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Efficiency of Wood Vinegar Mixed with Some Plants Extract against the Housefly (*Musca domestica* L.)

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***Abstract*—**The efficiency of wood vinegar mixed with each individual of three plants extract such as: citronella grass (*Cymbopogon nardus*), neem seed *(Azadirachta indica* A. Juss), and yam bean seed (*Pachyrhizus erosus* Urb.) were tested against the second instar larvae of housefly (*Musca domestica* L.). Steam distillation was used for extraction of the citronella grass while neem and yam bean were simple extracted by fermentation with ethyl alcohol. Toxicity test was evaluated in laboratory based on two methods of larvicidal bioassay: topical application method (contact poison) and feeding method (stomach poison). Larval mortality was observed daily and larval survivability was recorded until the survived larvae developed to pupae and adults. The study resulted that treatment of wood vinegar mixed with citronella grass showed the highest larval mortality by topical application method (50.0%) and by feeding method (80.0%). However, treatment of mixed wood vinegar and neem seed showed the longest pupal duration to 25 day and 32 days for topical application method and feeding method respectively. Additional, larval duration on treated *M. domestica* larvae was extended to 13 days for topical application method and 11 days for feeding method. Thus, the feeding method gave higher efficiency compared with the topical application method.

***Keywords*—**Housefly (*Musca domestica* L.), neem seed (*Azadirachta indica*), citronella grass (*Cymbopogon nardus*) yam bean seed (*Pachyrhizus erosus*), mortality.

I. Introduction

THE house fly, *Musca domestica* L. (Diptera: *Muscidae*), is an important insect pest of household and dairy farming that acts as a transmitter of many human and animal diseases [1], [2]. Chemical control of this pest has been dependent upon the insecticides and development of the insecticides resistance in house flies restricts this strategy. The house fly not only resisted insecticides which were used as spray, but also the insecticides mixed in the baits and having toxic side effects to humans and non-target organisms [3]. Plant extracts may be an alternative source of housefly control agent because they have been reported to show several bioactivities such as insecticidal, antifungal, and nematicidal activities because they pose little threat to the environment or to human health [4]. Therefore many plants have been reported for their potential insecticidal actions on different stages of *M. domestica* via crude extracts or extracted active compounds [5].

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Wood vinegar has been used for a variety of purposes, such as industrial, livestock, household and agriculture products. Wood vinegar improves soil quality, eliminates pests, plant growth regulator or plant growth inhibitor [6], [7]. Since the 1930's, wood vinegar has also been used in agriculture as a fertilizer and growth-promoting agent. The wood vinegar showed potential to control on housefly (*Musca domestica* L.) at 30% concentration [8].

Neem (*Azadirachta indica* A. Juss) belongs to family *Maliaceae* and is native to tropical South East Asia, including Thailand [9]. 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 or animals [10].

Yam bean (*Pachyrhizus erosus* Urb.) belongs to family *Fabaceae* and is a tuber crop which grows well in tropical and sub-tropical regions [11]. Yam bean is grown widely in the Northern part of Thailand. It is a papilionacar species, known as *Derris elliptica, Lonchocarpus utilis* and *L. urucu*. Rotenone is the most important toxic substance in Yam bean or Derris roots. It is formulated as pesticides and these toxic effects have not been reported in exposed humans or from ingestion. 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 [12].

Citronella grass (*Cymbopogon nardus* Rendle) belongs to family *Gramineae* and is the source of the commercial citronella oil. The complete oil is mainly used as an insect repellent for humans and pets. In Thailand a preration of crude citronella oil mixed with leaves of neem (*A. indica* A. Juss) and rhizomes of *Alpinia galanga* (L.) Willd. are 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 [13].

