

## BIOLOGICAL EFFECTS OF *PETIVERIA ALLIACEA* AND *FLUEGGAE VIROSA* ON THE LIFE CYCLE OF A DISEASE VECTOR (*MUSCA DOMESTICA*)

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### ABSTRACT

The direct effects of *Petiveria alliacea*, and *Flueggae virosa* were tested on the life cycle of *Musca domestica*. Leaf powders of *P. alliacea*, and *F. virosa* at various concentrations of 2, 5, 10, and 15 % (w/w) were added to the mixture of rice and fish paste and tested on the larval duration, pupation percent, pupal weight, pupal duration, and adult emergence percent. Diets treated with 2, 5, 10 and 15% concentration of *P. alliacea*, and *F. virosa* generally increased the duration of the third instar larva by 50, 75, 25, 75 and 75% in comparison with the control. Moreover, pupal duration was lengthened while total development time was increased by 15% concentration of *P. alliacea*, and *F. virosa*. In addition, a 15% concentration of *P. alliacea* and *F. virosa*, reduced the mean emergence of adults by 10.0, 9.0, 11.9, 11.9 and 13.8% including the corresponding weights of adult *M. domestica* by 13.9, 15.5, 19.4, 13.2 and 19.4% respectively. The powder materials of *P. alliacea* and *F. virosa* appear to have promising biological effects in controlling the developmental stages of *M. domestica*.

**KEYWORDS:** *Musca domestica*; *Petiveria alliacea*; *Flueggae virosa*

### INTRODUCTION

House fly, *Musca domestica* is the most common of all domestic flies and have been implicated in transmitting at least 65 diseases to humans, including typhoid fever, dysentery, cholera, poliomyelitis, yaws, anthrax, tularemia, leprosy and tuberculosis (Cohen, *et al.*, 1991; Ewald, *et al.*, 1991; Farag, *et al.*, 2013; Olsen, *et al.*, 1998; Wolff, *et al.*, 1969). This is possible because of their feeding habit and proximity to human habitation. The most commonly used methods to control for houseflies are sanitation, use of traps and insecticides, but in some instances, integrated fly control has been implemented. Because local plants product contains pharmacological components that can inhibit the developmental process of *M. domestica*, concerted efforts are now directed on local plants species and their chemicals in the control of *M. domestica*.

In general, plant chemicals have specific toxic, repellent and deterrent effects, and they are used to control *M. domestica* alone or with several other insect vectors of human diseases (Machtinger, *et al.*, 2015; Machtinger, *et al.*, 2015; Machtinger, *et al.*, 2015). The use of plant chemicals has been effective because insects such as *M. domestica* inhabits the municipal waste around human dwelling, and are not exposed to various forms of these phytochemicals. For this reason, essential oils from plants represent a main source of chemicals to test the potentiality of repellency/

deterrence and/or toxic effects on *M. domestica* (Khatte, 2012; Pohlit, *et al.*, 2011; Tsao, *et al.*, 1996). For example, a mixture of *Desmodium paniculatum* with tannins reduced percentage emergence and average weight of *M. domestica* (Littlefield *et al.*, 2011). Application of sublethal doses of thyme oil to *M. domestica* significantly decreased the longevity of both sexes, larva vitality and pupa survival (Pavela, 2007; Pavela, 2008; Pavela, 2011). Furthermore, *Ocimum basilicum*, *Gardenia jasmooides* and *Lantana camara* reduced the growth rate in the larvae of *M. domestica* (Ahmed, *et al.*, 2013; Guntharee, 2008). The aforementioned studies and several other studies (Pavela, 2008; Chauhan, *et al.*, 2015; Malik, *et al.*, 2007; Pinta, *et al.*, 2015; Ponnudura, *et al.*, 2015) suggest that local plants represent a major source of novel drugs to control *M. domestica*.

