International Journal of Mosquito Research

ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2022; 9(6): 131-139

www.dipterajournal.com
Received: 19-08-2022
Accepted: 22-09-2022

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Larvicidal activity of *Allium sativum* against *Aedes*, *Anopheles*, and *Culex* spp.: A systematic review

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DOI: https://doi.org/10.22271/23487941.2022.v9.i6b.649

Abstract
Over the years, mosquitoes have developed a resistance to synthetic household pesticides, and this caused a significant decline in standard protocols against vector-borne diseases, forcing the rest of the world to create alternative solutions. In response to the growing need for environmentally friendly alternatives for synthetic insecticides, this review was initiated to collect a valid information to authenticate and support the claims of the effectiveness of garlic (Allium sativum) as a toxic agent against common mosquito species. The gathered data showed that garlic possess toxic characteristics needed against mosquitoes, even at lower concentrations. The researchers concluded that garlic is undoubtedly potent against Anopheles and Culex spp. and could be a significant ingredient in future plant-based pesticides. These findings suggest the application of garlic in pest management and vector control programs.

Keywords: *Allium sativum*, *Aedes* spp., *Anopheles* spp., *Culex* spp., larvicidal activity, biopesticide

1. Introduction

Since 2014, major outbreaks of mosquito-borne diseases such as malaria and dengue endemically occurred in many countries, which caused the health systems to stagger. Currently, 219 million malaria cases are recorded, and 400,000 infections yearly. Medically, mosquitoes are known as vectors of various diseases that affect people worldwide. Mosquitoes transmit these diseases to people and some animals through biting. The infected humans can also carry the disease to an uninfected mosquito when bitten by the vector. While humans can be uninfected again once treated, mosquitoes are inherently capable of spreading the disease throughout their lifetime. Certain mosquito species carry diseases; these are mosquito vectors: *Aedes*, *Anopheles*, or *Culex* spp. The *Aedes* spp. is the causative agent for diseases: chikungunya, dengue, lymphatic filariasis, rift valley fever, yellow fever, and Zika virus. On the other hand, *Anopheles* mosquitoes are responsible for lymphatic filariasis and malaria, while *Culex* mosquitoes are responsible for Japanese encephalitis, West Nile fever, and lymphatic filariasis [1].

The Philippines experienced the worst year of the Dengue epidemic last 1998. As the fastest spreading vector-borne disease, dengue became endemic in the Philippines along with malaria, which is rampant in Palawan [2]. In a report by the Department of Health of the Philippines (DOH) last 2012, dengue has a five-year average case of 185,008 with a 0.39 fatality rate and 732 five-year average death. Given these statistics, the government launched the National Dengue Prevention and Control Program to ensure a Dengue-free Philippines and reduce the morbidity rate by at least 25% by 2022. Inclusions of this program are Surveillance, Case Management and Diagnosis, Integrated Vector Management (IVM), Outbreak Response, Health Promotion, Advocacy, and Research [3]. The government also launched the National Malaria Control and Elimination Program targeting a malaria-free Philippines by 2030. These two programs are in continuous progression as the DOH reaffirms its commitment to eradicating dengue and malaria in the Philippines through preventive and case management measures [4].

At present, mosquitoes have already developed a resistance to synthetic pesticides, which contributes to the struggle of fighting against vector-borne diseases [5]. The standard protocols against vector-borne diseases have come to decline; thus, researchers have taken the initiative to conduct a study on the effectiveness of garlic. Studies have proven that garlic essential oil (EO) is toxic, thus, recommending the use of garlic as a natural larvicide against mosquitoes. Promising results from clinical trials have proven garlic as a potential alternative to synthetic insecticides used at home [6, 7].

Garlic, scientifically known as *Allium sativum*, is a member of the vast family of onions (Amaryllidaceae) and is classified into the same genus in which onions, chives, leek, and shallots belong. Historically known as an ingredient and spice for household use, this wonder plant contains a significant nutritional value and serves a wide array of medicinal benefits that ancient civilizations recognized [8]. Garlic has proven curative and pesticidal properties. Due to the actions of several sulfur-containing compounds with potent biological activity, it has long been recognized as a bactericide. These substances also prevent the growth of fungi. Garlic has both repellant and biocidal effects, making it a good pesticide [9].

Using biopesticides instead of commercially available synthetic pesticides reduces the impact on public health and the environment. There is a high demand to produce organic pesticides that are natural, safe to use, and eco-friendly for protection against vector mosquitoes [10]. In line with this, this review aims to investigate the larvicidal properties of *Allium sativum*, particularly its toxicity against vector mosquitoes: *Aedes*, *Anopheles*, and *Culex* spp., as a more environmentally friendly and inexpensive substitute for synthetic pesticides available on the market.

2. Materials and Methods

This systematic review follows the 2020 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Guidelines for selecting studies. Literature review articles are obtained via online searches from international journal databases such as ScienceDirect, PubMed, and ProQuest. The keywords used to obtain the studies were 1) "larvicidal" OR "toxicity," 2) "mosquito," 3) "*Aedes*" "OR "*Anopheles*" OR "*Culex*," AND 4" "garlic" OR "*Allium sativum*."

