Efficiency of Wood Vinegar and Extracts from Some Medicinal Plants on Insect Control

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Udomporn Pangnakorn, Suwimol Kanlaya and Chumpon Kuntha: Efficiency of Wood Vinegar and Extracts from Some Medicinal Plants on Insect Control

ABSTRACT

The efficiency of wood vinegar and extracts from three of the medicinal plants such as: neem seed (*Azadirachta indica* A. Juss), citronella grass (*Cymbopogon nardus*) and yam bean seed (*Pachyrhizus erosus*) were tested on the 3rd instar larvae of mosquito (*Culex quinquefasciatus* Say). The raw wood vinegar was purified by standing method and filtering method. Seed of neem, citronella grass and yam bean were collected, cleaned and dried at 25°C for 3-4 days and powdered. Stream distillation was used for extraction the citronella grass while neem and yam bean were simple extracted by fermentation with ethyl alcohol. The toxicity test was evaluated under laboratory condition by using two methods namely Topical application method (contact poison) and Dropping method (stomach poison). The mortality of the mosquito larvae were observed daily for 3 days. The results revealed that the wood vinegar at the low concentration 2% by dropping method could kill 73.75%, 82.50% and 96.25% of mosquito larvae after 24, 48 and 72 hours respectively with significance different at p<0.05. The wood vinegar at the high concentration of 20% by topical application method could kill only 15.0% of mosquito larvae after 72 hours with significance different at p<0.05, while at the lower concentrations of 2%, 5% and 10% gave rather low mortality at 5%, 5% and 10% respectively. When the 2% of wood vinegar mixed with each individual extracted substances of neem seed or yam bean seed or citronella grass at ratio of 1:50 ml gave the larval mortality of 92.5%, 50.0% and 45.0% respectively at 72 hours after treated. While by dropping method all of the treatments gave 100% mortality of the mosquito larvae at 48 hours after treated.

Key words: Mosquito larva (*Culex quinquefasciatus* Say), wood vinegar, medicinal plants, neem seed (*Azadirachta indica*), citronella grass (*Cymbopogon nardus*) yam bean seed (*Pachyrhizus erosus*), mortality

Introduction

Wood vinegar is a byproduct from charcoal production. It is a liquid generated from the gas and combustion of fresh wood burning in an airless condition namely, Iwate kiln. When the gas from the combustion is cooled, it condenses into liquid. Wood vinegar has been used in a variety of processes, such as industrial, livestock, household and agriculture products. Wood vinegar improves soil quality, eliminates pests, accelerates plant growth, plant growth regulator or growth inhibiting (Apai and Thongdeethae, 2001). Since the 1930's, wood vinegar has also been used in agriculture as a fertilizer and growth-promoting agent. Raw wood vinegar has approximately 200 chemicals, such as acetic acid, formaldehyde, ethyl-valerate, phenol, methanol, tar, etc. The condensate consists of pyroligneous acid and a tarry residue, which will separate and settle upon cooling wood vinegar is slightly toxic to fish and very toxic to plants if too much is applied (Yoshimura and Hayakawa 1991).
Neem (Azadirachta indica A. Juss): family Maliaceae is native to tropical South East Asia, including Pakistan, Sri Lanka, Thailand, Malaysia and Indonesia [18]. Neem extracts contain a natural chemical called azadirachtin. The substance is found in all parts of the tree. The leaves are used effectively, though the chemical is much more concentrated in the fruit, especially in the seeds. Neem is easy to prepare and use, and is environmentally safe and not harmful to man and animals [17].

Yam bean (Pachyrhizus erosus Urb.) belongs to the family Leguminosae, subfamily Papilionoidea. This leguminous plant has species native from the Amazon region and from Mexico semiarid region [16], which grow well in tropical and sub-tropical regions [21]. Yam bean is grown widely in the Northern part of Thailand. Yam bean is a papilionacar specy, known as Derris elliptica, Lonchocarpus utilis and L. urucu. Rotenone is the most important toxic substance in Yam bean or Derris roots. Rotenone is colourless, crystalline solid, has low solubility in water but high solubility in acetone, ethyl acetate and chloroform. It is formulated as pesticides and for fish killing, both natural and commercial products. However, these toxic effects have not been reported in exposed humans or ingestion. The fatal case of Rotenone or Yam bean toxicity is very rare in humans. Yam bean seeds ingestion and toxicity to humans has occurred very rarely and no fatal case has occurred in Thailand and only one fatal case has been reported in the world [13].