Pharmacological studies have acknowledged the value of ethnomedicinal plants as prospective source of bioactive materials (Bala, *et al.*, 2015; Shrestha, *et al.*, 2015; Ullah, *et al.*, 2014). Extensive studies using advanced scientific techniques determined the medicinal properties of *P. alliacea* and *F. virosa* that could play a major role in the control of house fly (Christi, *et al.*, 2013; Kerdudo, *et al.*, 2015; Oliveira, *et al.*, 2011; Silva, *et al.*, 2015; Uruena, *et al.*, 2008). Compounds isolated from *P. alliacea* includes astilbin, myricitrin, engeletin (flavonoids), triterpenes,

daucosterol and lignoceric acid (Luz, et al., 2016). Dibenzyl trisulphide (DTS), is a lipophilic compound found in *P. alliacea* (Rosner, et al., 2001), and exhibits cytotoxic activity (Williams, et al., 2007). Moreover, *P. alliacea* contains dibenzyl trisulphide and dichloromethane with toxic pharmacological effects and insecticidal properties (Luz, et al., 2016; Hu, et al., 2016; Kisseih, et al., 2015; Szacon, et al., 2016). Compounds extracted from the leaves of *F. virosa* include virosecurinine, viroallosecurinine, norsecurinine, dihydronorsecurinine (viroisine) hordenine and N-methyltetrahydro- $\beta$ -carboline (Alam, et al., 2015; Al-Rehaily, et al., 2015; Chao, et al., 2016; Siddiqui, et al., 2015; Wang, et al., 2016; Zhang, et al., 2015; Zhang, et al., 2016; Zhang, et al., 2015; Zhang, et al., 2015). Moreover, *F. virosa* contains indolizidine alkaloids, which are the main isomers and derivatives of the highly toxic securinine (Zhang, et al., 2015). Several pharmacological properties of bergenin extracted from *F. virosa* are associated with inhibitory effect on growth of different vectors of human diseases (Ambika and Saravanan, 2016; El-Hawary, et al., 2016; Gu, et al., 2016; Kraujaliene, et al., 2016; Liu, et al., 2016; Shakeel, et al., 2016; Shakeel, et al., 2016; Yang, et al., 2016). In addition, the alkaloid virosecurinine is toxic with significant cytotoxicity properties (Chirkin, et al., 2015).

There are several ethnopharmacological evidences describing various pharmacological components of *P. alliacea* and *F. virosa* that could eliminate *M. domestica* (Pedersen, et al., 2009). However, because of lack of reliable experimental data, this knowledge has not been immersed into common medical practice to eliminate *M. domestica*. The goal of this study was to assess the direct effect of powder materials of *P. alliacea* and *F. virosa* on *M. domestica*, especially the stage in the life cycle of the disease vector where the effect is more potent. Here, we tested the hypothesis that the powder materials of *P. alliacea*, *F. virosa* could disrupts the developmental stages of *M. domestica* at specific concentrations. Our results show that at 15% concentration, powder materials of *P. alliacea* and *F. virosa* reduce the mean emergence of adults *M. domestica*. Moreover, the duration of the larval and pupal instars were lengthened with an increased total development time from egg to adult. There was a reduction in the mean emergence of adults and the corresponding weights of adult *M. domestica*. Our findings warrant continue toxicological and pharmacological testing on how the synergy between different components in *P. alliacea* and *F. virosa* could be developed to suppress the developmental stages and elimination of *M. domestica*.

## MATERIALS AND METHOD

Adult *M. domestica* used for this study were reared in the laboratory in order to obtain a self-sustaining colony. The insect was reared at  $27 \pm 2^\circ\text{C}$  and  $75 \pm 10\%$  relative humidity on a paste of grinded rice and fish mixed with water in the ratio 1:1:1.5 w/v. Two plant species of *P.*