Synergism is a special case of joint action where one has the effect of increasing potency of the other component of the mixture that their combined effect is greater than the amount of their individual effects [14]. This study was carried out to investigate the toxicity properties of the combination of wood vinegar and selected extracts from neem (*A. indica* A. Juss), yam bean seed (*P .erosus* Urb.) and citronella grass (*C. nardus*) against housefly larvae (*M. domestica* L.).

II. Materials and Methods

A. Preparation and Purification of Raw Wood Vinegar

Wood vinegar is made from burning waste wood in an Iwate kiln at 120-430°C. The smoke from carbonization is cooled by the outside air, and then the hot steam condensed into liquid is collected. It is called raw wood vinegar and must be purified before use by Standing method and Filtering method [7]. The purred wood vinegar was diluted at 10% concentrations and mixed with each individual extracted substance of neem seed or yam bean seed or citronella grass at ratio of 1:50 ml and were tested on the 1st instars larvae of house fly.

B. Plant Preparation

Fresh 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.

C. Plant Extraction

1. Steam Distillation Method

100 g dried sample of citronella grass were placed in a distillation flask with approximately much water and 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 was concentrated and mixed with wood vinegar for testing on the housefly larvae (*Musca domestica* L.).

2. Solvent Extraction

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

D. Insect Preparation

House fly, *Musca domestica* L. were collected from livestock cages in the campus of Naresuan University, Thailand, and were laboratory-reared with laid eggs on powdered of dog food and hatched at room temperature. Two-day old larvae (second instar) of house fly were identified and prepared for bioassay tests.

E. Larvicidal Bioassay

Topical application method use contact poison; the 1.0 µl droplet of each treatment of wood vinegar mixed with plants extract was dropped on the head area of the first instar of *M. domestica* larvae with a micro applicator, and then the larvae were transferred to a cup (10 larvae /cup) with 5 g powdered of dog food, for each treatment of wood vinegar mixed with the plants extract and control four replicates were maintained. After the treatment, behavioral and morphological changes were observed and mortality was recorded daily. Acute toxicity analysis was carried out after 24 hour and sub-acute toxicity analysis was carried out after 11 days.

Feeding assays use stomach poison; first instar *M. domestica* larvae were orally treated with different treatments of wood vinegar mixed with plants extract through piece of dog food. The wood vinegar 1.0 µl droplet of each treatment was dropped on 5 g powdered of dog food. After 24 hours, the larvae were fed daily with untreated dog food. Larval mortality was recorded in the larvae for 96 hours as described by [15].

After 96 hours, the surviving larvae from both methods were reared on untreated dog food. The growth development and metamorphosis were observed and recorded until the larvae developed to pupae and adults. Pupal mortality was calculated by subtracting the number of emerging adults from the total number of pupae. The percent adult emergence and deformities were also recorded. The time required for larvae to reach pupae and adult stages was recorded. Weight and shape of the insect were also recorded throughout the experimental duration.

F. Statistical Analysis

The significance of treatments was calculated by one way Analysis of Variance (ANOVA) and effective treatment was separated by the Duncan new multiple ranges test (DMRT). Differences between means were considered significant at $P< 0.05$.

III. Result

A. Larvicidal Activity of Mixed Wood Vinegar and Plants Extract against Musca domestica L.

According to the result showed that larval mortality had not reach to 50 percent occurred on treated *Musca domestica* L. larvae after 96 hour (3 days), but the survived larvae showed increasing percent mortality rate day after day until the surviving larvae developed to pupae. The unaffected pupae survived and developed to adults. In untreated control all emerged adults were healthy and had good morphological appearance. Therefore the last four days of larval mortalities before developing to pupa of *M. domestica* after application of wood vinegar mixed the three plants extracts are shown in Table I by topical application method and Table II by feeding method. In general, larval mortality increased with increasing the duration of exposure. At 13 days after treatment, the survival larvae in treatment of control (water) and 10% wood vinegar had developed to pupa. The treatment of mixed wood vinegar and citronella grass showed highest larval mortality (50%) with had significant differences ($P<0.05$) followed by mixed wood vinegar and neem seed and mixed wood vinegar and yam bean seed with 40.0% and 22.5% respectively for topical application. Similarly, the 50 mortality percent had not occurred on treated housefly larvae after 3 days by feeding method. Therefore, at the 11^{th} days after treatment, the survival larvae in treatment of control (water) had developed to pupa. The toxicities to housefly larvae resulted treatment of

