Termiticidal and Nematicidal activities of five extracts from Garlic (Allium sativum)

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Abstract. Garlic (Allium sativum Linn) has been known and used as therapeutic agents to prevent several pathologies diseases. The aim of this study is to evaluate the effects of five garlic extracts, namely, aqueous garlic extract (AGE), methanol garlic extract (MGE), ethyl acetate garlic extract (EAGE), and n-hexane garlic extract (HGE) against root-knot nematode, Meloidogyne sp. and termite, Nasutitermes sp. The nematicidal and termiticidal activities of five garlic extracts were performed at concentration of 10; 25; and 50%. The nematicidal assay revealed that aqueous garlic extract (AGE) and ethyl acetate garlic extract (EAGE) have higher activities against Meloidogyne sp at all concentrations tested at 5 h of time incubation with the percentages of viabilities between 4.67%-15.63% and 0%-10.23% respectively. Meanwhile, the termiticidal assay results showed that aqueous garlic extract (AGE) and ethyl acetate garlic extract (EAGE), and n-hexane garlic extract (HGE) have stronger activity against Meloidogyne sp at all concentrations tested at 8 h of time incubation with the percentages of viabilities of the extracts were 0%. Overall, the results showed that ethyl acetate garlic extract (EAGE) has highest activity against Meloidogyne sp, while the n-hexane garlic extract (HGE) has strongest activity against Nasutitermes sp.

Keywords: Garlic (Allium sativum Linn), Meloidogyne sp, and Nasutitermes sp.

1. Introduction

Root-knot nematodes are parasitic worms that capable to infect more than 5000 of plant species [1] and can cause declining of the quality and the quantity of agricultural products [2, 3]. In several countries, using of nematicidal compounds such as methyl bromide, ethylene dibromide, and dibromo chloropropane as insecticides have been banned due to their effects to the environmental and health [4, 5].

Termites, in addition, are pests and destructive insects and also become a very serious problem to agricultures and housing, particularly to materials containing cellulose. Nevertheless, termites are also capable destroying non-cellulosic materials such as asbestos and aspal bituminous [6, 7].
Currently, several natural products have been reported to possess nematicidal and termiticidal activity. *Myrtus communis* [8], *Fumaria palviflora* [3], *Arisaema erubescens* [9], and *Ailanthus altissine* were reported to have nematicidal activities. In addition, the essential oils from *Verbesina encelioides* [8], *Kadsura heteroclite* [10], *Origamun vulgare* and *Mentha pulegium*, *Foeniculum vulgare* and *Pimpinella anisum* have also been reported to have nematicidal activity against root-knot nematodes. Meanwhile, the essential oils from *Cymbopogon citratus*, *Eucalyptus globulus*, *Syzygium aromaticum*, *Origamun vulgare*, *Rosmarinus officinalis*, *Cinnamomum verum* and *Tymus vulgaris* have also been reported to have activity as termiticidal [4].

Several studies have been reported that extract of garlic (*Allium sativum*) and other *Allium* species are known to have antibacterial, antifungal, and nematicidal activities [11]. S-allylcysteine sulphoxide is an organosulfur compound, also known as allicin, is a major compound is believed to be responsible in some garlic activities such as antibiotics, antimicrobials, and antifungal. It has been known that garlic is to be rich in the content of several organosulfur compounds such as diallysulfide (DAS), diallyldisulfide (DADS), diallyltrisulfide (DATS), and vinylthiin. These organosulfur compounds have been reported as phytoprotectants and green pesticides [12]. The garlic extract also have been reported effective killing *Caenorhabditis elegans* worms. Meanwhile, the methanolic extract of garlic was reported effective against *Aphelenchoides sacchari* on mushrooms and *Aphelenchoides grayi* on carrots [11]. In the meantime, limited report about the activity of the garlic extract on termites especially on *Nasutitermes* species. The aim of this study is to evaluate the effects of five garlic extracts such as aqueous garlic extract (AGE), methanolic garlic extract (MGE), ethyl acetate garlic extract, EAGE, and n-hexane garlic extract (HGE) against root-knot nematode, *Meloidogyne* sp. and termites, *Nasutitermes* sp.

### 2. Materials and Methods

#### 2.1. Materials

Garlic (*Allium sativum*) was purchased from a traditional market, Lambaro, Aceh Besar district, Aceh Province, Indonesia. Ethanol, methanol, ethyl acetate, n-hexane, chloroform, paper disc, hydrochloric acid, FeCl₃ were purchased from PT. Kairos, Yogyakarta. Mg powder, Wagner, Dragendorf, and Mayer reagents were provided from Department of Chemistry, Universitas Syiah Kuala. The root-knot nematodes (*Meloidogyne* sp) were isolated from celery plant. The termites (*Nasutitermes* sp) were used in this study were collected from the ambarella plant (*Spondias* sp).