*alliacea* and *F. virosa* were collected, and the leaves were air dried and pulverized into powder. The powder materials were individually added into a mixture of rice and fish paste at concentrations of 2, 5, 10, and 15 % weight per weight (%w/w). Eggs laid by reared adults were used for the experiments in this study. Thirty eggs were placed in a bioassay made of plastic cup (4.5 cm  $\times$  8.5 cm) containing diets treated with different powder materials of *P. alliacea* and *F. virosa*. A mixture of rice and fish paste without plant powder was use as the control. Eggs in plastic containers were observed daily for the development of larva, pupa and emergence of adults. Precisely, we examined the larvae at daily basis to estimate larval duration, which was calculated as the intervals between the beginning of 1st instar larvae and pupation. The duration for each larva was monitored on daily basis until pupation. Thereafter, we counted the resulting pupae and determined their weights to calculate the percent of pupation including the mean pupal weight. The reduction in pupal weight, percentage of total pupae developed to adults was all determined. The emergence of successfully metamorphosed adults was determined and expressed in percentages.

## Data analysis

We expressed our results as mean values  $\pm$  standard error of mean (SEM) and statistical comparison of data was performed using descriptive statistics and Analysis of Variance (ANOVA)-repeated measures. Means were separated using Tukey test. All levels of significance were set at  $p < 0.05$ . We performed all statistical analysis using the SAS-based statistical package JMP (www.jmp.com; SAS, Cary, NC).

## RESULTS

The duration of egg, larva, and pupa and developmental time of *M. domestica* in different concentrations of *P. alliacea* is presented in Table 1. Eggs treated with 2, 5, 15 and 15% of *P. alliacea* hatched within 24 hours. Duration for the 1<sup>st</sup> larval instar in the various treated diets including control was 24 hrs. We observed a variation ( $2.00-2.50 \pm 0.29$  days) in the duration for the 2<sup>nd</sup> instar larval treated with diets, but this was not significantly different ( $P=0.19$ ). Similarly, there was no significant difference ( $P=0.54$ ) in the duration for the 3<sup>rd</sup> instar larva between 1.00 and  $1.50 \pm 0.29$  days. The development of eggs to adult stage varies from one concentration to the other. It ranges between  $10.50 \pm 0.29$  to  $11.25 \pm 0.25$  days while the duration for the control was 9.00 days. Mean percent emergence and adult weights in different concentrations of *P. alliacea* varied between  $90.82 \pm 2.15$  at 10% and  $81.67 \pm 2.15$  in 15% concentration (Table 2). The percentage emergence in the control diet was  $90.83 \pm 1.59$ . The weights of adults ranged between  $9.25 \pm 0.34$  and  $10.00 \pm 0.43$  mg with no significant difference ( $P > 0.05$ ). The mean weights for adults in the control diets were  $10.75 \pm 0.25$  mg.

Table 3 presents the duration of egg, larva, pupa

**Table 1:** Egg, Larva and Pupa duration and development time of *Musca domestica* maintained on diet n treated with different concentrations of *Petiveria alliacea*

Treatment (%)	Duration (days)					Development time (days)
	Eggs	Larval			Pupa	
		1 <sup>st</sup> instar	2 <sup>nd</sup> Instar	3 <sup>rd</sup> Instar		
2	1	1	2.00 <sup>a</sup>	1.50 ± 0.29 <sup>a</sup>	5.00 <sup>ab</sup>	10.50 ± 0.29
5	1	1	2.25 ± 0.25 <sup>a</sup>	1.50 ± 0.29 <sup>a</sup>	5.25 ± 0.48 <sup>ab</sup>	11.00 ± 0.58
10	1	1	2.00 <sup>a</sup>	1.25 ± 0.25 <sup>a</sup>	5.25 ± 0.25 <sup>b</sup>	10.50 ± 0.29
15	1	1	2.50 ± 0.29 <sup>a</sup>	1.50 ± 0.29 <sup>a</sup>	4.15 ± 0.15 <sup>b</sup>	11.25 ± 0.25
Control	1	1	2.00 <sup>a</sup>	1.00 <sup>a</sup>	4.00 <sup>a</sup>	9
			F=1.71	F=0.80	F=4.15	
			P=0.199	P=0.54	P=0.019	
			NS	NS	P<0.05	

**Table 2:** Mean percent emergence of adult and weights in different concentrations of *Petiveria alliacea* treated media.