In checking for eligibility included in this review, researchers chose articles that correspond to the aims and objectives of the study. Literature included topics published in journal articles from January 2012 to August 2022. The selected sources are studies regarding (1) the larvicidal activity of garlic, (2) clinical trials or experimental studies, and (3) mortality or toxicity against vector mosquitoes. This systematic review did not include studies that (1) evaluate the repellent activity of garlic; are (2) mini-reviews or systematic reviews; (3) surveys or prospective; (4) grey literature or unpublished articles; (5) book chapters; (7) studies on patient treatment; and (7) non-English articles.

The identified articles were screened and evaluated based on their title, abstract, and full text using the inclusion and exclusion criteria. Seven authors extracted information such as (1) Author and Year of Publication, (2) Component/Constituent Used, (3) Test Organism, and (4) Key Findings. The other two authors were assigned to check the accuracy of each piece of data gathered.

The literature search was individually done by five authors on the databases using the given search terms. The first and second rounds of screening were done by two independent authors and one independent author, respectively, based on the title and abstract. The final screening was done by two authors based on the full-text articles to ensure that the eligibility criteria were followed. In case of conflicts, whether to include an article, a third author is included for consensus. Although not a criterion for excluding an article, the included articles were further evaluated by two authors for risk of bias using the JBI critical appraisal tool for randomized controlled trials. A point is given for each specific criterion an article fulfils. The corresponding score for each criterion, raw score, percentage, and risk of each article included are shown in Table S1.

3. Results

Out of 91 articles identified, only nine studies were eligible for this systematic review (Fig. 1). The summary of the data extracted from each article included is shown in Table 1. Four studies evaluated the toxicity of garlic against *Culex* spp., five studies against *Aedes* spp., and no studies about *Anopheles* spp. were included.

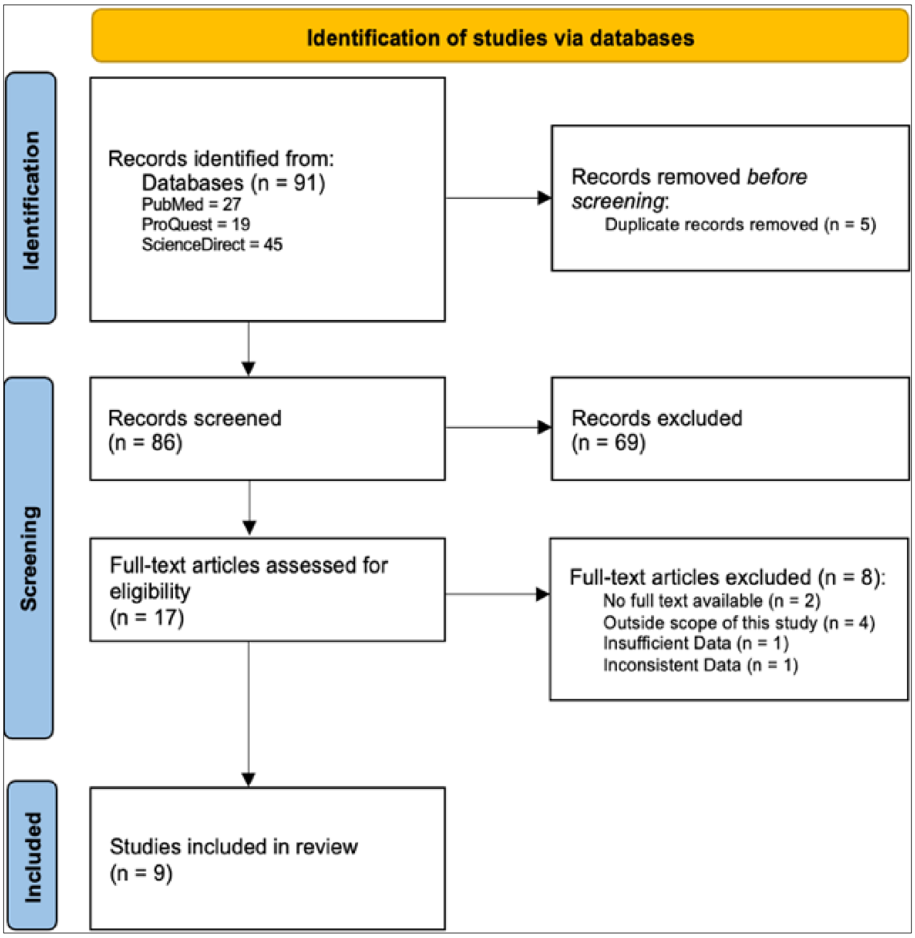

Fig 1: Study Selection Flowchart

3.1 Garlic Phytochemical Screening

Two studies used Gas Chromatography-Mass Spectrometry (GC-MS) analysis to identify the primary active components in garlic bulbs EO. Diallyl disulfide and diallyl trisulfide were the two primary compounds that both studies identified [6, 11]. These results are consistent with the study of Mahanta and Khanikor but differ in the material they used since the study used a mixture of *Allium sativum* (bulbs) + *Citrus paradisi* (peels) and *Allium sativum* (bulbs) + *Citrus paradisi* (leaves). Still, the study recovered diallyl trisulfide and diallyl disulfide as the primary components, with garlic yielding the highest amount of the two compounds than the other components tested [12].

