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

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

K Khairan[1,2,3]*, Aulina[4], N Yusra[4], C N Eriana[4], M Bahi[4], S Syaukani[5], R Sriwati[6] and C Jacob[7]

[1] Department of Pharmacy, Universitas Syiah Kuala, Banda Aceh, 23111, Indonesia
[2] Pusat Riset Obat Herbal, Universitas Syiah Kuala, Banda Aceh, 23111, Indonesia
[3] Atsiri Research Centre, Universitas Syiah Kuala, Banda Aceh, 23111, Indonesia
[4] Department of Chemistry, Universitas Syiah Kuala, Banda Aceh, 23111, Indonesia
[5] Department of Biology, Universitas Syiah Kuala, Banda Aceh, 23111, Indonesia
[6] Department of Plant Diseases, Universitas Syiah Kuala, Banda Aceh, 23111,Indonesia
[7] School of Pharmacy, Universitaet des Saarlandes, Saarbruecken, 66123, Germany

*E-mail: khairankhairan@unsyiah.ac.id

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 *Melodogyne 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), ethyl acetate garlic extract (EAGE), and *n*-hexane garlic extract (HGE) have stronger activity against *Melodogyne 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 have nematicidal activities. In addition, the essential oils from *Verbesina encelioides* [8], *Kadsura heteroclite* [10], *Origanum vulgare* and *Mentha pulegium*, *Foeniculum vulgare* and *Pimpinella anisum* have also been reported have nematicidal activity against root-knot nematodes. Meanwhile, the essential oils from *Cymbopogon citratus*, *Eucalyptus globulus*, *Syzygium aromaticum*, *Origanum 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), diallyltetrasulfide (DATTS), and vinyldithiins. 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 meantimes, limited report about the activity of the garlic extarct 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, $FeCl_3$ were purchased from PT. Kairos, Yogjakarta. Mg powder, Wagner, Dragendorf, and Mayer reagents were provided from Department of Chemistry, Universitas Syiah Kuala. The *root-knot nematodes* (*Melodogyne 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.

$$Viability\ (\%) = \frac{t_3}{t_0}\ x\ 100 \qquad Viability\ (\%) = \frac{t_5}{t_0}\ x\ 100$$

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.

$$Viability\ (\%) = \frac{t_4}{t_0}\ x\ 100 \qquad Viability\ (\%) = \frac{t_8}{t_0}\ x\ 100$$

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	Phytochemical contents			
	AGE[a]	MGE[b]	EAGE[c]	HGE[d]
Alkaloids	+	-	+	+
Flavonoids	-	+	+	-
Saponins	+	+	+	-
Tannins	-	-	-	-

[a]*aqueous garlic extract* (AGE), [b]*methanol garlic extract* (MGE), [c]*ethyl acetate garlic extract* (EAGE), [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. 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 currator (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_o.