Treatment (%)	Mean percentage Emergence	Mean weights (mg)
		(m ± S.E)
2	85.00 ± 2.15	10.00 ± 0.43
5	85.00 ± 2.15	9.92 ± 0.16
10	90.82 ± 4.91	9.25 ± 0.55
15	81.67 ± 2.15	9.25 ± 0.34
Control	90.83 ± 1.59	10.75 ± 0.25

**Table 3:** Egg, Larva and Pupa duration and development time of *Musca domestica* maintained on diet treated with different concentrations of *Flueggae virosa*.

Treatment (%)	Duration (days)					Development time (days)
	Eggs	Larval			Pupa	
		1 <sup>st</sup> instar	2 <sup>nd</sup> instar	3 <sup>rd</sup> instar		
2	1	1	2.00 <sup>b</sup>	1.50 ± 0.29 <sup>a</sup>	5.00 ± 0.41 <sup>a</sup>	10.50 ± 0.29
5	1	1	2.50 ± 0.29 <sup>b</sup>	1.75 ± 0.25 <sup>a</sup>	4.75 ± 0.25 <sup>a</sup>	10.75 ± 0.25
10	1	1	2.00 <sup>b</sup>	1.75 ± 0.25 <sup>a</sup>	4.75 ± 0.25 <sup>a</sup>	10.50 ± 0.29
15	1	1	1.25 ± 0.25 <sup>a</sup>	1.75 ± 0.25 <sup>a</sup>	4.75 ± 0.25 <sup>a</sup>	9.75 ± 0.25
Control	1	1	2.00 <sup>b</sup>	1.00 <sup>a</sup>	4.00 <sup>a</sup>	9
			F=6.85	F=1.96	F=2.03	
			P=0.02	P=0.15	P=0.14	
			P<0.05	NS	NS	

and developmental time of *M. domestica* in different concentrations of *F. virosa*. Eggs treated with diets mixed 2, 5, 10 and 15 % concentrations of *F. virosa*, including the control hatched within 24 h. The duration for the 1<sup>st</sup> larval instar in the various diets as well as the control was 24 h. The 2<sup>nd</sup> instar larval duration vary, ranging between 1.25 ± 0.25 and 2.50 ± 0.29 days, and the variation was significantly differently (P=0.02). There was no significant difference in the duration of the 3<sup>rd</sup> instar (P=0.15). Total days of development from egg to adult stage ranged between 9.75 ± 0.25 and 10.75 ± 0.25 days. Development from egg to adult stage was 9.00 days for the control. The mean percent emergence and adult weights in different concentrations of *F. virosa* is presented in Table 4. The percentage emergence ranges between 81.65 ± 4.19 in 2%, 84.10 ± 4.41 in 5%. It was 90.83 ± 1.59 in the control. There was no significant difference (P>0.05) in the weights of the emerged adults (9.08 ± 0.63 and 9.58 ± 0.63 mg). Adult weight in the control was 10.75 ± 0.25.

**Table 4:** Mean percent emergence and adult weights in different Concentrations of *Flueggae virosa* treated media.

Treatment (%)	Mean percentage	Mean weights
	Emergence	(mg) (m ± S.E)
2	81.65 ± 4.19	9.17 ± 0.63
5	84.10 ± 4.41	9.58 ± 0.37
10	81.67 ± 6.16	9.17 ± 0.62
15	82.50 ± 4.59	9.08 ± 0.63
Control	90.83 ± 1.59	10.75 ± 0.25

**DISCUSSION**

Three major findings arise from the experiments in this study. First, we found that eggs of *M. domestica* placed on all diets treated with leaf powders of *P. alliacea* and *F. virosa* in 2, 5, 10 and 15 % concentrations including control hatched within 24 h. Second, we found a prolongation of the larval and pupal instars resulted in an increase in the total development time from egg to adult in all the treated media, except for the control condition. Third, 15% concentration of *F. virosa* reduced the mean

emergence of adults and the corresponding weights of adult *M. domestica*. Finally, and very interesting, we observed that some of the larvae of *alliacea* and *F. virosa* died as larval-pupal intermediates and some pupae also failed to emerge as adults. This finding indicates that some of the plant powders contain components that probably disrupt the biological activities of the life cycle of *M. domestica*. Taken together, these results suggest that at a higher concentration of 15% or above, *P. alliacea* and *F. virosa* may represent an efficient biological approach of controlling *M. domestica*.