Different compounds were observed in two other studies [13, 14]. In the first study, active compounds were analyzed using GC-MS and High-Performance Liquid Chromatography (HPLC). Present compounds in *A. sativum* were 9-Octadecenamide, (Z)-, trisulfide, di-2-propenyl, and Isochiapin B %2<; [13] while in the second study, screening using organic solvents was able to detect the presence of alkaloids, flavonoids, cardiac glycosides, terpenes, steroids, and resin in garlic leaf extracts [14].

3.2 Analysis of the Larvicidal Activity of Garlic

All studies included evaluated the larvicidal activity of garlic. Studies showed that components and composition of garlic, such as leaves (aqueous extract), essential oils, and emulsions based on garlic essential oils, are highly toxic to mosquito larvae [6, 11-18].

According to two studies, garlic was highly potent against the larvae of *Ae. Albopictus* [15, 16]. Three studies also studied the activity of garlic against various stages *Ae. aegypt*i larvae [17, 18, 11]. Results were observed at 24 hrs post-treatment (PT); among the three studies, the highest toxicity was shown in the former study, with garlic EOs having the least LC_{50} against the third instar larvae of *Ae. Aegypti* [17] followed by diallyl trisulfide against the fourth instar larvae of *Ae. Aegypti* [11].

At 24 hrs PT, the highest toxicities were observed by using garlic leaf aqueous and ethanolic extracts against the fourth instar larvae of *Culex quinquefasciatus* and garlic bulb EOs against the late third instar larvae of *Culex restuans* [14, 6]. The ovicidal activity was also noted against the egg rafts of either *Cx. pipiens* or *Cx. restuans*. The experiment was observed for seven days at five ppm, resulting in a 0% hatch rate and survival among the egg rafts [6]. Results are summarized in Table 2.

3.3 Garlic Toxicity against Adult Mosquitoes

Three studies included testing against adult mosquitoes [11, 12, 13]. At 24 hrs PT, garlic Eos exhibited the least LC_{50} and diallyl trisulfide with the highest LC_{50} against adult *Ae. Aegypti* [11]. The results are supported in another study which stated that *Allium sativum* possessed a high adulticidal activity against *Cx. Quinquefasciatus* but with a higher dose [12]. *A. sativum* EO caused mortality but is one of the least effective EO against *Cx. Pipiens* [13].

3.4 Toxicity of Garlic in O/W Emulsion

Only one study evaluated the effect of garlic Oil-in-Water (O/W) emulsions [17]. The low solubility of garlic EO in water affects its toxicity against mosquitoes. Compared to the LC_{50} of garlic EO, garlic in O/W emulsions produced greater toxicity against the third instar larvae of *Ae. aegypti*. LC_{50} from Garlic/Amylose-Hex-Am powder, Garlic/Amylose-Hex-Am solution, Garlic/Sodium palmitate powder, and Garlic/Sodium palmitate solution emulsion resulted in a 0.71 ppm decrease from the garlic treatment. Encapsulating garlic oil in emulsifiers increases its pesticidal activity and stability in an aqueous environment [17].

