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Effect of Wood Vinegar for Controlling on Housefly (*Musca domestica* L.)

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***Abstract*—**Raw wood vinegar was purified by both standing and filtering methods. Toxicity tests were conducted under laboratory conditions by the topical application method (contact poison) and feeding method (stomach poison). Larvicidal activities of wood vinegar at four different concentrations (10, 15, 20, 25 and 30 %) were studied against second instar larvae of housefly (*Musca domestica* L.). Four replicates were maintained for all treatments and controls. Larval mortality was recorded up to 96 hours and compared with the larval survivability by two methods of larvicidal bioassay. Percent pupation and percent adult emergence were observed in treated *M. domestica.* The study revealed that the feeding method gave higher efficiency compared with the topical application method. Larval mortality increased with increasing concentration of wood vinegar and the duration of exposure. No mortality was found in treated *M. domestica* larvae at minimum 10% concentration of wood vinegar through the experiments. The treated larvae were maintained up to pupa and adult emergence. At 30% maximum concentration larval duration was extended to 11 days in *M. domestica* for topical application method and 9 days for feeding method. Similarly the pupal durations were also increased with increased concentrations (16 and 24 days for topical application method and feeding method respectively at 30% concentration) of the treatments.

***Keywords*—**Housefly (*Musca domestica* L.), wood vinegar, mortality, topical application, feeding

I. Introduction

THE house fly, *Musca domestica* L. (Diptera: Muscidae), is a well-known cosmopolitan pest of both farm and home. House flies are always found in association with humans or activities of humans including on postharvest crop or food with many pathogens, such as enterohemorrhagic *Escherichia coli, Yersinia pseudotuberculosis*, and *Salmonella spp.* [8]. Chemical methods of control, principally involving the judicious use of insecticides as premise-sprays or fly baits, play a key role in comprehensive pest management programs designed to minimize the impact of house fly infestations. Methods for house fly control inside commercial establishments are extremely limited. Application of pesticide fogs or surface residuals is rarely permitted, and granular sugar/toxicant scatter baits cannot be used [15]. However, a limited number of chemical classes are represented with these products, some with mammalian safety issues.

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Furthermore, there are an increasing number of reports in the literature describing house fly populations that exhibit varying levels of resistance to currently available insecticide classes, including pyrethrins and pyrethroids, organophosphates and carbamates, fiproles, insect growth regulators, avermectins, and organochlorines .[4], [10], [12] ,[14]. Consequently, new insecticides that are not subject to resistance or cross-resistance with existing chemicals are continually needed for successful pest management practices, principally to mitigate or delay the onset of resistance and preserve the effectiveness of older chemical classes.

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 for a variety of purposes, such as industrial, livestock, household and agriculture products. Wood vinegar improves soil quality, eliminates pests, accelerates plant growth, plant growth regulator or growth inhibiting [3], [17]. 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 compounds, 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 [23].

This study was carried out to investigate the toxicity of wood vinegar against housefly larvae (*Musca domestica* L.).

II. Materials and Methods

A. Preparation and purification of raw wood vinegar:

Wood vinegar is made from burning waste wood in a charcoal kiln (or Iwate kiln). The wood is burnt at 120-430°C. The smoke from carbonization is cooled by the outside air when passing through the chimney to produce pyroligneous liquor. The hot steam condensed into liquid is collected. It is called raw wood vinegar and must be purified before use by two methods.

1. Standing method

The raw wood vinegar is stored for 3 months to allow siltation. The vinegar is yellow like a vegetable oil. After setting, 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.

2. Filtering method

Charcoal was broken into small pieces, soaked with water

and placed on a funnel. Then the wood vinegar after standing method was poured through the charcoal. The wood vinegar after filtering method was diluted with water in various concentrations and was tested on the 1st instars larvae of house fly. The wood vinegar was formulated and dose-response bioassays were conducted to measure toxicity to housefly larvae.

B. Treatments and concentrations

Different concentrations of wood vinegar 10%, 15%, 20%, 25% and 30% were formulated by water and were tested against first instar larvae of housefly in the laboratory.

C .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.