Citronella grass (Cymbopogon nardus) family: Gramineae It is grown commercially in Sri Lanka, India, Burma, Indonesia and Java. The plant is the source of the commercial citronella oil. Citronella oil distilled from the leaves of C. nardus is very widely used aroma material in perfumery and cosmetic. The complete oil is mainly used as an insect repellent for humans and pets and applied in soap, detergents, household insecticides and technical products. In Thailand a preration of crude citronella oil mixed with leaves of neem (Azadirachta indica A. Juss) and rhizomes of Alpinia galanga (L.) Willd. is applied as a bio-pesticide in agriculture. The United States Environmental Protection Agency considers oil of citronella as a biopesticide with a non-toxic mode of action (EPA 1993).

Mosquito (Culex quinquefasciatus Say) (Diptera: Culicidae) is widely distributed in tropical and sub-tropical areas of the world [15]. The life cycle of Cx. quinquefasciatus mosquito is complete metamorphosis and consists of four developmental stages namely, egg, larvae, pupa and adult. Cx. quinquefasciatus active only at night and may be found resting during the day around houses, chicken houses and outbuilding. They are known as one of the important vectorresin transmission of bancroftian filariasis. They are also considered to be another important vector of encephalitis virus [15]. At present human prefer to use natural products derived from plants to prevent mosquito. Particularly, the herbal repellency against mosquitoes and products developed from them are environment-friendly with less harmful than synthetic and caused many undesirable side effects to human [14]. Most of Asian people are familiar to use crude extract substances from medicinal plants to avoid from agricultural and medical insects biting, especially mosquitoes [2]. The volatile oils from various plant species such as neem [19], citronella grass [10] were also studies on their effectiveness of repellents against mosquitoes.

This study was carried out to investigate the toxicity properties of wood vinegar and selected each extracts from neem (A. indica A. Juss), yam bean seed (P. erosus Urb.) and citronella grass (C. nardus) with mixed the wood vinegar against mosquito larvae (Cx. quinquefasciatus Say).

Materials and methods

Plant Preparation:

Fresh of neem seed, citronella grass and yam bean seed were collected, cleaned and air dried at 25 C° for 3-4 days and powdered for sample extraction.

Plant Extraction:

Steam Distillation Method:

100 g dried sample of citronella grass were placed in a distillation flask with approximately much water and was heated on heating mantle at about 100°C. The flask was allowed to boil for 5 hours until the distillation was completed. The distillate was collected in a separating funnel in which the aqueous portion was separated from the volatile oil. The volatile oil was collected and kept in a stoppered cylinder at 4°C and were concentrated and mixed with wood vinegar for testing on the larvae of mosquitoes (Culex quinquefasciatus Say).

Solvent Extraction:

The simple extraction by dried sample of neem seed or yam bean seed were fermented with ethyl alcohol 95% ( ratio 1:5), the containers were covered with Para film and left for 3 days which the solvent are need to continuous blend. After 3 days the solution was flitted to remove the ethyl alcohol. The extracted plant substances were kept in container cover with aluminum foils under temperature at 4 °C.

Preparation and Purification Method of Raw Wood Vinegar:
Wood vinegar is made from burning fresh wood in a charcoal kiln (or Iwate kiln). The woods were burned at 120-430°C. The smoke from carbonization was cooled by the outside air when passing through the chimney occurs to produce pyroligneous liquor. The hot steams condensed into liquid were collected. It is called raw wood vinegar and necessary to pure before using.

**Standing Method:**

The raw wood vinegar was leave for 3 months to become silted. The vinegar will turn yellow like vegetable oil. After which, it will turn light brown and the tar will become silted. The top content will be light, clear oil. Remove the tar and light oil, as well as the dark brown translucent oil and the remainder will be sour vinegar.

**Filtering Method:**

Charcoal was broken into small piece, soaked with water and place on funnel. Then the wood vinegar after standing method was poured through the charcoal. Therefore the wood vinegar after filtering method were diluted in various concentrations or mixed with the extracted plants. In this study, the treatments were formulated in ratio of wood vinegar: extracted plant =1:50 cc. and were tested on the 3rd instars of mosquito larvae.

**Insect Preparation:**

Mosquito Eggs (*Cx. quinquefasciatus* Say) were collected from pond around campus in Naresuan University, Thailand and were laboratory-reared and were hatched at room temperature. Three-day old larvae (3rd instar) of mosquito were identified and prepared for bioassay tests.

**Larvicidal Bioassay:**

**Topical Application Method:**

Is contact poison, the substances 1.0 µl droplet of each treatments were dropped on head area of the 3rd instar of mosquito larvae with micro applicator, and then the larvae were moved to the cup (10 larvae /cup) with 50 ml of water for observation mortality.

**Dropping Method:**

Is stomach poison, the substances 5 ml droplet of each treatments were dropped on the cup with 50 ml of water, then the 3rd instar larva of mosquito was placed on the cup (10 larvae /cup). Each treatment was carried out in 4 replicates.