the mixed wood vinegar and citronella grass, the mixed wood vinegar and neem seed and the mixed wood vinegar and yam bean produced a significant difference ($P<0.05$) with mortalities rate at 80.0%, 67.5%, and 50.0 % respectively for feeding method. Hence, the feeding method showed a higher mortality of *M. domestica* larvae than the topical application method. Although the mixed wood vinegar with all of the plants extracts did not show insecticidal properties to tested *M. domestica* larvae. However, the tested of mixed wood vinegar and plants showed some inhibitory effects on growth and metamorphosis activity and survival of first instar larvae of *M. domestica*. Among the tested formulations, the mixed wood vinegar and citronella grass showed the highest effect in both bioassay methods (50.0 % for topical application method and 80.0% for feeding method) but the mixed wood vinegar and neem seed is a potent inhibitor of larval development. It shows late developed activities of the first instar housefly larvae.

TABLE I
LARVICIDAL ACTIVITY OF MIXED WOOD VINEGAR AND PLANTS EXTRACT AGAINST THE HOUSEFLY LARVAE (*MUSCA DOMESTICA* L.) BY TOPICAL APPLICATION METHOD

Treatment	Larval mortality (%)			
	Days			
	10	11	12	13
Control (water)	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
Wood vinegar 10%	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
WV:yam bean seed	15.0^{a}	20.0^{ab}	22.5^{ab}	22.5^{ab}
WV:neem seed	25.0^{ab}	32.5^{b}	35.0^{b}	40.0^{b}
WV:citronella grass	40.0^{b}	42.5^{b}	45.0^{b}	50.0^{b}
C.V. (%)	46.8	39.6	49.2	35.9
F-test	*	*	*	*

* = significant difference, Means in the followed by the same letter are not significantly different at 5% level by DMRT
Mark**:** WV=Wood Vinegar

TABLE II
LARVICIDAL ACTIVITY OF MIXED WOOD VINEGAR AND PLANTS EXTRACT AGAINST THE HOUSEFLY LARVAE (*MUSCA DOMESTICA* L.) BY FEEDING METHOD

Treatment	Larval mortality (%)			
	Days			
	8	9	10	11
Control (water)	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
Wood vinegar 10%	22.5^{ab}	27.5^{ab}	30.0^{ab}	32.5^{ab}
WV: yam bean seed	42.5^{ab}	45.0^{ab}	50.0^{bc}	50.0^{bc}
WV: neem seed	52.5^{b}	57.5^{b}	62.5^{bc}	67.5^{bc}
WV: citronella grass	65.0^{b}	70.0^{b}	77.5^{c}	80.0^{c}
C.V. (%)	55.8	48.6	63.6	62.2
F-test	*	*	*	*

* = significant difference, Means in the followed by the same letter are not significantly different at 5% level by DMRT
Mark**:** WV=Wood Vinegar