Our finding that the *P. alliacea* and *F. virosa* generally prolonged the second and third larval duration of *M. domestica* is supported by other studies (Tsao, et al., 1996; Singh and Upadhyay, 1993) using other plants. Moreover, *P. alliacea* and *F. virosa* treated diets prolonged the pupa duration. Similar observation was reported for other plants by Assar (Assar, 2003) as application of *Atriplex inflata* powder prolonged the duration of the pupa. Furthermore, *Artemisia monosperma*, *Conyza dioscoridis*, *Eichhornia crassipes*, *Clerodendron inerme*, *Clocasia antiqorum*, and *Farestia aegyptia* lengthened the pupa duration of *M. domestica* (Bakr, et al., 2003). The prolongation of the larval and pupal instars in our current study resulted in the observed increase in total development time from egg to adult in all the treated media. A delay in reproductive development appears to subsequently results in a decrease in total fecundity of the females and male to female contact. It is also possible that the delayed development could be as a result of delayed moulting process since the total number of days of development is shorter in the control diet.

The effect of the powder materials of *F. virosa* on the life cycle of *M. domestica* vary with concentrations and a 15% concentration resulted in a decrease in the developmental time, suggesting that an increase in concentration beyond 15 % may prevent normal post-embryonic development and the emergence of adults. The percentage of adult emergence from treated diets at all concentrations was less than the control condition. Some larvae of *P. alliacea* and *F. virosa* died as larval-pupal intermediates while some pupae also failed to emerge as adults. It is possible that *P. alliacea* and *F. virosa* powder materials contain desirable primary or secondary pharmacological components, which elicit biological activities that inhibit larval/pupal transformation and pupal eclosion hindrances.

All parts of *F. virosa* including the leaf, root and stem contain indolizidine alkaloids, mainly isomers and derivatives of the highly toxic securinine. Several pharmacological properties of bergenin from *F. virosa* are associated with inhibitory effect on growth of different vectors of human disease including *Trypanosoma brucei* (Muthaura, et al., 2011). In addition, the alkaloid virosecurinine is toxic and showed significant cytotoxicity properties (Weinreb, 2009). *P. alliacea* contains dibenzyl trisulphide and dichloromethane that has lipophilic

metabolites with toxic pharmacological effects. A direct contact of the powder materials of *F. virosa* and *P. alliacea* could disrupt pupal transformation by absorption, translocation and a possible movement through the membranes to the site of action (Ujváry, et al., 1992). This might have contributed to the observed reduction in the emergence of adults.

Diets treated at different concentrations of leaf powders of *P. alliacea* and *F. virosa* lengthened the duration of the larval and pupal instars, increased total development time from egg to adult, and reduce the mean emergence of adults and the corresponding weights of *M. domestica*. This finding indicates that the leaf powders materials of *P. alliacea* and *F. virosa* can be used to control *M. domestica* by inhibiting the developmental process. Since the mean emergence of adults is reduced, the direct consequence is the shortening of the life span resulting in larval-pupal intermediates.

In this study, we investigated the effect of *P. alliacea* and *F. virosa* on *M. domestica* and the possibility of using these materials as larvicides, pupacides to control a vector of many human diseases by a direct contact with the vector or its breeding places. Our findings suggest that a direct contact of the powdered form of *P. alliacea* and *F. virosa* affected the normal development of the different developmental stages of *M. domestica*. For decades, pharmacognostic and ethnobotanical studies have focused in the search of a single plant drug isolation, assuming that one drug is responsible for all plant biological activity. Our findings suggest a possibility of synergy between different components in *P. alliacea* and *F. virosa* that show great promise in suppressing developmental stages of *M. domestica* of *M. domestica*.

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