Table 1: Summary of the Included Studies

Reference	Component/ Constituent Used	Test Organism	Key Findings	Risk of Bias*
[13]	Garlic EO	Early fourth larvae and 3-4 days old adult *Cx. pipiens*	Garlic was labelled as H (highly effective), with 95-100% mortality, against the fourth instar larvae of *Cx. pipiens* observed after 48 hours PT. In this study, phytochemical analysis by GC-MS and HPLC revealed active compounds in *Allium sativum*, this includes 9-Octadecenamide, (Z)- (29.07%), Trisulfide, di-2-propenyl (14.86), and I B %2< (8.63%).	L
[15]	Garlic rhizome (1% garlic oil in acetone solution)	Third instar larvae of A*e. albopictus*	Garlic caused high mortality in the larval stages of *Ae. albopictus.* 50% mortality was observed after more than two days.	L
[16]	Garlic crude extract, Garlic AgNPs	Third and fourth instar larvae of *Ae. albopictus*	All AgNPs counterparts of the plant extracts exhibited greater activity against *Ae. albopictus* in terms of mortality.	L
[14]	Garlic leaves, aqueous extracts, garlic leaves ethanolic extracts	Fourth instar larvae of *Cx. quinquefasciatus*	100% larval mortality against the fourth instar larvae of *Cx. quinquefasciatus* was observed after three days. The ethanolic extracts induced significant mortality against *Cx. quinquefasciatus* than aqueous extracts of the plant species studied. The presence of alkaloids, flavonoids, cardiac glycosides, terpenes and steroids, and resin in garlic leaves extracts was obtained using organic solvents.	L
[12]	Garlic EO in Dimethyl Sulphoxide (DMSO), Garlic bulb EO in acetone, Garlic bulb EO + *Citrus paradisi* leaves EO, Garlic bulb EO + *Ocimum sanctum* leaves EO, Garlic bulb EO + *Citrus grandis* (peels) EO, Garlic bulb EO + *Citrus grandis* (leaves) EO, Garlic bulb EO + *Mentha piperita* (leaves) EO, Garlic bulb EO + Temephos	Third instar larvae and 4-5 days old adult female of *Cx. quinquefasciatus*	At volume ratios 1:1, 1:2, 2:1, and 3:1, binary mixtures of garlic EO and oils from leaves of *Citrus paradisi*, *Ocimum sanctum*, and *Citrus grandis* showed a synergistic effect against third instar larvae of *Cx. quinquefasciatus.* Meanwhile, *Martha piperita* leaves EO only showed a synergistic effect on ratios 2:1 and 3:1, and an antagonistic effect on 1:1 and 1:2. Both EOs from *Citrus grandis* and *Martha piperita* leaves showed no effect on the 1:3 volume ratio. On the adulticidal assay, EOs from garlic bulbs along with *Citrus paradisi* peels showed a synergistic effect on ratios 1:1 and 1:2. EOs from garlic bulbs and *Aegle marmelos* leaves showed a synergistic effect on ratios 1:2 and 1:3, and antagonistic effect on ratio 3:1. Binary mixture of EOs of garlic bulbs and *Eucalyptus maculata* leaves showed a synergistic effect on ratio 1:1, and an antagonistic effect on ratios 2:1 and 3:1. Using GC-MS analysis, major active constituents of 1:1 binary mixture of *Allium sativum* (bulbs) + *Citrus paradisi* (peels) oil mixture were discovered. These components are iallyl trisulfide (34.98%), diallyl disulfide (32.15%), and Beta citronellol (12.21%). On the 1:1 binary mixture of *Allium sativum* (bulbs) + *Citrus paradisi* (leaves) oil mixture, major compounds were found to be diallyl disulfide (32.75%), citronellal (23.64%), and diallyl trisulfide (16.455).	M
[6]	Garlic bulb EO, Allyl disulphide, Garlic bulb EO + Asafoetida stem and roots EO	Late third instar larvae and egg rafts of *Cx. pipiens* and *Cx. restuans*	The larvae of *Culex restuans* were more sensitive to garlic EO than *Culex pipiens.* Allyl disulphide, compound from garlic and asafoetida mixture, is highly toxic to the larval stage of both *Culex* spp. Garlic and its major component, allyl disulphide, were able to inhibit the growth of *Cx. pipiens* and *Cx. restuans* eggs. The two EOs worked antagonistically with each other. Major compounds found in GC-MS analysis of garlic EO are allyl disulphide (49.13%), diallyl trisulfide (31.08%), and diallyl tetrasulfide (11.01%).	M
[17]	Garlic EO, Garlic/Amylose-Hex-Am powder, Garlic/Sodium palmitate powder, Garlic/Amylose-Hex-Am solution, Garlic/Sodium palmitate solution	Late third instar larvae of *Ae. aegypti*	Garlic oil amylose emulsion increased the toxicity of garlic against the third instar larvae of *Ae. aegypti.*	M

[18]	Garlic bulb acetone extracts, Garlic AgNPs	Second and third instar larvae of *Ae. aegypti*	Garlic bulb extract requires a higher concentration at all exposure times than garlic silver nanoparticles to kill 50% of the population. At 250 ppm, 100% mortality was observed after 48 hrs for both larval instars using garlic AgNPs.	M
[11]	Garlic bulb EO, Diallyl Disulfide, Diallyl Trisulfide, Diallyl Disulfide + Limonene, Diallyl Disulfide + Temephos	Fourth instar larvae and 3-4 days old adult *Ae. aegypti*	At all exposure times, garlic EO is more toxic against the fourth instar larvae of *Ae. aegypti* than adult *Ae. aegypti.* Using GC-MS, two major compounds were identified from garlic EO namely, diallyl disulfide (8.51%) and diallyl trisulfide (7.75%). Diallyl disulfide and diallyl trisulfide compounds showed an antagonistic effect with each other.	L

*L: Low-Risk; M: Moderate Risk; H: High-Risk

3.5 Garlic Efficacy in Binary Mixtures

Among the three studies that evaluated the efficacy of garlic in binary mixtures [6, 11, 12], two studies claimed that garlic combined with different substances could improve its toxicity. Synergistic effect was observed when diallyl disulfide was mixed with other compounds, such as eucalyptol on a smaller scale and limonene and temephos on small and large populations of the fourth instar larvae of *Ae. aegypti* [11]. Garlic EOs mixed with oils from leaves of *Citrus paradisi*, *Ocimum sanctum*, and *Citrus grandis* against the third instar larvae of *Cx. quinquefasciatus* at volume ratios 1:1, 1:2, 2:1, and 3:1 also exhibited the same effect [12]. These combinations showed synergistic effect, thus making the mixture more potent against mosquitoes than using garlic alone. However, in the same study, the two primary compounds screened, diallyl disulfide and diallyl trisulfide, were combined and tested against the third instar larvae of *Ae. aegypti* and showed antagonistic effects, although used in equal ratios [11]. The same effect when the EOs of garlic and asafoetida are mixed and used against the third instar larvae of *Cx. pipiens* and *Cx. restuans* [6].