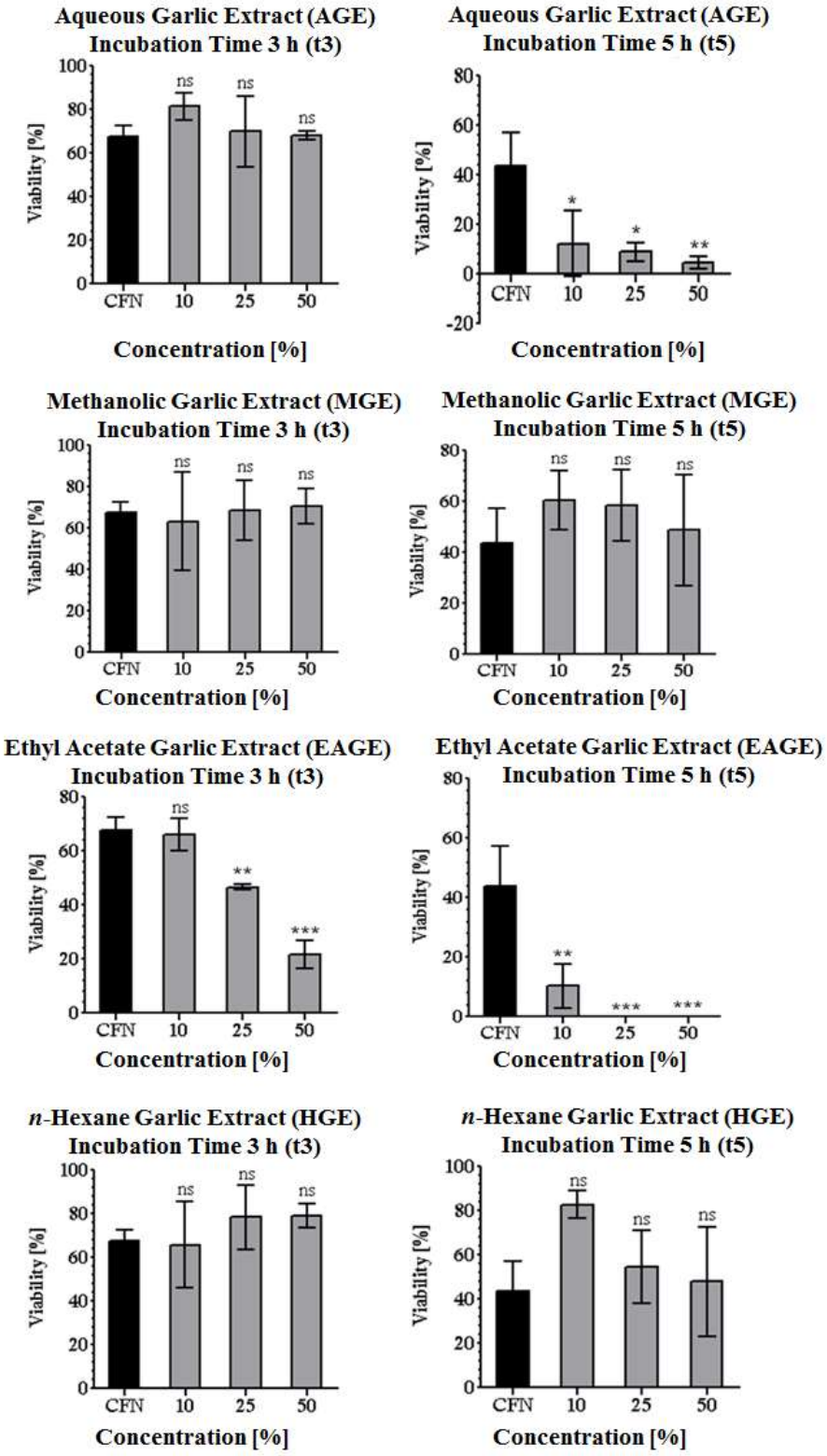

Figure 2. The percentage of viability of garlic extracts against *Meloidogyne sp* after 3 and 5 hours of time incubation. In this assay, the significance of data is expressed on control carbofuran (CFN). Data significance are: ns = p≥0,05, * = p <0.05, ** = p <0,01, and *** = p <0.001. Data were analysed 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 [%]	The average of percentage of viability (%)				
	Incubation at 3 hours (t_3)				
	Control[e]	AGE[a]	MGE[b]	EAGE[c]	HGE[d]
10		81,38	63,24	66,15	65,81
25	67,59	69,89	68,55	46,54	78,37
50		68,18	70,62	21,62	79,12
Concentration of extract [%]	Incubation at 5 hours (t_5)				
	Control[e]	AGE[a]	MGE[b]	EAGE[c]	HGE[d]
10		15,63	60,35	10,23	82,75
25	43,79	8,96	58,17	0	54,39
50		4,67	48,71	0	47,86

[a]*aqueous garlic extract* (AGE), [b]*methanol garlic extract* (MGE), [c]*ethyl acetate garlic extract* (EAGE), [d]*n-hexane garlic extract* (HGE), [e]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.*

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 [%]	The average of percentage of viability (%)				
	Incubation at 4 hours (t_4)				
	Control[e]	AGE[a]	MGE[b]	EAGE[c]	HGE[d]
10	66,66	36,11	42,59	50,83	6,66
25		8,33	54,23	28,51	0
50		8,33	46,66	25,13	0
Concentration of extract [%]	Incubation at 8 hours (t_8)				
	Control[e]	AGE[a]	MGE[b]	EAGE[c]	HGE[d]
10	2,66	0	37,55	0	0
25		0	26,98	0	0
50		0	0	0	0

[a]*aqueous garlic extract* (AGE), [b]*methanol garlic extract* (MGE), [c]*ethyl acetate garlic extract* (EAGE), [d]*n-hexane garlic extract* (HGE), [e]positive control (butox).

