Evaluation of larvicidal activity of the essential oil of *Allium macrostemon* Bunge and its selected major constituent compounds against *Aedes albopictus* (Diptera: Culicidae)

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**Abstract**

**Background:** During the screening programme for new agrochemicals from Chinese medicinal herbs and local wild plants, the essential oil of dried bulbs of *Allium macrostemon* Bunge (Liliaceae) was found to possess larvicidal activity against mosquitoes. The aim of this research was to determine the larvicidal activity of the essential oil and its major constituent compounds against the larvae of the Culicidae mosquito, *Aedes albopictus*.

**Methods:** Essential oil of *A. macrostemon* was obtained by hydrodistillation and analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The activity of the essential oil and its two major constituents were evaluated, using World Health Organization (WHO) procedures, against the fourth instar larvae of *Ae. albopictus* for 24 h and larval mortalities were recorded at various essential oil/compound concentrations ranging from 9.0 - 150 μg/ml.

**Results:** The essential oil of *A. macrostemon* exhibited larvicidal activity against the early fourth instar larvae of *Ae. albopictus* with an LC₅₀ value of 72.86 μg/ml. The two constituent compounds, dimethyl trisulfide and methyl propyl disulfide possessed strong larvicidal activity against the early fourth instar larvae of *Ae. albopictus* with LC₅₀ values of 36.36 μg/ml and 86.16 μg/ml, respectively.

**Conclusion:** The results indicated that the essential oil of *A. macrostemon* and its major constituents have good potential as a source for natural larvicides.

**Keywords:** *Allium macrostemon*, *Aedes albopictus*, Larvicidal activity, Methyl propyl disulfide, Dimethyl trisulfide

**Background**

Females of many species of mosquitoes consume blood from living vertebrates, including humans. They are an important public health concern around the world. In the process of feeding on blood, some of them transmit extremely harmful human diseases, such as yellow fever, dengue fever, malaria, several forms of encephalitis, filariasis and chikungunya [1]. Millions of deaths occur globally each year due to mosquito-borne diseases with a disproportionate effect on children and elders in developing countries [2]. Mosquitoes also cause allergic responses on humans that include local skin and systemic reactions such as angioedema [3]. The yellow fever mosquito (*Aedes aegypti* L.) and the Asian tiger mosquito (*Ae. albopictus* Skuse) are the two main species of mosquito responsible for dengue fever and malaria in China [4]. Mosquito larvae and pupae are currently controlled by the usage of synthetic chemical insecticides organophosphates (e.g. temephos, fenthion, malathion), and insect growth regulators (e.g. diflubenzuron, methoprene). Their repeated use has disrupted the natural biological control systems, sometimes resulting in the widespread development of resistance as well as undesirable effects on non-target organisms, toxic residues in food, workers’ safety,
and high cost of procurement [5]. These problems have warranted the need for developing alternative strategies using ecofriendly products. From this point of view, botanical pesticides, including essential oils, are promising since they are effective, environmentally friendly, easily biodegradable and often inexpensive. Moreover, herbal sources provide a lead for discovering new insecticides [5]. It is suggested that many essential oils and constituent compounds derived from various essential oils can exert toxic activity against mosquito species [6-11]. During our mass screening program for new agrochemicals from wild plants and Chinese medicinal herbs, essential oil of dried bulbs of *Allium macrostemon* Bunge (Family: Liliaceae) was found to possess larvicidal activity against the Asian tiger mosquito, *Ae. albopictus*.

The dried bulbs of *A. macrostemon* are well known as the traditional Chinese medicine “Xie Bai”, which is used for the treatment of thoracic pain, stenocardia, heart asthma and diarrhea [12]. The bulbs and leaves, eaten as part of the diet, are emmenagogue, nervine and tonic. When added to the diet on a regular basis, they help to reduce blood cholesterol levels, act as a tonic to the digestive system and also tonify the circulatory system [12]. In the previous reports, various furostanol saponins, steroidal saponins and spirostane saponins have been isolated and identified from the plant [13-26]. Chemical composition of essential oil of *A. macrostemon* has been analyzed previously [27-29]. Juice of this plant is used as a moth repellent [13]. However, a literature survey has shown that there is no report on insecticidal activity of the essential oil of *A. macrostemon* and its constituent compounds against mosquitoes, thus we decided to investigate the larvicidal activity of the essential oil of *A. macrostemon* and its major constituents against the Asian tiger mosquito.

**Methods**

**Plant material**

Air-dried bulbs of wild *A. macrostemon* (5 Kg, collected during August, 2012, from Baicheng city, Jilin Province, 44.57°N latitude and 121.50° E longitude) were purchased from the Anguo Medicinal Herb Market, Hebei 071200, China. The plant sample was identified by Dr Liu QR (College of Life Sciences, Beijing Normal University, Beijing 100875, China) and a voucher specimen (CMH-XieBai-Liaoning-2012-06) was deposited at the Department of Entomology, China Agricultural University, Beijing, China. The sample was ground to powder using a grinding mill (Retsch Muhle, Haan, Germany) and was subjected to hydrodistillation using a modified Clevenger-type apparatus for 6 h and extracted with *n*-hexane. Anhydrous sodium sulphate was used to remove water after extraction. Essential oils were stored in airtight containers in a refrigerator at 4°C for subsequent experiments.