D. Larvicidal bioassay

Topical application method use contact poison; the wood vinegar 1.0 µl droplet of each treatment 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 concentration of wood vinegar treatments 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 concentrations of wood vinegar through piece of dog food. The wood vinegar 5.0 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 [1].

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.

E. 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 $P< 0.05$.

F. Larvicidal activity of wood vinegar against Musca domestica L.

Results of larval mortalities due to the effect of wood vinegar are shown in Table I by topical application method and Table II by feeding method. In general, larval mortality increased with increasing concentration of wood vinegar and the duration of exposure. It showed that no larval mortality occurred on *Musca domestica* larvae by topical application method after 96 hours (3 days); except the 25% and 30% concentrations had non significant differences ($P<0.05$) with mortality rate of 2.5 and 5.0 percent respectively. Particularly, no mortality was found in treated *M. domestica* larvae with 10% concentration of wood vinegar through the experimental. Whereas the mortality had not occurred on housefly larvae by feeding method after 48 hours (2 days); except the 25% and 30% of concentration produced a significant difference ($P<0.05$) with mortality rate of 5.0 and 12.5 percent respectively. After 3 days the surviving larvae showed increasing percent mortality rate day after day until the surviving larvae developed to pupae. At 11 days after treatment, the survival larvae in treatment of control (water) and 10% concentration had developed to pupa. Whereas the toxicities to housefly larvae resulted 15%, 20%, 25% and 30% of concentration were also significant difference ($P<0.05$) with mortalities rate at 10, 25.0, 32.5 and 47.5 percent respectively (Table I). Similarly, at 9 days after treatments, 30.0, 37.5, 45.0, 52.5 and 72.5 percent larval mortalities were recorded in wood vinegar concentration of 15%, 20%, 25% and 30% respectively (Table II). Hence, the feeding method showed a higher mortality of housefly larvae than the topical application method. Wood vinegar is a potent inhibitor of larval development it shows late developed activities of the first instar housefly larvae. Pupae were responsible for the activities and also had significant activity. All the tested wood vinegar did not show insecticidal properties to *M. domestica* larvae. However the tested wood vinegar showed some inhibitory effects on growth and metamorphosis activity and survival of first instar larvae of *M. domestica*. As the concentration increased, the observed mortality also increased. Among the tested formulations, the 30% concentration showed the highest effect in both bioassay methods (47.5 percent for topical application method and 72.5 percent for feeding method).

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

Treatment	Larval mortality (%)										
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11
Control(water)	0.0	0.0	0.0	0.0	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
wood vinegar 10%	0.0	0.0	0.0	0.0	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
wood vinegar 15%	0.0	0.0	0.0	5.0	5.0^{ab}	5.0^{ab}	7.5^{ab}	7.5^{ab}	10.0^{ab}	10.0^{ab}	10.0^{a}
wood vinegar 20%	0.0	0.0	0.0	5.0	7.5^{ab}	10.0^{c}	12.5^{b}	15.0^{b}	20.0^{b}	20.0^{bc}	25.0^{b}
wood vinegar 25%	0.0	0.0	2.5	7.5	15.0^{ab}	20.0^{c}	27.5^{c}	27.0^{cd}	32.5^{cb}	32.5^{cd}	32.5^{b}
wood vinegar 30%	0.0	0.0	5.0	12.5	17.5^{b}	27.5^{c}	30.0^{c}	35.0^{d}	40.0^{d}	42.5^{d}	47.5^{c}
C.V. (%)	-	-	36.5	47.2	49.6	64.4	67.3	59.8	54.3	49.8	27.3
F-test	-	-	ns	ns	*	*	*	*	*	*	*