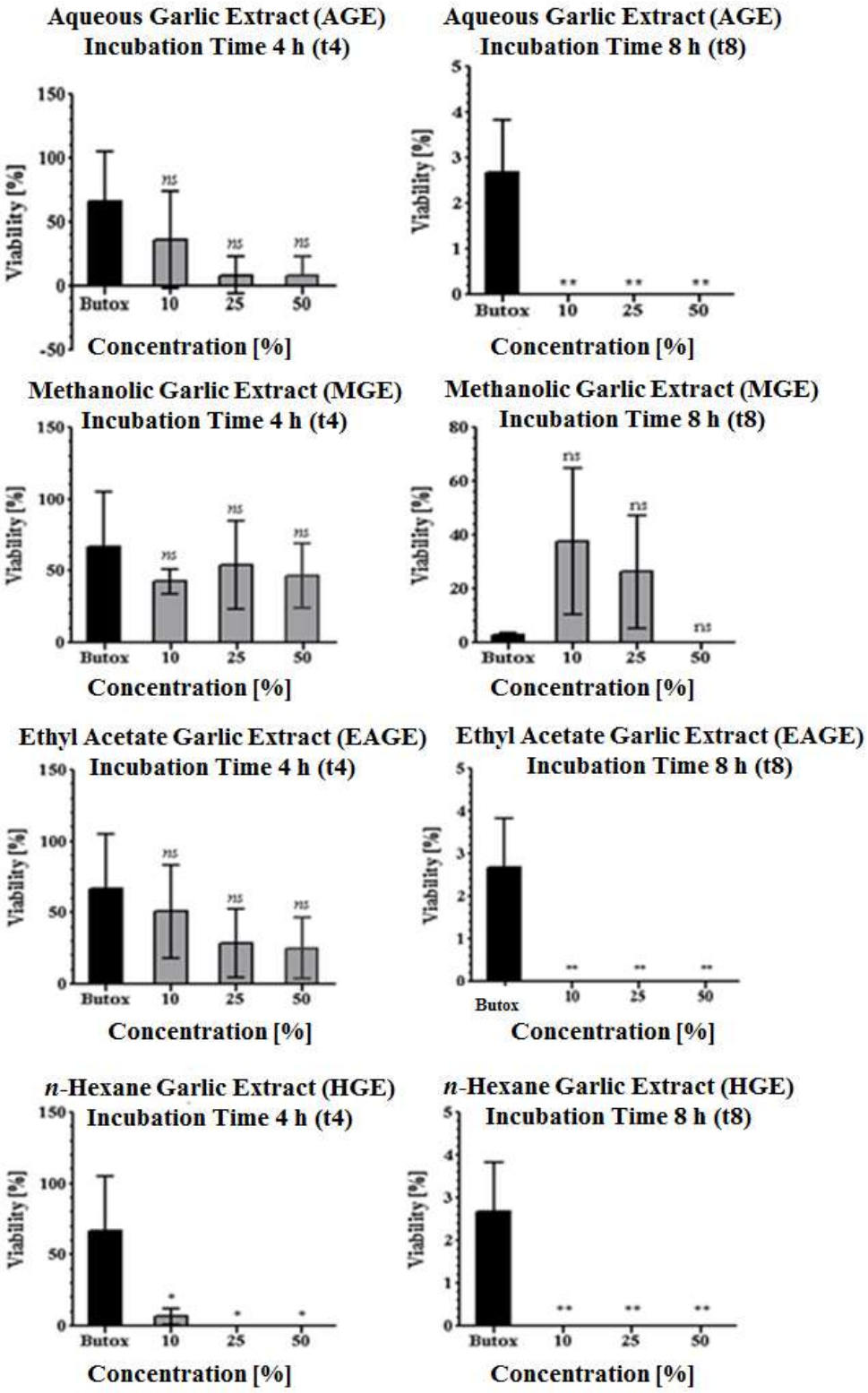

Figure 4. The percentage of viability of garlic extracts against *Nasutitermes sp* after 4 and 8 hours of time incubation. In this assay, the significance of data is expressed on control butox. Data significance are: ns = p≥0,05, * = p <0.05, ** = p <0,01, and *** = p <0.001. Data were analysed using 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.*

5. Acknowledgement

We thank the Scientific Research Support Fund of the Ministry of Higher Education and Scientific Research-Indonesia for the funding.