The concentrations assayed were 2.0%, 5.0%, 10.0% and 20.0% of wood vinegar by topical application method and were 0.5%, 1.0%, 1.5% and 2.0% by dropping method. The 2.0% of wood vinegar was selected to mix up with each individual extracted substances of neem seed or yam bean seed or citronella grass at ratio of 1:50 ml and were tested on the 3rd instar mosquito larva both topical application method and dropping method. The mortality was observed daily for 24, 48 and 72 hours within 3 days. When control mortality occurred on the experimental test, the mortality was corrected by Abbott’s formula [1]:

\[
\text{% Mortality} = \frac{\text{test mortality} - \text{control mortality}}{100 - \text{control mortality}} \times 100
\]

**Results:**

The efficacy test of wood vinegar by topical application method is shown on Table 1. The result showed that toxicity to mosquito larvae (*Cx. quinquefasciatus* Say) at 20% of concentration was highly efficient with mortality rate of 15%. The lower concentration from 2% until 10% showed only 5.0% to 10.0% of mortality against on mosquito larvae. Particularly, after 24 hours had no effected on mortality of mosquito larvae. While the toxicities to mosquito larvae by dropping method resulted that at 2% of concentration was highly significant different (P < 0.05) with mortality rate of 96.25 % when compared to the other treatments (Table 2).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Mortality</th>
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<tbody>
<tr>
<td>1. control</td>
<td>0</td>
</tr>
<tr>
<td>2. wood vinegar 2%</td>
<td>1.25</td>
</tr>
<tr>
<td>3. wood vinegar 5%</td>
<td>3.75</td>
</tr>
<tr>
<td>4. wood vinegar 10%</td>
<td>7.50</td>
</tr>
<tr>
<td>5. wood vinegar 20%</td>
<td>15</td>
</tr>
</tbody>
</table>

ns = non significant; * = significant different, means in the followed by the same letter are not significantly different at 5% level by DMRT.
Table 2: Toxicities of wood vinegar against on mosquito larva (*Culex quinquefasciatus* Say) by dropping method

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hour</td>
</tr>
<tr>
<td>1. control</td>
<td>0.00</td>
</tr>
<tr>
<td>2. wood vinegar 0.5%</td>
<td>3.75</td>
</tr>
<tr>
<td>3. wood vinegar 1%</td>
<td>10.00</td>
</tr>
<tr>
<td>4. wood vinegar 1.5%</td>
<td>16.25</td>
</tr>
<tr>
<td>5. wood vinegar 2%</td>
<td>73.75</td>
</tr>
</tbody>
</table>

ns = non significant; * = significant different, means in the followed by the same letter are not significantly different at 5 % level by DMRT

Table 3: Toxicities of wood vinegar mixed with extracted substance from medicinal plants against on mosquito larvae (*Culex quinquefasciatus* Say) by topical application method

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hour</td>
</tr>
<tr>
<td>1. control</td>
<td>0</td>
</tr>
<tr>
<td>2. wood vinegar : water (1 ml : 50 ml)</td>
<td>5</td>
</tr>
<tr>
<td>3. wood vinegar : citronella grass (1 ml : 50 ml)</td>
<td>10</td>
</tr>
<tr>
<td>4. wood vinegar : yam bean seed (1 ml : 50 ml)</td>
<td>12.5</td>
</tr>
<tr>
<td>5. wood vinegar : neem seed (1 ml : 50 ml)</td>
<td>37.5</td>
</tr>
</tbody>
</table>

ns = non significant; * = significant different, means in the followed by the same letter are not significantly different at 5 % level by DMRT

Table 4: Toxicities of wood vinegar mixed with extracted substance from medicinal plants against on mosquito larva (*Culex quinquefasciatus* Say) by dropping method

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hour</td>
</tr>
<tr>
<td>1. control</td>
<td>0</td>
</tr>
<tr>
<td>2. wood vinegar : water (1 ml : 50 ml)</td>
<td>85</td>
</tr>
<tr>
<td>3. wood vinegar : citronella grass (1 ml : 50 ml)</td>
<td>95</td>
</tr>
<tr>
<td>4. wood vinegar : yam bean seed (1 ml : 50 ml)</td>
<td>100</td>
</tr>
<tr>
<td>5. wood vinegar : neem seed (1 ml : 50 ml)</td>
<td>100</td>
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</tbody>
</table>

ns = non significant; * = significant different, means in the followed by the same letter are not significantly different at 5 % level by DMRT

The toxicity test of wood vinegar mixed with the extracted substances from neem seed or yam bean seed or citronella grass against on larvae of mosquito by topical application method are shown Table 3. Treatments of wood vinegar mixed with neem seed showed highly significance different (P < 0.05) of percent mortality at 92.5% after 72 hours followed by wood vinegar mixed with yam bean seed, wood vinegar mixed with citronella grass and only wood vinegar of percent mortality at 50.0%, 45.0% and 37.5% respectively. While the toxicities to mosquito larvae by dropping method resulted that all of treatments showed highly efficiency with mortality of mosquito larvae to 100% after 72 hours. Particularly, the treatment of wood vinegar mixed with neem seed and wood vinegar mixed with yam bean seed showed mortality at 100% within 24 hour after testing (Table 4).