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

TABLE II
LARVICIDAL ACTIVITY OF WOOD VINEGAR AGAINST HOUSEFLY LARVAE (MUSCA DOMESTICA L.) BY FEEDING METHOD

Treatment	Larval mortality (%)								
	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day8	Day9
Control(water)	0.0	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
wood vinegar 10%	0.0	0.0^{a}	2.5^{a}	5.0^{ab}	10.0^{ab}	15.0^{b}	22.5^{b}	25.0^{b}	30.0^{b}
wood vinegar 15%	0.0	0.0^{a}	5.0^{a}	10.0^{abc}	17.5^{b}	22.5^{bc}	30.0^{b}	32.5^{bc}	37.5^{bc}
wood vinegar 20%	0.0	0.0^{a}	7.5^{a}	15.0^{bc}	22.5^{b}	27.5^{c}	32.5^{b}	40.0^{bc}	45.0^{bc}
wood vinegar 25%	0.0	5.0^{b}	12.5^{a}	17.5^{c}	25.0^{b}	30.0^{c}	40.0^{b}	47.5^{c}	52.5^{c}
wood vinegar 30%	0.0	12.5^{c}	25.0^{b}	35.0^{c}	45.0^{c}	52.5^{d}	60.0^{c}	67.5^{d}	72.5^{d}
C.V. (%)	-	32.1	36.2	46.7	52.0	29.1	37.0	35.9	33.2
F-test	-	*	*	*	*	*	*	*	*

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

G. 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 wood vinegar. The treated larvae were maintained up to adult emergence. Due to the effect wood vinegar insect development was interrupted and caused some mortality in the pupal stage. By topical application method, 100, 90, 75, 67.5 and 52.5 percent pupicidal activities of *M. domestica* were recorded in 10%, 15%, 20%, 25% and 30% of concentration respectively. Whereas in the feeding method, 70, 62.5, 55, 47.5 and 27.5 percent pupicidal activities were recorded in 10%, 15%, 20%, 25% and 30% concentrations respectively(Table III). Similarly, by topical application method, 100, 90, 75, 67.5 and 52.5 percent adult emergence of *M. domestica* were recorded in 10%, 15%, 20%, 25% and 30% concentrations respectively. Whereas in the feeding method, 70, 62.5, 55, 47.5 and 27.5 percent adult emergence were recorded in 10%, 15%, 20%, 25% and 30% concentrations respectively (Table IV).

At 10% concentration and in untreated control, less than 10 days of period of pupa were recorded in *M. domestica* by topical application method (Table III). The unaffected pupae developed into adults. In untreated control all emerged adults were healthy and had good morphological appearance. At 30% maximum concentration larval duration was extended to 11 days in *M. domestica* for topical application method and 9 days for feeding method (Table I & Table II). Similarly the pupal durations were also increased with increased concentrations (16 and 24 days for topical application method and feeding method respectively at 30% concentration) of the treatments. Average survival durations of pupa as shown on Table III, due to effect of wood vinegar at 10%, 15%, 20%, 25% and 30% were recorded respectively as 9, 10, 12, 13 and 16 days for topical application method and 12, 15, 18, 21 and 24 days for feeding method.

TABLE III
COMPARISON OF PERCENTAGE SURVIVING PUPAE OF HOUSEFLY (*MUSCA DOMESTICA* L.) AFTER TREATMENT WITH WOOD VINEGAR BY TOPICAL APPLICATION AND FEEDING METHODS

Treatment	Topical application		Feeding method	
	pupation (%)	duration of pupa(day)	pupation (%)	duration of pupa(day)
Control(water)	100.0^{d}	7	100.0^{d}	8
wood vinegar 10%	100.0^{d}	9	70.0^{c}	12
wood vinegar 15%	90.0^{c}	10	62.5^{bc}	15
wood vinegar 20%	75.0^{b}	12	55.0^{bc}	18
wood vinegar 25%	67.5^{b}	13	47.5^{b}	21
wood vinegar 30%	52.5^{a}	16	27.5^{a}	24
C.V. (%)	16.2		21.8	
F-test	*		*	

* = significant difference, means (in the followed by the same letter) are not significantly different at 5% level by DMRT

TABLE IV
COMPARISON OF THE PERCENTAGE SURVIVING ADULTS OF HOUSEFLY (*MUSCA DOMESTICA* L.) AFTER TREATMENT WITH WOOD VINEGAR BY TOPICAL APPLICATION AND FEEDING METHODS

Treatment	Adult emergence (%)	
	Topical application	Feeding method
Control(water)	100.0^{d}	100.0^{d}
wood vinegar 10%	100.0^{d}	70.0^{c}
wood vinegar 15%	90.0^{c}	62.5^{bc}
wood vinegar 20%	75.0^{b}	55.0^{bc}
wood vinegar 25%	67.5^{b}	47.5^{b}
wood vinegar 30%	52.5^{a}	27.5^{a}
C.V. (%)	16.2	21.8
F-test	*	*