References

[1] Jaouannet M, Magliano M, Arguel M J, Gourgues M, Evangelisti E, Abad P and Rosso M N 2013 The root-knot nematode calreticulin Mi-CRT is a key effector in plant defence suppression *Molecular Plant-Microbe Interactions* **26** 97–105.

[2] Dang Q L, Kim W K, Nguyen C M, Choi Y H, Choi G J, Jang K S, Park M S, Lim C H, Lu N H and Kim J 2011 Nematicidal and antifungal activities of annonaceous acetogenins from *Annona squamosa* against various plant pathogens *Journal of Agricultural and Food Chemistry* **59** 11160–11167.

[3] Naz I, Saifullah and Khan M R 2013b Nematicidal activity of nonacosane-10-ol and 23a-Homostigmast-5-en-3β-ol isolated from the roots of *Fumaria parviflora* (Fumariaceae) *Journal of Agricultural and Food Chemistry* **61** 5689–5695

[4] Ntalli N G, Ferrari F, Giannakou I and Menkissoglu-Spiroudi U 2011 Synergistic and antagonistic interactions of terpenes against *Meloidogyne incognita* and the nematicidal activity of essential oils from seven plants indigenous to Greece *Pest Management Science* **67** 341–351

[5] Andrés M F, Gonzalez-Coloma A, Sanz J, Burillo J and Sainz P 2012 Nematicidal activity of essential oils: A review *Phytochemistry Reviews* **11** 371–390

[6] Bultman J D, Beal R H and Ampong F F K 1979 Natural resistance of some tropical African woods to coptotermes formosanus shiraki *Journal of Forest Products* **29** 46-51

[7] Verma M, Sharma S and Prasad R 2009 Biological alternatives for termite control: a review *International Bio deterioration & Bio degradation* **63** 959-972

[8] Oka Y 2012 Nematicidal activity of *Verbesina encelioides* against the root-knot nematode *Meloidogyne javanica* and effects on plant growth *Plant Soil* **355** 311–322

[9] Du S S, Zhang H M, Bai C Q, Wang C F, Liu Q Z, Liu Z L, Wang Y Y and Deng Z W 2011 Nematicidal flavone-glycosides against the root-knot nematode (*Meloidogyne incognita*) from *Arisaema erubescens* Tubers *Molecules* **16** 5079–5086.

[10] Li H Q, Bai C Q, Chu S S, Zhou L, Du S S, Liu Z L and Liu Q Z 2011 Chemical composition and toxicities of the essential oil derived from *Kadsura heteroclita* stems against *Sitophilus zeamais* and *Meloidogyne incognita Journal of Medicinal Plants Research* **5** 4943–4948.

[11] Block E 2010 *Garlic and other Allium: The lore and the science* (Cambridge, United Kingdom: The Royal Society of Chemistry)

[12] Jacob C 2006 A scent of therapy: pharmacological implications of natural products containing redox-active sulfur atoms *Natural Product Reports* **23** 851-863

[13] Mahesar H, Bhutto M A, Khand A A and Narejo N T 2010 Garlic used as an alternative medicine to control diabetic mellitus in alloxan-induced male rabbits *Pak. J. Physiol* **6** 1 39-41

[14] Harborne J B 1987 *Phytochemical method: A Guide to Modern Techniquues of Plant Analysis* (Bandung: Bandung Institute Technology Press)

[15] Huang YH, Mao ZC and Xie BY 2016 Chinese leek (*Allium tuberosum* Rottler ex Sprengel) reduced disease symptom caused by root-knot nematode *Journal of Integrative Agriculture* **15** 2 364–372

[16] Agrios G 2007 *Teknologi bahan alam* (Bandung: Institut Teknologi Bandung Press)
[17] Krishna K, Grimaldi, David A, Krishna, Valerie, Engel and Michael S 2013 Treatise on the Isoptera of the world *Bulletin of the American Museum of Natural History* **377** 1–2704.
[18] Beccaloni, George, Eggleton, Paul and Zhang ZQ ed 2013 *Order Blattodea, animal biodiversity: An outline of higher-level classification and survey of taxonomic Richness* **1** 46–48.
[19] Il Kwon P, Ju-Yong P, Kyung Hee K I M, Kwang S C, In Ho C, Chul S K and Sang C S 2005 Nematicidal activity of plant essential oils and components from garlic (*Allium sativum*) and cinnamon (*Cinnamomum verum*) oils against the pine wood nematode (*Bursaphelenchus xylophilus*) *Nematology* **7** 5 767-774