B. Pupicidal Activity and Adult Emergence

Tables III and IV show the pupal mortalities, duration of pupa, and adult emergence of *M. domestica* when treated with the mixed wood vinegar and each of three medicinal plants. Pupae were responsible for the activities and also had significant activity. According to, no mortality was found in treated *M. domestica* at treatment of wood vinegar 10% through the experiment by topical application method. The treated larvae were maintained up to adult emergence. However the effect of these treatments *M. domestica* development was interrupted and caused some mortality in the pupal stage. Pupicidal activities of *M. domestica* in treatment the mixed wood vinegar and yam bean, the mixed wood vinegar and neem seed and the mixed wood vinegar and citronella grass were recorded in 77.5%, 60.0% and 50.0% respectively. Whereas in the feeding method, the treated *M. domestica* in treatment of the mixed wood vinegar and yam bean, the mixed wood vinegar and neem seed and the mixed wood vinegar and citronella grass showed percent of pupation at 67.5%, 50.0%, and 32.5% respectively. Although the mixed wood vinegar and citronella grass shows the highest affect to developed activities of the housefly pupae, but the mixed wood vinegar and neem seed is a potent inhibitor of pupa development. It shows the highest duration of pupa at 25 days, followed by the mixed wood vinegar and citronella grass, the mixed wood vinegar and yam bean and 10% wood vinegar with average survival durations of pupa at 20, 17, and 8 days respectively by topical application method. Similarly, the mixed wood vinegar and neem seed shows the highest duration of pupa at 32 days, followed by the mixed wood vinegar and citronella grass, the mixed wood vinegar and yam bean and 10% wood vinegar with survival durations of pupa at 26, 21, and 11 days respectively by feeding method (Table III). Percent adult emergence of *M. domestica* presented in treatment of 10% wood vinegar, the mixed wood vinegar and yam bean, the mixed wood vinegar and neem seed and the mixed wood vinegar and citronella grass were recorded as 100%, 77.5%, 60.0% and 50.0% respectively for topical application method. Whereas, percent adult emergence of *M. domestica* in treatment of 10% wood vinegar, the mixed wood vinegar and yam bean, the mixed wood vinegar and neem seed and the mixed wood vinegar and citronella grass were recorded as 67.55%, 50.0%, 32.5% and 20.0% respectively for feeding method (Table IV).

TABLE III
COMPARISON OF PERCENTAGE PUPICIDAL ACTIVITY OF HOUSEFLY (*MUSCA DOMESTICA* L.) AFTER TREATMENT OF MIXED WOOD VINEGAR AND PLANTS EXTRACT BY TOPICAL APPLICATION AND FEEDING METHODS

Treatment	Topical application method		Feeding method	
	pupation (%)	duration of pupa (days)	pupation (%)	duration of pupa (days)
Control (water)	100.0^{b}	6	100.0^{c}	7
Wood Vinegar 10%	100.0^{b}	8	67.5^{bc}	11
WV: yam bean seed	77.5^{ab}	17	50.0^{ab}	21
WV: neem seed	60.0^{a}	25	32.5^{ab}	32
WV: citronella grass	50.0^{a}	20	20.0^{a}	26
C.V. (%)	25.9		53.1	
F-test	*		*	

* = significant difference, Means in the followed by the same letter are not significantly different at 5% level by DMRT
Mark**:** WV=Wood Vinegar

TABLE IV
COMPARISON OF THE PERCENTAGE ADULT EMERGENCE OF HOUSEFLY (*MUSCA DOMESTICA* L.) AFTER TREATMENT OF MIXED WOOD VINEGAR AND PLANTS EXTRACT BY TOPICAL APPLICATION AND FEEDING METHODS

Treatment	Adult emergence (%)	
	Topical application method	Feeding method
Control (water)	100.0[b]	100.0[c]
Wood Vinegar 10%	100.0[b]	67.5[bc]
WV: yam bean seed	77.5[ab]	50.0[ab]
WV: neem seed	60.0[a]	32.5[ab]
WV: citronella grass	50.0[a]	20.0[a]
C.V. (%)	25.9	53.1
F-test	*	*

* = significant difference, Means in the followed by the same letter are not significantly different at 5% level by DMRT
Mark: WV=Wood Vinegar

IV. DISCUSSION

In this investigation three plants extract mixed with wood vinegar were tested against larval stage of housefly (*Musca domestica* L.) under laboratory condition by two methods of bioassay: topical application method (contact poison) and feeding method (stomach poison). Among the selected plants citronella grass (*Cymbopogon nardus*) is considered the most potential insecticidal activity and followed by neem seed (*Azadirachta indica* A. Juss) and yam bean seed (*Pachyrhizus erosus* Urb.). Similarly, Pangnakorn et al. [8] reported that feeding method gave higher efficiency compared with topical application method. The basis for toxicity by topical application of plant extracts to house flies has been fairly documented [16], and may indicate possible neurotoxic action of the active constituents of the plant species that is mainly related to the acetycholinesterase and octopaminergic levels or the active constituents may transform the alcohol present into the fly body into the corresponding esters [17].