The EOs from *A. sativum* (bulbs) and *C. paradisi* (leaves) were evaluated but showed no improvement when used together or individually against the third instar larvae of *Cx. quinquefasciatus*. Results changed when the binary mixture was tested against adult *Cx. quinquefasciatus*; it was noted that both EOs worked synergistically at 1:1 and 1:2 volume ratios [12]. Table 3 summarizes the effects of garlic in a binary mixture.

3.6 Synthesis of Silver Nanoparticles (AgNPs) from Garlic and its Activity against Mosquitoes

Increasing agrochemical resistance has impacted the efficacy of frequently used chemicals against common vectors such as *Ae. albopictus*. Garlic is rich in secondary metabolites, which are responsible for reducing and capping silver ions, making garlic an excellent plant material for AgNP synthesis [18]. The AgNPs made from plant material are remarkably effective against the larval stage of various mosquito species [16]. The latter studied the effects of garlic crude extract and garlic green synthesized AgNPs to produce an environmentally friendly and economically alternative biopesticide. The evaluation of AgNPs from garlic against the third and fourth instar larvae of *Ae. albopictus* revealed that treatment with garlic-based AgNPs displayed even higher toxicity than garlic extract [16]. Against *Ae. aegypti* wherein at maximum ppm and 48 hrs PT, AgNPs from garlic bulb extract resulted in 100% mortality for both second and third instar larvae, and 99% and 98% mortality for the second and third instar larvae, respectively. The ability of AgNPs to penetrate the mosquito's exoskeleton allows it to function more effectively than garlic EOs [18].

4. Discussion

The findings from this systematic review presented that garlic is toxic to vector mosquitoes. Garlic was highly potent against the third instar larvae of *Ae. albopticus* [15, 16]. However, among the 11 plant extracts evaluated, garlic crude extract was the least effective when compared with bakain and neem extracts against the third and fourth instar larvae.[16] A higher dose was also observed against larvae and adult *Cx. quinquefasciatus* [12]. This study was countered when researchers observed the least LD_{50} against the fourth instar larvae of *Cx. quinquefasciatus* [14].

Table 2: Larvicidal and Adulticidal Activity of Garlic

S No.	Component/ Constituent Used	Target Organism	Results
1[13]	Garlic EO	Early fourth larvae of *Cx. pipiens* (n = 60)	LT50 = 13.95 hours
		3-4 days old adult *Cx. pipiens*	LC50 = 15.57 ppm
2[15]	Garlic rhizome (1% garlic oil in acetone solution)	Third instar larvae of A*e. albopictus* (n = 540)	At 24 hrs, LC50 = 66.5 mg/L At 48 hrs, LC50 = 56.8 mg/L At 72 hrs, LC50 = 46.5 mg/L LT50 = 55.11 hours
3[16]	Garlic crude extract	Third instar larvae of *Ae. albopictus* (n = 300)	At 24 hrs, LC50 = 13.610 g/dL At 48 hrs, LC50 = 10.932 g/dL At 72 hrs, LC50 = 5.744 g/dL
		Fourth instar larvae of *Ae. albopictus* (n = 300)	At 24 hrs, LC50 = 15.166 g/dL At 48 hrs, LC50 = 12.239 g/dL At 72 hrs, LC50 = 7.894 g/dL
	Garlic AgNPs	Third instar larvae of *Ae. albopictus* (n = 300)	At 24 hrs, LC50 = 1.915 g/dL At 48 hrs, LC50 = 1.303 g/dL At 72 hrs, LC50 = 0.810 g/dL

