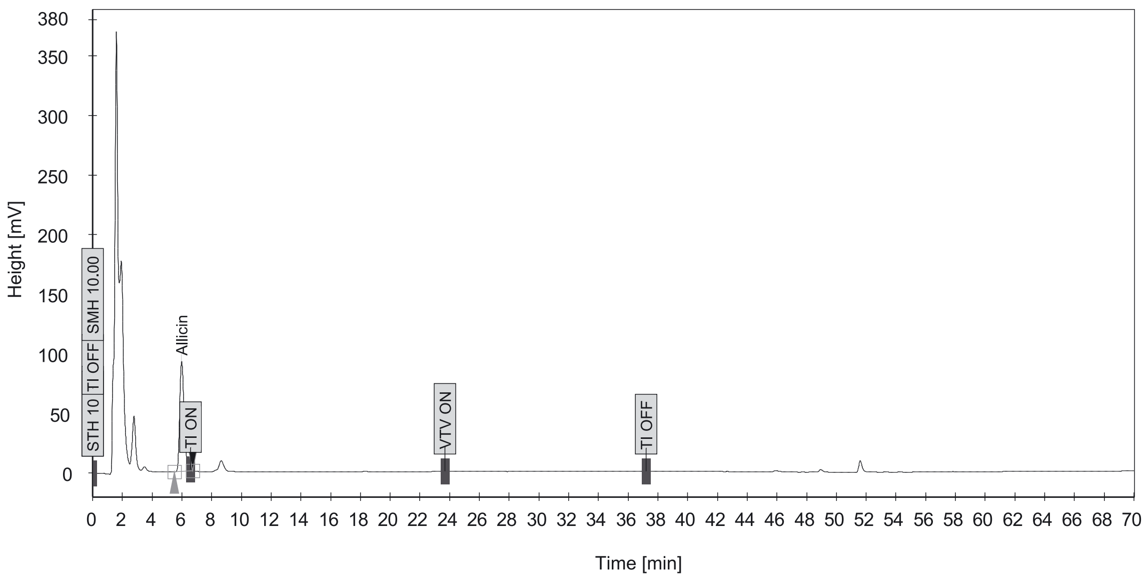
lic juice, indicating the effectiveness of the juice in offering protection to the stored maize grains. While the untreated control grains lost over 8% of their original weight, on average, the grains treated with GAG and GUN only lost a negligible < 0.5% of their average weight after 60 days.

HPLC analysis of *A. sativum*

The HPLC chromatograms depicting the amount of allicin in the freshly prepared garlic juice is shown in figures 4 and 5. The amount of allicin in GUN and GAG was found to be 1.88 mg/ml and 3.50 mg/ml, respectively.

Discussion

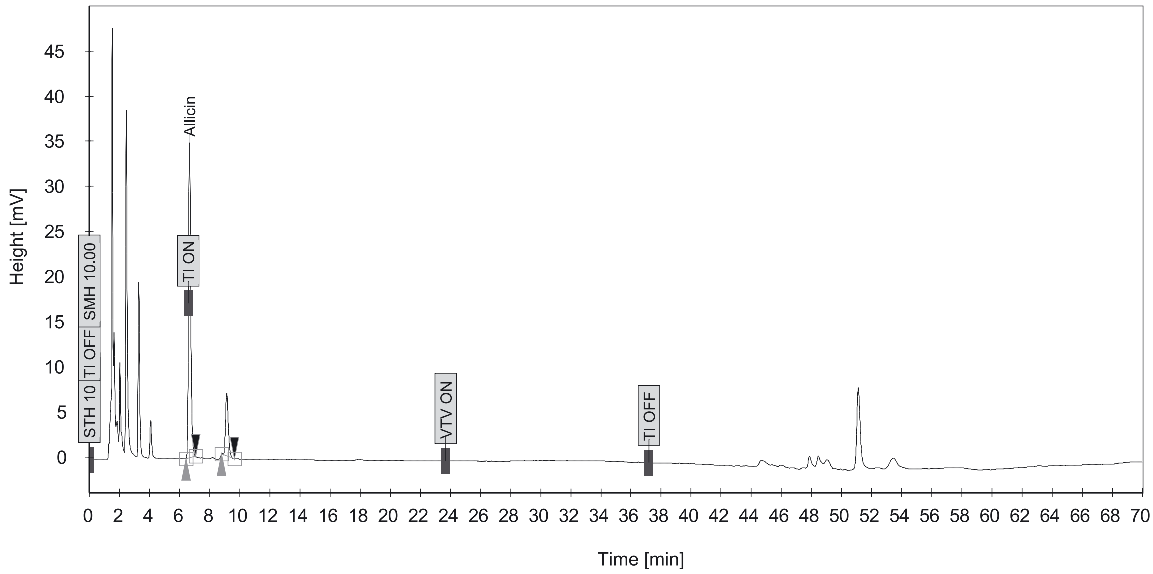
To the best of our knowledge, no study has been carried out on the use of fresh garlic juice as a biopesticide against *S. zeamais.* A search of the leading electronic database, Scopus, returned no hits for each of the search terms "garlic juice weevil", "garlic juice *Sitophilus*", and "garlic juice *Sitophilus zeamais*". Therefore, this work most likely represents a remarkable shift from the conventional approach to studying the use of biologically active compounds from garlic in crop protection. Importantly, given that allicin is volatile and unstable, and upon production rapidly decomposes to other breakdown products like