#### 2.2. Preparation of garlic simplicial

Fresh of garlic were peeled, washed with tap water and weighed as much as 500 g and then dried at room temperature (25-29 °C) for 4 weeks to produce the simplicial of garlic. This simplicial then used to prepare of garlic extracts.

#### 2.3. Preparation of aqueous garlic extract (AGE)

One hundred grams of fresh garlic was destructed in 150 mL of sodium chloride solution (0.9%) using a mixer at a maximum speed for 10 minutes. The solution was then filtered using cheesecloth to obtain the filtrate of garlic. The filtrate was centrifuged at 4000 rpm for 10 minutes in cold condition to obtain the aqueous garlic extract (AGE). The extract was dissolved in 100 mL of sodium chloride solution (0.9%) to give a concentration of AGE of 1000 mg/ml. This extract was then stored at -20°C for further analysis [13].

#### 2.4. Preparation of garlic extracts

Three hundred grams of the simplicial of garlic was macerated with 1500 mL of n-hexane for 7 days at room temperature. The macerate was filtered to obtain a macerate of garlic hexane, while the residue was macerated successively using 1500 mL of ethyl acetate and methanol respectively to obtain the macerates of ethyl acetate and methanol. All organic macerates evaporated using vacuum rotary
evaporator to produce crude extracts of *n*-hexane (HGE), ethyl acetate (EAGE), and methanol (MGE). All the extracts then stored at -20°C for further analysis [13, 14].

2.5. Phytochemicals Screening of Garlic Extracts
The methods described by Harborne [14] were used for the phytochemical test. The phytochemical screening of aqueous garlic extract (AGE), methanol garlic extract (MGE), ethyl acetate garlic extract, EAGE), and *n*-hexane garlic extract (HGE) in this study including alkaloids, flavonoids, saponins, and tannins.

2.6. Nematicial Assay
2.6.1. Isolation of *Melodogyne* sp. The rounded parts (also called *gall*) from the root of celery were removed and weighed as much as 24 grams. Furthermore, prepared 24 of test tubes and inserted one gram of *gall* to each of test tube, then added 5 ml of sterile water for each test tube to obtain the suspension of *Melodogyne* sp. The suspension then filtered and counted the number of nematodes using a nemacytometer under a microscope.

2.6.2. Activity of *Meloidogyne* sp. The nematicidal activity of the aqueous garlic extract (AGE), methanol garlic extract (MGE), ethyl acetate garlic extract, EAGE), and *n*-hexane garlic extract (HGE) against *Melodogyne* sp was performed based on method described by Huang et al.[15] with a slight modification. Briefly, each of garlic extract was prepared at various concentrations of 10; 25; and 50% and 2 mL of each concentration of the extract was placed in the test tube and stand for 5 minute to evaporate the solvent. Furthermore, 1 mL of suspension of *Melodogyne* sp inserted into the test tube containing of the extract, then incubated for 3 and 5 hours. After incubation, the percentage viability of *Melodogyne* sp was counted to determine the viability of the nematodes. The viability of the nematodes of each concentration is expressed in the percentage of viability (%) of *t*3 and *t*5. In this assay, the test were performed in three replicates, and the curator (carbofuran) used as a positive control. The percentage of viability is expressed as the equation below.

\[ \text{Viability (\%)} = \frac{t_3 - t_0}{t_0} \times 100 \]

2.7. Termiticidal Assay
The termiticidal activities of aqueous garlic extract (AGE), methanol garlic extract (MGE), ethyl acetate garlic extract, EAGE), and *n*-hexane garlic extract (HGE) were performed against *Nasutitermes* sp. Briefly, each of garlic extract was prepared at various concentrations of 10; 25; and 50%. Two mL of each concentration of extract are sprayed using foliar spray above the petri dish that filled with wet-cotton (as moisturizer) and termites (10 termites per petri dish). The petri dish then incubated for 4 and 8 hours. After incubation, the viability of *Nasutitermes* sp was counted to determine the viability of termites. The viability of the termites of each concentration is expressed in the percentage of viability (%) of *t*4 and *t*8. In this assay, the test were performed in three replicates, and curator (carbofuran) used as a positive control. The percentage of viability is expressed as the equation below.

\[ \text{Viability (\%)} = \frac{t_4 - t_0}{t_0} \times 100 \]