All of the treatments did not evoke an immediate mortality response among the treated larvae; larval mortality was recorded until the surviving larvae developed to pupae. Only the treatment of mixed wood vinegar and citronella grass showed larval mortality reach to 50 percent by topical application method. For that reason larval mortality were recorded and revealed only the last four days before pupation as shown on Tables I and II (13 days by topical application method and 11 days by feeding method). The treatment of mixed wood vinegar and citronella grass, pupation occurred less because the larvae died after extended period in the instar. It indicated that *M. domestica* larvae were highly susceptible to stomach poisoning and the pupa duration elongation was increased. In this study, prolongation of the larval duration with tested plants was similar to [18] reported that using *Melia azedarac* stated that larvae observed to pupate faster as their environment increased in toxicity in *M. domestica*. This is clearly a self-preservation mechanism since the pupal form is less susceptible to the environment.

It should be noted that pupation at treatment of mixed wood vinegar and citronella grass occurred less than treatment of mixed wood vinegar and neem seed because pupal morality also occurred before the adult emergence. But duration of pupa at the treatment of mixed wood vinegar and neem seed was delayed more than all of the other treatments both method of topical application and feeding. Schmutterer [19] reported that azadirachtin showed larval and pupal duration elongation and reduced longevity similar to the test compound. The benefit of elongation is that housefly larvae numbers are reduced due to longer life cycle and would decrease the vectorial capacity of houseflies. Also a number of *M. domestica* adults exposed to some neem products failed to come out from the puparium [20].

There are many researcher investigated the potency of several plant extracts and some commercial insecticides against the house fly. Samarasekera et al. [21] used *Cinnamomum zeylanicum* bark and *Cymbopogon citratus* oils showed good knockdown and mortality against adult *M. domestica*. Additional, the exposure to several plant substances causes delayed larval development through decreased growth rates [22]. Hence the organic compounds in mixed wood vinegar and some plants extract that toxicity can be utilized the joint action toxicity resulting from mixing botanical extracts with wood vinegar.

Generally, the introduction of synergists in a pest control programme therefore could be of great benefit economically as well as ecologically, thereby reducing the cost of increasing toxicity of a given treatment [23]. However, synergism between synthetic insecticides and phytochemicals appears to be more common than among different phytochemicals [24].

Synergism might be due to phytochemicals inhibiting the insect ability to use detoxifying enzymes against synthetic chemicals. Islam and Aktar [25] reported that the mixtures of indigenous plant extracts with synthetic pyrethroid insecticide(s) are more effective than the insecticide or plant extracts alone, and such synergistic mixtures may be. Identifying these synergist compounds within mixtures may lead to the development of more effective biopesticides as well as the use of smaller amounts in the mixture to achieve satisfactory levels of efficacy [26].

V. CONCLUSION

Accordingly, botanical insecticides based on natural compounds from plants, are expected to be a possible alternative. Many plant extracts have shown potential insecticidal activity against houseflies. The results of this study investigation reveal the broad spectrum toxic properties of the tested plants extracts mixed with wood vinegar against the larval stage of *Musca domestica*. However, further studies need to be conducted to evaluate the mode of action and cost efficacy of these materials under practical field conditions. Synergistic action with mixed wood vinegar and plants extract determined in the present study could be exploited for integrated pest management (IPM) programs.

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