Discussion:

This study tested the wood vinegar and extracts from three of the medicinal plants susceptibility of the 3rd instar larvae of mosquito (*Culex quinquefasciatus* Say), in the laboratory based on two methods of testing, topical application method (contact poison) and dropping method (stomach poison). The study revealed that dropping method gave high efficiency to the larvae than topical application method. Although the higher concentration of the wood vinegar between 2.0 % to 20.0 % were applied by topical application method, meantime the lower concentration of wood vinegar of percent mortality at 0.5% to 2.0% were applied by dropping method but high mortality occurred on the this method. Moreover when the 2.0% of wood vinegar was selected to mix up with each individual extracted substance of the three of plants and were tested on the mosquito larvae (*Cx quinquefasciatus* Say). The results revealed that susceptibility of mosquito larvae with mortality rate ranging from 37.5% to 92.5% by topical application method but mortality rate at 100 % by dropping method. Based on the standard WHO contact test using discriminating dosage under controlled temperatures, the ability of mosquitoes to have survived the diagnostic dose after 24 hours is indicative of resistance in population, as defined by percentage mortality in the test population [4]. However, the adulticidal activity determined by topical application revealed that five essential oils from celery (*Apium gravellens*), caraway (*Carum carvi*), zedoary (*Curcuma zedoaria*), long peper (*Piper longum*) and Chinese star anise (*Illicum verum*) a promising adulticidal efficacy against mosquito *A. aegypti* [5]. Correspondingly, larvicidal activity of 8 of the 11 plants extracts from nine...
Juss suggest that the extracts of from neem environment [9]. In this way, the results obtained suggest that the extracts of *A. grandifolia* and *M. setosa* are promising as larvicides against *A. aegypti* larvae and could be useful in the search for new larvicidal natural compounds [6].

The use of plant extracts in insects control is an alternative pest control method forminimizing the noxious effects of some pesticidal compoundson wildlife, livestock, nontarget insect species and the noxious effects of some pesticidal compounds on wildlife, livestock, nontarget insect species and the

environment [9]. In this way, the results obtained suggest that the extracts of from neem (*A. indica* A. Juss), yam bean seed (*P. erosus* Urb.) and citronella grass (*C. nardus*) mixed with the wood vinegar are promising as larvicides against *Cx. quinquefasciatus* Say. Moreover, these results could be useful in the search for newer, more selective, and biodegradable larvicidal natural compounds. Corbel et al. [7] demonstrated the excellent intrinsic toxicity, measured by topical application against laboratory-reared *A. aegypti* adults, of some pyrethroids and organophosphates, including bifenthrin, permethrin, and temephos. When new insecticides are developed, there is a concern regarding the cross-resistance that may be developed if they have a similar mode of action to well-established insecticides [20]. Correspondingly, some plant extracts or phytochemicals have been reported to be highly effective against insecticide-resistant insect pests [11,3]. Unlike synthetic insecticides, the evolution of insect resistance to plant-based products has not been reported. The literature review on the biological effects of neem products demonstrated no cases of arthropod resistance to these agents [12]. This finding corresponded to the study of Schmutterer [18] which stated the failure in an attempt to select for resistance against neem products in the laboratory. The absence of resistance to the products of botanical origin among mosquitoes might be due to their bioactive constituents, which are mixtures of various related compounds with different modes of action and, hence, the development of resistance to such products is somewhat difficult. In addition, the low frequency, short period, and small scales of applying plant-derived insecticides in vector control programs also serve to lower the development of resistance in a mosquito population [18,21].

Conclusion:

The need for search and development of environmental safe, biodegradable and low cost natural products, which can be widely use by individual and communities in specific situation. This study investigated efficiency of wood vinegar and extracts from three plant species, which show potential as alternatives for developing and producing in an effective strategy used to control mosquito larvae. Consequently, because Thailand possesses a large diversity of medicinal plants that have potential and a valuable source of biologically active substances, the commercial exploitation of plant-derived products would contribute towards the country’s economic development.

Acknowledgement

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References