3. Results and discussion
3.1. Phytochemicals Screening of Garlic Extracts
The phytochemical screening of garlic extracts obtained was presented in Table 1. The table shows that aqueous garlic extract (AGE) contain alkaloids and saponins. Methanol garlic extract (MGE) contains flavonoids and saponins. Ethyl acetate garlic extract (EAGE) contains alkaloids, flavonoids, and saponins. The *n*-hexane garlic extract (HGE) contain alkaloids.
Table 1. The results of phytochemical screening of the garlic (Allium sativum) extracts.

| Phytochemical constituent | AGE\textsuperscript{a} | MGE\textsuperscript{b} | EAGE\textsuperscript{c} | HGE\textsuperscript{d} |
|---------------------------|-------------------------|----------------------|-----------------------|----------------------|
| Alkaloids                 | +                       | -                    | +                     | +                    |
| Flavonoids                | -                       | +                    | +                     | -                    |
| Saponins                  | +                       | +                    | +                     | -                    |
| Tannins                   | -                       | -                    | -                     | -                    |

\textsuperscript{a}aqueous garlic extract (AGE), \textsuperscript{b}methanol garlic extract (MGE), \textsuperscript{c}ethyl acetate garlic extract (EAGE), \textsuperscript{d}n-hexane garlic extract (HGE), (+) = present of the secondary metabolites; (-) = absent of the secondary metabolites.

3.2. Nematicidal activity of garlic extracts against Meloidogyne sp

Garlic extract has been reported to be effective to killing of Caenorhabditis elegans and root-knot nematodes. Meanwhile, the methanol garlic extract are reported effective killing of Aphelenchoides sacchari in mushroom and Aphelenchoides grayi in carrot [11].

![Figure 1](image)

**Figure 1.** Isolation of root-knot nematodes Meloidogyne sp from gall of celery plant (Apium graveolens).

Garlic has been known rich of organosulfur compounds such as alliin, allicin, and some polysulfanes. These compounds have been known high activity against various types of diseases such as cancer diseases and its tendency as phytoprotectants and as “green pesticides”. In this study, the activity of garlic extracts were tested against root-knot nematode Meloidogyne sp that isolated from gall of celery (Apium graveolens)(see figure 1).

*Meloidogyne sp* is belongs to the family of Heteroderidae, order of Tylenchida, and Phylum of Nematodes [16]. *Meloidogyne sp* also named as parasitic nematodes, this nematode is found in gall in the root of plant of celery (gall is swelling part on the root of plant). This nematode is harmful in celery plant, and can cause plant disease such as wither, dwarf, and eventually the plant will be death within a few days.

The activity of garlic extracts are presented in table 2 and figure 2. In this assay, the garlic extracts were prepared in various concentrations of 10; 25; and 50%, and the cururat (carbofuran) was used as a positive control. Carbofuran is a nematicidal compound is commonly used for root-knot nematodes. In this study, the viability of nematodes of each concentration is expressed in the percentage of viability. The number of nematodes that live after 3 and 5 hours of time incubation are expressed as \( t_3 \) and \( t_5 \), while the number of nematodes living at the 0 hour is expressed as \( t_0 \).
Figure 2. The percentage of viability of garlic extracts against *Meloidogyne sp* after 3 and 5 hours of time incubation. In this assay, the significance of data is expressed on control carbofuran (CFN). Data significance are: ns = p≥0.05, * = p <0.05, ** = p <0.01, and *** = p <0.001. Data were analysed using Prism 5 software.
The nematicidal activity showed that ethyl acetate garlic extract (EAGE) had a higher activity or had a lower of percentage viability compared with AGE, HGE, and MGE at all of time incubation tested. The results also showed that EAGE more active than positive control (almost three time in activity compared with carbofuran as positive control). Meanwhile, the activity of EAGE at concentrations of 25 and 50% can kill all nematodes. Overall, the activity level of garlic extracts against root-knot nematodes of *Meloidogyne sp* is as follows EAGE>AGE>MGE>HGE.