* = significant difference, means (in the followed by the same letter) are not significantly different at 5% level by DMRT

III. DISCUSSION

This study tested the susceptibility of the first instar larvae of House fly, *Musca domestica* L. to wood vinegar in the laboratory based on two methods of testing : topical application method (contact poison) and feeding method (stomach poison). Different concentrations of wood vinegar did not evoke an immediate mortality response among the treated larvae. For that reason larval mortality was recorded 96 hour after the treatments. The study revealed that the feeding method gave higher efficiency compared with the topical application method. Following [1] larval mortality was recorded after 96 hours. Toxicity studies of wood vinegar indicated that less than 50 per cent larval mortality occurred 96 hours after the treatment and pupal morality also occurred before the adult emergence. At lower concentration, pupation was delayed less than a higher concentration. At higher concentration, pupation occurred less because the larvae died after extended period in the instar. Higher concentration of wood vinegar indicated that *M. domestica* larvae were highly susceptible to stomach poisoning and the pupa duration elongation was increased.

Generally most of the botanical insecticides including commercial products caused delayed mortalities in insect pests as reported by many investigators. In the last 20 years, natural phytocompounds are used in the development of natural pesticides and these natural pesticides interfere with the growth, development and metamorphosis of insects [2]. Leatemia and Isman [9] reported that high concentrations of plant extracts caused high mortality of larvae even though only very small portions of the leaf discs were consumed. Schmutterer [21] reported that developmental effects on insects caused by azadirachtin are attributed to disruption of endocrine events. Also azadirachtin showed larval and pupal duration elongation and reduced longevity similar to the test compound. According to Sharma and Seth [19] adult deformities might be due to disruption of the neuroendocrine regulation of molting. Schmutterer [21] reported that developmental effects on insects caused by azadirachtin are attributed to disruption of endocrine events.

Generally, insect development and differentiation are controlled by hormones [5]. During larval and pupal stages, ecdysteroids and juvenile hormones play major role in moulting and metamorphosis. Hoffmann and Lorenz [7] pointed out that the toxic plant compounds can alter or modify ecdysteroid titer in insect haemolymph due to a blockage of release of prothoracicotropic hormone from the brain-corpus cardiacum complex. Hence the organic compounds in wood vinegar that mimic hormone analogues can be utilized in insect pest control programmes. Additional, Hummelbrunner and Isman [6] have reported that the exposure to several plant substances causes delayed larval development through decreased growth rates. Typically azadirachtin had a detrimental effect on larval growth and development, and prolonged the larval duration [13]. Murugan [16] also observed increased larval and pupal duration and decreased

life span of adult male and female. Neem oils were used as insecticides, antifeedants, repellants and disruptants of normal growth; Disruption of growth are the main characteristics of pest control; neem is being used in the field at lower concentrations than those originally recommended [18]. Similarly, Singh *et al.*, [21] reported that stem-bark of *Mundulea sericea* is used for controlling house-flies as an insecticide; this plant is found in South India. Deguelin was isolated from *M. sericea* and it is most commonly used as insecticide in Africa and South America [11].

IV. Conclusion

The need is for research and development of environmentally safe, biodegradable and low cost natural products which can be widely used by individuals and communities in specific situations. This study investigated the efficiency of wood vinegar against housefly. It showed potential biological activities such as, larvicidal, pupicidal, and adult deformities against *M. domestica*. As a result *M. domestica* larvae tested with feeding activity were more susceptible than the *M. domestica* larvae tested with topical application method. It showed potential inhibitor and larvicidal activities in *M. domestica* L. The effect of wood vinegar at various concentrations was chronic also. The treated insect species showed deformities at larval, pupal and adult stages. After the treatment, development efficiency, metamorphosis, and growth were highly reduced depending on the concentrations. Due to the effects of wood vinegar, larval and pupal durations were elongated, the development was inhibited, and emerged adults' life span was decreased. The studies of this promising natural product may bring new leads in developing future pesticides.

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