**Bioassays**

Range-finding studies were run to determine the appropriate testing concentrations. Concentrations of 150.0, 75.0, 37.5, 18.5, and 9.0 μg/ml of the crude extract/compound were tested. The larval mortality bioassays were carried out according to the test method of larval susceptibility as suggested by the World Health Organization [31]. Twenty larvae were placed in glass beaker with 250 ml of aqueous suspension of test material at various concentrations and an emulsifier (DMSO) was added in the final test solution (less than 0.05%). Five replicates were run simultaneously per concentration and with each experiment, a set of concentration.
controls using 0.05% DMSO and untreated sets of larvae in tap water, were also run for comparison. The two constituents, dimethyl trisulfide (98%) and methyl propyl disulfide (97%) were purchased from Aladdin Industrial Inc. (Shanghai, China). For comparison, commercial chlorpyrifos [purchased from National Center of Pesticide Standards (8 Shenliao West Road, Tiexi District, Shenyang 110021, China)] was used as a positive control. The toxicity of chlorpyrifos was determined at 5, 2.5, 1.25, 0.6, and 0.3 μg/ml. The assays were placed in a growth chamber (L16:D9, 26–27°C, 78–80% relative humidity). Mortality was recorded after 24 h of exposure and the larvae were starved within this period. The percentage of mortality was corrected for control mortality using Abbott’s formula. Results from all replicates for the pure compounds/ oil were subjected to probit analysis using the PriProbit Program V1.6.3 to determine LC50 values and their 95% confidence intervals [32].

Results and discussion

Essential oil chemical composition

The yield of yellow essential oil of *A. macrostemon* was 0.56% (v/w based on dry weight) while its density was 0.92 g/ml. A total of 16 components from the essential oil of *A. macrostemon* were identified, accounting for 98.27% of the total oil. The principal constituents of *A. macrostemon* essential oil were methyl propyl disulfide (47.2%) and dimethyl trisulfide (37.2%) followed by diallyl thiosulfinate (3.3%) and 1,3-dithiane (2.6%) (Table 1). Monoterpenoids only represented 3 of the 16 compounds, corresponding to only 0.29% of the whole oil, while 13 of the 16 constituents were S-containing compounds (97.98% of the crude essential oil). A large variation in the chemical profiles of the *A. macrostemon* essential oil in this study was observed from those of previous reports [27-29]. For example, dimethyl trisulfide (21.0%), methyl propyl trisulfide (18.6%), methyl propyl disulfide (17.0%) and isopropyl propyl disulfide (10.6%) were the major compounds in the essential oil of *A. macrostemon* harvested from Shenyang, Liaoning province, China [27]. However, the essential oil of *A. macrostemon* collected from Jilin province, China mainly contained dimethyl trisulfide (29.98%), methyl allyl trisulfide (29.01%), methyl allyl disulfide (6.87%), and methyl propyl trisulfide (5.74%) [28], while the essential oil of *A. macrostemon* obtained from Sichuan province, China contained methyl allyl trisulfide (20.73%), dimethyl trisulfide (16.01%), dimethyl tetrasulfide (9.25%), and dimethyl disulfide (5.62%) [29]. The results above suggest that there are great variations in chemical composition of essential oil of *A. macrostemon*. This variation could be due to analytic methods, population, different parts used, harvest time and local, climatic and seasonal factors as well as storage duration of medicinal herb. Thus, further studies on plant cultivation and essential oil standardization are needed.

### Table 1 Chemical constituents of the essential oil of *Allium macrostemon*

| Peak no. | Compound                          | RIa | RIb | Content (%) |
|----------|-----------------------------------|-----|-----|-------------|
| 1        | Diallyl sulfide                   | 848 | 848 | 0.34        |
| 2        | Allyl isothiocyanate              | 890 | 890 | 0.51        |
| 3        | Methyl allyl disulfide            | 915 | 912 | 1.58        |
| 4        | α-Pinene                          | 937 | 939 | 0.14        |
| 5        | Methyl propyl disulfide           | 950 | 946 | 47.23       |
| 6        | Dimethyl trisulfide               | 975 | 972 | 37.18       |
| 7        | β-Pinene                          | 980 | 979 | 0.08        |
| 8        | 1,3-Dithiane                     | 1027| 1027| 2.57        |
| 9        | Limonene                          | 1029| 1024| 0.07        |
| 10       | Diallyl disulfide                 | 1077| 1076| 1.52        |
| 11       | Methyl allyl trisulfide           | 1134| 1133| 0.68        |
| 12       | Methyl propyl trisulfide          | 1168| 1168| 1.27        |
| 13       | Dimethyl tetrasulfide             | 1214| 1212| 0.59        |
| 14       | Diallyl trisulfide                | 1296| 1289| 0.52        |
| 15       | Diallyl thiosulfinate             | 1325| 1324| 3.29        |
| 16       | Allyl methyl tetrasulfide         | 1386| 1384| 0.82        |
|          | Total identified                  |     |     | 98.39       |
|          | Monoterpenoids                    |     |     | 0.29        |
|          | S-Containing compounds            |     |     | 98.10       |

*Identification by co-injection of authentic compounds; *RI, retention index as determined on a HP-5MS column using a homologous series of n-hydrocarbons; bRI, retention index reported in the literature [30,33].