		Fourth instar larvae of *Ae. albopictus* (n = 300)	At 24 hrs, LC50 = 2.542 g/dL At 48 hrs, LC50 = 1.665 g/dL At 72 hrs, LC50 = 1.222 g/dL
4[14]	Garlic leaves aqueous extracts	Fourth instar larvae of *Cx. quinquefasciatus* (n = 10/concentration)	At 24 hrs, LD50 = 2.10±0.08 mg/mL At 48 hrs, LD50 = 1.33±0.044 mg/mL At 72 hrs, LD50 = 1±0.032 mg/mL
	Garlic leaves ethanolic extracts	Fourth instar larvae of *Cx. quinquefasciatus* (n = 10/concentration)	At 24 hrs, LD50 = 0.79±0.022 mg/mL At 48 hrs, LD50 = 0.65±0.053 mg/mL At 72 hrs, LD50 = 0.60±0.031 mg/mL
5[12]	Garlic bulb EO in DMSO	Third instar larvae of *Cx. quinquefasciatus* (n = 20/replica/conc.)	At 24 hrs, LC50 = 18.23 ppm
	Garlic bulb EO in acetone	4-5 days old adult female *Cx. quinquefasciatus* (n = 20/replica/conc.)	At 24 hrs, LD50 = 0.5 µg/cm2
6[6]	Garlic bulb EO	Late third instar larvae of *Cx. pipiens* (n = 20/container)	At 24 hrs, LC50 = 7.5 ppm
		Late third instar larvae of *Cx. restuans* (n = 20/container)	At 24 hrs, LC50 = 2.7 ppm
		Egg rafts of either *Cx. pipiens* or *Cx. restuans* (n = 52)	After 7 days at 5 ppm, Hatch and Survived = 0 (0.0%)
	Allyl disulphide	Late third instar larvae of *Cx. pipiens* (n = 20/container)	At 24 hrs, LC50 = 12.5 ppm
		Late third instar larvae of *Cx. restuans* (n = 20/container)	At 24 hrs, LC50 = 9.4 ppm
		Egg rafts of either *Cx. pipiens* or *Cx. restuans* (n = 52)	After 7 days at 11 ppm, Hatched and Survived = 0 (0.0%)
7[17]	Garlic EO	Late third instar larvae of *Ae. aegypti* (n = 180)	At 24 hrs, LC50 = 7.95 ppm
	Garlic/Amylose-Hex-Am powder	Late third instar larvae of *Ae. aegypti* (n = 180)	At 24 hrs, LC50 = 6.62 ppm
	Garlic/Sodium palmitate powder	Late third instar larvae of *Ae. aegypti* (n = 180)	At 24 hrs, LC50 = 7.24 ppm
	Garlic/Amylose-Hex-Am solution	Late third instar larvae of *Ae. aegypti* (n = 180)	At 24 hrs, LC50 = 5.97 ppm
	Garlic/Sodium palmitate solution	Late third instar larvae of *Ae. aegypti* (n = 180)	At 24 hrs, LC50 = 6.29 ppm
8[18]	Garlic bulb acetone extract	Second instar larvae of *Ae. aegypti* (n = 20/beaker)	At 6 hrs, LC50 = 365.83 ppm At 12 hrs, LC50 = 289.74 ppm At 24 hrs, LC50 = 228.74 ppm At 36 hrs, LC50 = 196.45 ppm At 48 hrs, LC50 = 108.42 ppm
		Third instar larvae of *Ae. aegypti* (n = 20/beaker)	At 6 hrs, LC50 = 415.08 ppm At 12 hrs, LC50 = 339.46 ppm At 24 hrs, LC50 = 262.42 ppm At 36 hrs, LC50 = 224.14 ppm At 48 hrs, LC50 = 129.11 ppm
	Garlic AgNPs	Second instar larvae of *Ae. aegypti* (n = 20/beaker)	At 6 hrs, LC50 = 222.02 ppm At 12 hrs, LC50 = 175.57 ppm At 24 hrs, LC50 = 115.03 ppm At 36 hrs, LC50 = 69.12 ppm At 48 hrs, LC50 = 44.77 ppm
		Third instar larvae of *Ae. aegypti* (n = 20/beaker)	At 6 hrs, LC50 = 258.07 ppm At 12 hrs, LC50 = 208.50 ppm At 24 hrs, LC50 = 120.75 ppm At 36 hrs, LC50 = 91.88 ppm At 48 hrs, LC50 = 62.82 ppm
9[11]	Garlic bulb EO	Fourth instar larvae of *Ae. aegypti* (n = 60)	At 24 hrs, LC50 = 16.19 ppm At 48 hrs, LC50 = 7.57 ppm At 72 hrs, LC50 = 7.57 ppm
		3-4 days old adult *Ae. aegypti* (n = 30)	At 24 hrs, LC50 = 120.16 ppm At 48 hrs, LC50 = 66.35 ppm At 72 hrs, LC50 = 29.68 ppm
	Diallyl disulfide	Fourth instar larvae of *Ae. aegypti* (n = 60)	At 24 hrs, LC50 = 16.29 ppm At 48 hrs, LC50 = 14.86 ppm At 72 hrs, LC50 = 14.65 ppm
		3-4 days old adult *Ae. aegypti* (n = 30)	At 24 hrs, LC50 = 166.02 ppm At 48 hrs, LC50 = 94.86 ppm At 72 hrs, LC50 = 49.50 ppm
	Diallyl trisulfide	Fourth instar larvae of *Ae. aegypti* (n = 60)	At 24 hrs, LC50 = 10.53 ppm At 48 hrs, LC50 = 9.87 ppm At 72 hrs, LC50 = 8.40 ppm
		3-4 days old adult *Ae. aegypti* (n = 30)	At 24 hrs, LC50 = 298.07 ppm At 48 hrs, LC50 = 123.08 ppm At 72 hrs, LC50 = 90.30 ppm

The varying results may be due to factors such as plant components, method of preparation, target organism, and bioassay preparation. Regardless, garlic appears to have high ovicidal, larvicidal, and adulticidal activity against mosquito species. This activity is thought to be caused by diallyl disulfide and diallyl trisulfide, present in garlic [6, 11].

