

# Asian Pacific Journal of Tropical Disease

journal homepage: <http://www.apjtc.com>



Original article

<https://doi.org/10.12980/apjtd.7.2017D7-200>

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## Powdered leaf extracts of *Harungana madagascariensis*, *Margaritaria discoidea*, and *Antigonon leptopus* disrupt larva and pupa stages of a tropical disease vector

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### ARTICLE INFO

#### Article history:

Received 1 Sep 2017

Received in revised form 21 Sep 2017

Accepted 29 Sep 2017

Available online 29 Nov 2017

#### Keywords:

*Harungana madagascariensis*

*Margaritaria discoidea*

*Antigonon leptopus*

Malaria

Vector

Tropical disease

### ABSTRACT

**Objective:** To determine the specific effect of leaf powder of *Harungana madagascariensis* (*H. madagascariensis*), *Margaritaria discoidea* (*M. discoidea*), and *Antigonon leptopus* (*A. leptopus*) on the life cycle of *Musca domestica* (*M. domestica*).

**Methods:** We tested the effects of the powder extracts of *H. madagascariensis*, *M. discoidea*, and *A. leptopus* on the life cycle of housefly, *M. domestica*. Adult flies were collected from a refuse dump, maintained under constant conditions: temperature of  $(27 \pm 2)$  °C and a relative humidity of  $75\% \pm 10\%$ . Flies were sustained on a mixture of ground rice, ground fish, and water at a ratio of 1:1:1.5 (w/v). Plant leaves were dried and crushed into powder and mashed into paste at various concentrations of 2%, 5%, 10% and 15% (w/w). Different life stages of *M. domestica* were treated with different concentrations of the powdered extracts.

**Results:** *H. madagascariensis*, *M. discoidea*, and *A. leptopus* extended the duration of the 3rd instar larva by 25%, 50% and 75% respectively in comparison to the control. Moreover, the pupal duration and total development time were lengthened while the mean emergence of adult and the corresponding weight of *M. domestica* were reduced.

**Conclusions:** Leaf powders of *H. madagascariensis*, *M. discoidea*, and *A. leptopus* appear to have promising biological effects in controlling the developmental stages of *M. domestica* especially if the plants powder concentrations are used above 15%.

## 1. Introduction

Housefly *Musca domestica* (*M. domestica*) belongs to the family Muscidae (order Diptera). About 90 percent of all flies occurring in human habitations are houseflies. Once a foremost nuisance and danger to public health in towns, houseflies are still a problem wherever decomposing organic waste and trash are allowed to accumulate. Houseflies may transmit on their feet millions of microorganisms that could spread at least 65 diseases to humans, including typhoid fever, dysentery, cholera, poliomyelitis, yaws, anthrax, tularemia, leprosy, and tuberculosis due to their feeding habit and proximity to human habitation[1,2]. Warm typical

conditions are normally optimal for the maturity of the housefly and it can complete its lifecycle within 7–10 days, with several generations in subtropical and tropical regions[1,3]. Garbage, manure, and similar wastes that cannot be made inaccessible to flies can be treated with larvicidal douses or dusts. Residual insecticidal sprays are effective against flies for several weeks; however, some houseflies developed resistance to certain insecticides, such as dichlorodiphenyltrichloroethane (DDT). Due to the built-up resistance, efforts have been put into new control methods which include local plants species and their chemicals that contain pharmacological components that can inhibit the development process of *M. domestica*.

Application of sublethal doses of thyme oil to *M. domestica* decreased significantly longevity of both sexes. Larva vitality and pupa survival were also affected by treating females with thyme oil. Plant extracts of *Calotropis procera*, *Acacia nilotica* and *Cassia senna* have been shown to have insecticidal activity

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The journal implements double-blind peer review practiced by specially invited international editorial board members.

on *Culex pipiens*[4]. It has been shown that *Ocimum basilicum*, *Gardenia jasmioides* and *Lantana camara* are very effective in delaying the growth of larvae of *M. domestica*[5]. Another study shows that *Bambusa multiplex*, *Tadehagi triquetrum*, and *Uraria crinita* are efficient in repulsing and killing the larvae of *Chrysomya megacephala*[6]. A mixture of *Desmodium paniculatum* with tannins reduced percentage emergence and average weight of *M. domestica*[7,8]. The above-mentioned studies and several other studies suggest that local plants represent a major source of innovative drugs to control *M. domestica*.

In spite of the short lifespan of the housefly, there have been very few studies using biodegradable insecticides of plant origin. Pharmacological studies have recognized the value of ethno medicinal plants as potential source of bioactive materials[8-11]. It is against this background that the powders of leaves *Margaritaria discoidea* (*M. discoidea*), *Antigonon leptopus* (*A. leptopus*), and *Harungana madagascariensis* (*H. madagascariensis*) were tested on the life cycle of *M. domestica*. Extensive studies have identified active ingredients with insecticidal properties in *M. discoidea*, *A. leptopus* and *H. madagascariensis*[12-14] that could play a major role in the control of housefly. For instance, *M. discoidea* were discovered to have many alkaloids including phyllochrysin (a central nervous system stimulant) and securinine[15,16]. Oral administration of an aqueous extract at various concentrations showed no acute toxicity in rats and no adverse change in behavior, suggesting that it may be safe for pharmacological uses. The aqueous extract of *M. discoidea* stem bark was investigated for its anti-inflammatory and analgesic activities in animal models (rats). The extract reduced significantly the formation of oedema induced by carrageenan and histamine, and had a good analgesic effect, with the results comparable to those of indomethacin, the reference drug used in the study.