Peak results:

Index	Name	Time [min]	Quantity [ug/ml]	Height [mV]	Area [mV.min]	Area [%]
1	Allicin	5.983	188.32	92.2	31.7	100.000
Total			188.32	92.2	31.7	100.000

Fig. 4. HPLC chromatogram for GUN allicin determination. The peak for allicin in diluted garlic juice (1 : 10) was detected at 5.98 min. The amount of allicin was determined to be 1.88 mg/ml corresponding to an allicin concentration of 11.60 Mm



Peak results:

Index	Name	Time [min]	Quantity [ug/ml]	Height [mV]	Area [mV.min]	Area [%]
1	Allicin	6.658	35.93	35.0	6.1	80.606
Total			35.93	41.9	7.5	100.000

Fig. 5. HPLC chromatogram for GAG allicin concentration determination. The peak for allicin in diluted garlic juice (1 : 100) was detected at 6.66 min. The amount of allicin in the freshly prepared garlic juice was determined to be 3.5 mg/ml corresponding to an allicin concentration of 21.57 Mm

ajoene and the vinyldithiins (Apitz-Castro *et al.* 1983; Block *et al*. 1984; Block 1985; Voigt and Wolf 1986; Iberl *et al*. 1990), we propose that allicin could not have been solely and principally responsible for the insecticidal effects reported in previous works investigating the exposure of agricultural pests to garlic essential oil. As comprehensively discussed by Staba *et al.* (2001), any of a number of treatments/processings of garlic such as freeze drying, steam distillation, oil maceration, ethanolic extraction, and low temperature drying, results in the production of complex mixtures including allicin, diallyldisulfides, diallytrisulfides, allyl methyl trisulfides, ajoene, and vinyldithiins.

The results show that although GUN and GAG belong to the same species, they exert slightly different effects on *S. zeamais* – a difference which can be reasonably attributed to their different allicin contents. Our results are similar to the work of Hamed *et al.* (2012) which recorded mortality rates ranging from 78–100% following 3–14 days of exposing *S. oryzae* adults to garlic essential oils. In addition to using garlic essential oils, other similar works such as those by Adedire and Ajayi (1996), and Arannilewa *et al.* (2006) also employed solvents like petroleum ether and ethanol as extraction vehicles thus making direct and accurate comparisons improbable.

Garlic's pungent smell has been attributed to the presence of organosulfur compounds such as allicin and diallyldisulfide (DADS) in the edible allium (Bautista *et al*. 2005). It has been suggested that garlic's pungency contributes to its toxicity in weevils by disrupting regular respiratory events (Adedire and Ajayi 1996). Furthermore, the structures of allicin and DADS are similar to that of allylisothiocyanate, which apart from lending wasabi and other mustard plants their pungency, induces pain and inflammation by activating the TRPA1 (Transient Receptor Potential Ankyrin-1) ion channel in neuronal cells (Fahey *et al*. 2001; Jordt *et al*. 2004; Wang and Woolf 2005). Work with neuronal cell cultures have also provided molecular evidence suggesting that allicin is the main sulfur compound in garlic that excites allylisothiocyanate-sensitive sensory neurons as well as activates TRPA1 and the related TRPV1 ion channels (Bautista *et al*. 2005; Macperson *et al*. 2005) which are present in pain-sensing neurons. Induction of pain could have significantly contributed to insect mortality by causing considerable stress to the maize weevils. Finally, allicin has been shown to be readily membrane permeable, and thus able to rapidly penetrate cellular compartments in biological systems (Miron *et al.* 2000). This attribute is of immense essence to the use of garlic juice as a biopesticide.

Conclusions

The present findings suggest that freshly prepared garlic juice which has allicin as its main biologically active compound, possesses a potentially vital insecticidal effect on *S. zeamais* when compared with the control. Thus, garlic offers significant promise for combating the threat posed by maize weevils to farmers in developing countries. This work is of major importance because it shows how small scale farmers plagued by the challenge of not being able to afford the conventional pesticides on the market. With no need for the more complex and sophisticated production of essential oils, this work's simplicity expressly lends zest to the overarching essence of providing a quick and easy solution to the problem of pest infestation in third world countries. There is need for further investigations to identify the other garlic juice constituents (apart from allicin) with toxic effects on *S. zeamais*, and to elucidate the precise mechanisms by which they exert their insecticidal effects.

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