Diallyl disulfide, an organosulfur compound derived from garlic, displays several biological activities such as anti-inflammatory by regulating the immune cells, anti-cancer by inhibiting cancer cell movement, antimicrobial by lowering virulence factor production, and antioxidant by activating enzymes responsible for detoxification [19]. Both compounds are thiosulfinate breakdown products that are toxic to insects [20].

Despite its positive effect on improving patient health, its compounds are highly potent for dengue mosquitoes. The study found that LC_{50} (at 24 hrs PT) of the compounds range from 10.53 ppm to 16.29 ppm against the fourth instar larvae of *Ae. Aegypti* [11]. This result is significantly lower than the LC_{50} of garlic bulb acetone extracts against the third instar larvae of the same species [18]. The following results suggest that chemicals found in garlic EOs may be a source of insecticidal molecules. However, this claim needs further investigation to clarify the compounds' mechanism of action. Although it is believed that both compounds are responsible for the toxic activity against mosquitoes, it was later revealed that both compounds, when combined, work antagonistically, which suggests that both compounds should be used individually. The antagonistic effect observed between the two active compounds of garlic may be due to competition for the same target site [11]. Additional studies are required to confirm why these two compounds work less than we expect them to. Alongside these findings, there are garlic binary mixtures that work synergistically. Garlic (bulb) EOs combined with oils from *C. paradisi* (leaves), *O. sanctum* (leaves), and *C. grandis* (leaves), at a 1:1 volume ratio, against the third instar larvae of *Cx. quinquefasciatus* manifest this effect [12]. Diallyl disulfide mixed with eucalyptol and limonene also manifest this effect against the fourth instar larvae of *Ae. aegypti* [11].

The lipophilic compounds of garlic affect its activity when immersed in water. Thus, O/W emulsion is preferred for aqueous applications since it improves the solubility and stabilization of lipophilic compounds. This method distributes the particles equally throughout the continuous phase (aqueous environment) [21]. The use of amylose complex efficiently protects the EO from oxidation, and improves thermal stability and aqueous phase dispersion, thus, decreasing the required concentration for bulk applications [17].

Another feature of garlic that is useful for its insecticidal activity is its AgNPs. Researchers studied silver for years because of its physicochemical properties: antibacterial, antifungal, antiviral, and anti-inflammatory activities. Green-synthesized AgNPs are much more environmentally-friendly and economical to produce. The few green sources to extract AgNPs are bacteria and plant extracts [22]. These AgNPs form due to the ability of garlic's secondary metabolites to reduce silver ions. Using nanoparticles lessens the environmental effect of such vector controls by only targeting the mosquito and not the other organisms in the environment [23]. Higher efficacies were shown when using green-synthesized AgNPs against vector mosquitoes outperforming their pure botanical garlic extract counterparts [16, 18]. The presence of insecticidal components in the AgNPs of *Allium sativum* caused higher mortality for the third and fourth instar *Ae. albopictus* compared with its sole botanical extracts [16]. This result implies that AgNPs from garlic are far more effective and practical due to the lower concentration required than using garlic extracts as a control measure for mosquitoes.

Table 3: Efficacy in Binary Mixture against Different Mosquito Species

<table>
<tr><th>Reference</th><th>Ratio</th><th>Target Organism</th><th>Component A</th><th>Component B</th><th>Effect*</th></tr>
<tr><td rowspan="26">[12]</td><td rowspan="10">1:1</td><td rowspan="6">Third instar larvae of Cx. quinquefasciatus</td><td rowspan="26">Allium sativum (bulbs) EO</td><td>C. paradisi (leaves) EO</td><td>S</td></tr>
<tr><td>O. sanctum (leaves) EO</td><td>S</td></tr>
<tr><td>C. grandis (peels) EO</td><td>N</td></tr>
<tr><td>C. grandis (leaves) EO</td><td>S</td></tr>
<tr><td>Mentha piperita (leaves) EO</td><td>A</td></tr>
<tr><td>Temephos</td><td>S</td></tr>
<tr><td rowspan="4">4-5 days old adult female Cx. quinquefasciatus</td><td>C. paradisi (peels) EO</td><td>S</td></tr>
<tr><td>Aegle marmelos (leaves) EO</td><td>N</td></tr>
<tr><td>Eucalyptus maculata (leaves) EO</td><td>S</td></tr>
<tr><td>Malathion</td><td>S</td></tr>
<tr><td rowspan="10">1:2</td><td rowspan="6">Third instar larvae of Cx. quinquefasciatus</td><td>C. paradisi (leaves) EO</td><td>S</td></tr>
<tr><td>O. sanctum (leaves) EO</td><td>S</td></tr>
<tr><td>C. grandis (peels) EO</td><td>N</td></tr>
<tr><td>C. grandis (leaves) EO</td><td>S</td></tr>
<tr><td>M. piperita (leaves) EO</td><td>A</td></tr>
<tr><td>Temephos</td><td>N</td></tr>
<tr><td rowspan="4">4-5 days old adult female Cx. quinquefasciatus</td><td>C. paradisi (peels) EO</td><td>S</td></tr>
<tr><td>A. marmelos (leaves) EO</td><td>S</td></tr>
<tr><td>E. maculata (leaves) EO</td><td>N</td></tr>
<tr><td>Malathion</td><td>N</td></tr>
<tr><td rowspan="6">2:1</td><td rowspan="6">Third instar larvae of Cx. quinquefasciatus</td><td>C. paradisi (leaves) EO</td><td>S</td></tr>
<tr><td>O. sanctum (leaves) EO</td><td>S</td></tr>
<tr><td>C. grandis (peels) EO</td><td>N</td></tr>
<tr><td>C. grandis (leaves) EO</td><td>S</td></tr>
<tr><td>M. piperita (leaves) EO</td><td>S</td></tr>
<tr><td>Temephos</td><td>S</td></tr>
</table>