### Table 2 Larvicidal activity of the essential oil of *Allium macrostemon*

| Treatment       | LC50 (μg/ml) (95% CL) | LC95 (μg/ml) (95% CL) | Slope ± SD | Chi-square value |
|-----------------|-----------------------|-----------------------|------------|-----------------|
| Essential oil   | 72.86 (66.02-78.67)   | 132.84 (126.76-145.17)| 2.72 ± 0.27| 7.73*           |
| Dimethyl trisulfide | 36.36 (33.23-39.64) | 55.65 (49.43-61.27) | 2.61 ± 0.26| 10.60*          |
| Methyl propyl disulfide | 86.16 (79.53-91.43) | 144.16 (130.22-155.82)| 2.59 ± 0.25| 8.94*           |
| Chlorpyrifos    | 1.86 (1.71-2.05)      | 6.65 (6.21-7.48)      | 0.87 ± 0.01| 3.13*           |

*Significant at P < 0.05 level.
(Table 2). The commercial insecticide, chlorpyrifos showed larvicidal activity against the mosquitoes with a LC50 value of 1.86 μg/ml, thus the essential oil of *A. macrostemon* was 39 times less toxic to *Ae. albopictus* larvae compared with chlorpyrifos. However, compared with the other essential oils/extracts in the literature, essential oil of *A. macrostemon* exhibited stronger or the same level of larvicidal activity against *Ae. albopictus* larvae, e.g., essential oils of *Eucalyptus urophylla* (LC50 = 95.5 μg/ml) [34]; essential oils of *Achillea millefolium* (LC50 = 211.3 μg/ml), *Helichrysum italicum* (LC50 = 178.1 μg/ml), *Foeniculum vulgare* (LC50 = 142.9 μg/ml) [35]; ethanol extract from *Cryptomeria japonica* (LC50 = 93.8 μg/ml) [36], and ethanol extract of *Knema attenuata* (LC50 = 141 ppm) [37] as well as a solution of 80% ethanol extract of fresh garlic (*Allium sativum*) [38]. Among the two constituent compounds, methyl propyl disulfide exhibited almost the same level of larvicidal activity as the essential oil and dimethyl trisulfide possessed stronger larvicidal activity than the crude oil against *Ae. albopictus* larvae. Moreover, compared with chlorpyrifos, dimethyl trisulfide exhibited 19 times less toxicity against *Ae. albopictus* larvae (Table 2).

In the previous reports, the two constituents exhibited antimicrobial activities against several bacteria and fungi as well as nematodes [39-42]. There are numerous reports on insecticidal activity of sulfide-containing compounds against pests including the two constituent compounds. For example, dimethyl trisulfide was demonstrated to exhibit toxicity to African cowpea bruchid (*Bruchidius atrolineatus*) [43]. Dimethyl trisulfide and methyl propyl disulfide also exhibited larvicidal activity against the yellow fever mosquito (*Ae. aegypti*) [44,45]. The above findings suggest that the essential oil and the two major constituent compounds show the potential to be developed as possible natural larvicides for the control of mosquitoes.

It seems that this plant is quite safe for human consumption because it has been used as a common vegetable or spice in Chinese food [46] and also as a medicinal herb for the treatment of thoracic pain, stenocardia, heart asthma and diarrhea [12]. Dimethyl trisulfide and methyl propyl disulfide can be used as food additives and flavours in China. However, no information on toxicity of the essential oil and the isolated constituents to humans are available. Thus, to develop a practical application for the essential oil and its constituents as novel insecticides, further research into the safety of the essential oil/compounds to humans is needed. Additional studies on the development of formulations are also necessary to improve the efficacy and stability and to reduce costs. Moreover, field evaluation and further investigations on the effects of the essential oil and its constituent compounds on non-target organisms are necessary.

**Conclusions**

The essential oil of *Allium macrostemon* bulbs and its two major constituents demonstrate strong larvicidal activity against *Aedes albopictus* mosquito larva. Our results suggested that the essential oil of *A. macrostemon* and the two major constituents may be recommended effectively in mosquito control program but needs to be further evaluated for safety in humans and to enhance their activity.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

ZLL designed the work and supervised the manuscript. XCL, QL and LZ performed experiments, interpretation of data and drafted the manuscript. All authors read and approved the final version of the manuscript.

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