### Table 2. The percentage viability of garlic extracts towards *Meloidogyne sp* after 3 and 5 hours of time incubation.

| Concentration of extract [%] | Incubation at 3 hours (t3) | The average of percentage of viability (%) |
|-----------------------------|---------------------------|------------------------------------------|
| Control                     | AGE<sup>a</sup>           | MGE<sup>b</sup>                          | EAGE<sup>c</sup> | HGE<sup>d</sup> |
| 10                          | 81,38                     | 63,24                                   | 66,15            | 65,81          |
| 25                          | 67,59                     | 69,89                                   | 46,54            | 78,37          |
| 50                          | 68,18                     | 70,62                                   | 21,62            | 79,12          |

| Concentration of extract [%] | Incubation at 5 hours (t5) | The average of percentage of viability (%) |
|-----------------------------|---------------------------|------------------------------------------|
| Control                     | AGE<sup>a</sup>           | MGE<sup>b</sup>                          | EAGE<sup>c</sup> | HGE<sup>d</sup> |
| 10                          | 15,63                     | 60,35                                   | 10,23            | 82,75          |
| 25                          | 43,79                     | 8,96                                    | 0                | 54,39          |
| 50                          | 4,67                      | 48,71                                   | 0                | 47,86          |

<sup>a</sup>aqueous garlic extract (AGE), <sup>b</sup> methanol garlic extract (MGE), <sup>c</sup> ethyl acetate garlic extract (EAGE), <sup>d</sup>n-hexane garlic extract (HGE), <sup>e</sup> positive control (carbofuran).

#### 3.3. Termiticidal activity of garlic extracts against *Nasutitermes sp*

In this study, the activities of garlic extracts were tested against termites. Termites are widely known as pests because they can damage buildings especially wooden buildings and agricultures products and cause many economic losses [6, 7].

![Figure 3. The termite species of *Nasutitermes spp.*](image-url)
The termite used in this study is species of *Nasutitermes* or *Nasutitermes* sp (see figure 3). *Nasutitermes* sp is type of insects and a member of the Isopteran order or order of Blattodea [17, 18]. In this study, the activity of garlic extracts was done by spraying garlic extracts at various concentrations (10, 25 and 50%) by using foliar spray. In this assay, butox was used as positive control, butox or deltametrin is a compound used to kill a parasitic organisms also called as ectoparasiticide and also used as antitermite drug and as insecticide. The activity of garlic extracts on *Nasutitermes* sp are listed in table 3 and the graph of viability of Nasutitermes are presented in figure 4.

Table 3 indicated that the hexane garlic extracts (HGE) has highest activity against *Nasutitermes* sp compared with other garlic extracts at 4 hours of time incubation. Meantime, at 8 hours of time incubation all of garlic extracts tested shows can killing all the termites, even more active than positive control. The activity level of garlic extracts against Nasutitermes sp is as follows HGE> AGE> EAGE> EGE.

The termiticidal activity of garlic extracts is presumably caused by the presence of organosulfur compounds, alkaloids, flavonoids, and saponins compound contained in garlic. The phytochemicals screening results showed that garlic extracts contains alkaloids, flavonoids and saponins (Table 1). The previous studies have been suggested that flavonoids [19] and alkaloids [7] were able to kill of *Carinatermes* sp.

**Table 3.** The percentage viability of garlic extracts towards *Meloidogyne sp* after 3 and 5 hours of time incubation.

| Concentration of extract [%] | Incubation at 4 hours (*t*₄) | Incubation at 8 hours (*t*₈) |
|-------------------------------|-------------------------------|-------------------------------|
|                               | Controlᵃ | AGEᵇ | MGEᵇ | EAGEᶜ | HGEᵈ | Controlᵃ | AGEᵇ | MGEᵇ | EAGEᶜ | HGEᵈ |
| 10                             | 36,11    | 42,59 | 50,83 | 6,66   | 0     | 0         | 37,55 | 0     | 0     | 0     |
| 25                             | 66,66    | 8,33  | 54,23 | 28,51  | 0     | 2,66      | 26,98 | 0     | 0     | 0     |
| 50                             | 8,33     | 46,66 | 25,13 | 0      | 0     | 0         | 0     | 0     | 0     | 0     |

ᵃaqueous garlic extract (AGE),ᵇmethanol garlic extract (MGE),ᶜethyl acetate garlic extract (EAGE),ᵈn-hexane garlic extract (HGE),apositive control (butox).
Figure 4. The percentage of viability of garlic extracts against *Nasutitermes* sp after 4 and 8 hours of time incubation. In this assay, the significance of data is expressed on control butox. Data significance are: ns = p≥0.05, * = p <0.05, ** = p <0.01, and *** = p <0.001. Data were analysed using Prism 5 software.
4. Conclusion
The phytochemical screening showed that all the garlic extracts contain general secondary metabolites of flavonoids, alkaloids, and saponins. The nematicidal assay showed that ethyl acetate garlic extracts (EAGE) has highest activity against *Meloidogyne sp*. Meanwhile, the termiticidal assay revealed that *n*-hexane garlic extract (HGE) has strongest activity against *Nasutitermes sp*.

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