		4-5 days old adult female *Cx. quinquefasciatus*		*C. paradisi* (peels) EO	N
				A. marmelos (leaves) EO	N
				E. maculata (leaves) EO	A
				Malathion	A
	1:3	Third instar larvae of *Cx. quinquefasciatus*		*C. paradisi* (leaves) EO	S
				O. sanctum (leaves) EO	S
				C. grandis (peels) EO	S
				C. grandis (leaves) EO	N
				M. piperita (leaves) EO	N
				Temephos	S
		4-5 days old adult female *Cx. quinquefasciatus*		*C. paradisi* (peels) EO	N
				A. marmelos (leaves) EO	S
				E. maculata (leaves) EO	N
				Malathion	N
	3:1	Third instar larvae of *Cx. quinquefasciatus*		*C. paradisi* (leaves) EO	S
				O. sanctum (leaves) EO	S
				C. grandis (peels) EO	N
				C. grandis (leaves) EO	S
				M. piperita (leaves) EO	S
		4-5 days old adult female *Cx. quinquefasciatus*		*C. paradisi* (peels) EO	N
				A. marmelos (leaves) EO	A
				E. maculata (leaves) EO	A
				Malathion	N
[6]	1:1	Late third instar larvae of *Cx. pipiens*	Garlic EO	Asafoetida EO	A
		Late third instar larvae of *Cx. restuans*		Asafoetida EO	A
[11]		Fourth instar larvae of *Ae. aegypti*	Diallyl disulfide	Diallyl trisulfide	A
				Eudesmol	A
				Eucalyptol	S
				Eugenol	A
				Limonene	S
				α- pinene	N
				Temephos	S
			Diallyl trisulfide	Eudesmol	A
				Eucalyptol	N
				Eugenol	A
				Limonene	N
				α- pinene	N
				Temephos	A

*S: Synergistic; A: Antagonistic; N: No effect

Although this study primarily focused on the toxicity activity of *Allium sativum* against *Aedes*, *Anopheles*, and *Culex* spp., the researchers also found studies reporting its repellent activity against mosquitoes. Oils from garlic demonstrated a significant repellent activity at the initial time points, implicating that garlic odor reduced attraction to human odor [24]. The repellent activity of anti-mosquito patches made from garlic, lemongrass, and marigold extracts show no significant difference from commercialized mosquito patches, making them both effective in repelling mosquitoes. However, the former is more economical, as the ingredients are natural and available everywhere [25]. Nevertheless, using garlic directly on the skin is not recommended since it has various side effects, some of which can cause skin irritations and a notably unpleasant odor [26].

5. Conclusion

A total of nine articles contain sufficient information about the topic of this systematic review. This review summarizes garlic's effective and potential vital components that can be utilized as a future alternative pesticide ingredient. Garlic is more than just a common food ingredient. Not only is it known for being an antioxidant, antimicrobial or anti-inflammatory agent, but it also possesses anti-mosquito properties. The articles revealed that diallyl disulfide and diallyl trisulfide were the most active compounds assumed that contribute to garlic's toxicity against mosquitoes. Additionally, garlic extracts, EOs, and silver nanoparticles from the said plant species have shown mortality for both *Aedes* and *Culex* spp. Furthermore, this study suggests using garlic in O/W emulsion for treating an aqueous environment, the breeding source of mosquitoes, to maximize its insecticidal activity.

The use of garlic does not leave any waste as all its components are toxic against various stages of a mosquito's life cycle. Furthermore, it gives the possibility of producing an environment-friendly alternative to synthetic pesticides. Using garlic is a sustainable way to improve the critical situation of vector-borne diseases. Future studies regarding the activity of garlic against *Anopheles* spp. are needed to conclude that garlic is potent for all kinds of mosquitoes.

6. Ethical statement: Not applicable

7. Conflict of interest: None

8. Funding source: None

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