Similarly, several ethnopharmacological studies identified different pharmacological components of *H. madagascariensis*, *M. discoidea*, and *A. leptopus* extracts that could eradicate *M. domestica*[17]. The objective of this study was to determine the specific effect of leaf powder of *H. madagascariensis*, *M. discoidea*, and *A. leptopus*, including which stage in the life cycle of the disease vector is the effect more potent. In this study, we assessed the effects of different concentrations of leaf powder of *H. madagascariensis*, *M. discoidea*, and *A. leptopus* on the developmental stages of *M. domestica* including length of time to emergence and weight at emergence.

## 2. Materials and methods

In this study, the adult houseflies used were reared in the laboratory at a temperature of  $(27 \pm 2)$  °C and relative humidity of  $75\% \pm 10\%$  on a paste containing ground rice, fish and water in a ratio of 1:1:1.5 w/v (weight per volume). *H. madagascariensis*, *M. discoidea* and *A. leptopus* were collected air-dried, and crushed into powder; the powdered leaves were individually added into the rice and fish paste at concentrations of 2%, 5%, 10% and 15% w/w (weight per

weight). The eggs laid by the nurtured adult flies were used in this study. Thirty eggs were placed in a bioassay made of a plastic cup ( $4.5 \text{ cm} \times 8.5 \text{ cm}$ ) containing the ground rice and fish paste mixed with the individual powdered leaves. A control experiment without the powdered leaves was set up to detect change and comparison. The development of the eggs into the larva, pupa and adult stage were observed in the plastic cup. The development of the 1st, 2nd and 3rd instar larvae and pupal stages were recorded in days and also the adult weights were recorded at emergence.

Statistical analysis was performed using the SAS (Statistical Analysis System) based statistical package JMP. We performed all experiments in quadruplicate to reduce error. Comparison of means was done using analysis of variance (ANOVA) while means were separated using the Turkey's range test. Level of significance was set at  $P < 0.05$ .

## 3. Results

The egg, larva and pupa duration and developmental time of *M. domestica* treated at different concentrations of *H. madagascariensis* is shown in Table 1. Eggs introduced into 2%, 5%, 10% and 15% of *H. madagascariensis* treated diets including control hatched within 24 h. Similarly, duration of the 1st instar larva in the various diets as well as the control was 24 h. There was variation in the duration of the 2nd instar larva on the treated media, ranging between  $(1.25 \pm 0.25)$  and  $(2.25 \pm 0.25)$  days and were significantly different from one another ( $P < 0.05$ ). There was no significant difference in the duration of the 3rd instar larva ( $P = 0.25$ ) with minimum and maximum number of days at 1.00 and  $(1.75 \pm 0.25)$  days, respectively. The entire days of development from egg to adult stage varied from one concentration to the other with no significant difference between them  $(9.25 \pm 0.48$  to 11.00 days) while the duration of the control was 9.00 days.

**Table 1**

Egg, larva and pupa duration and development time of *M. domestica* treated with different concentrations of *H. madagascariensis*.

| Treatment (%) | Duration (days) |            |                   |                   |                      | Development time (days) |
|---------------|-----------------|------------|-------------------|-------------------|----------------------|-------------------------|
|               | Eggs            | Larval     |                   |                   | Pupa                 |                         |
|               |                 | 1st Instar | 2nd Instar        | 3rd Instar        |                      |                         |
| 2             | 1.00            | 1.00       | 2.00 <sup>b</sup> | $1.75 \pm 0.25^a$ | $5.25 \pm 0.25^a$    | 11.00                   |
| 5             | 1.00            | 1.00       | $2.25 \pm 0.25^b$ | $1.25 \pm 0.25^a$ | $4.75 \pm 0.25^{ab}$ | $10.25 \pm 0.25$        |
| 10            | 1.00            | 1.00       | 2.00 <sup>b</sup> | $1.25 \pm 0.25^a$ | $4.25 \pm 0.25^{ab}$ | $9.50 \pm 0.29$         |
| 15            | 1.00            | 1.00       | $1.25 \pm 0.25^a$ | $1.25 \pm 0.25^a$ | $4.25 \pm 0.25^{ab}$ | $9.25 \pm 0.48$         |
| Control       |                 |            | 2.00 <sup>b</sup> | 1.00 <sup>a</sup> | 4.00 <sup>a</sup>    | 9.00                    |
|               |                 |            | $F = 5.75$        | $F = 1.50$        | $F = 5.00$           |                         |
|               |                 |            | $P = 0.05$        | $P = 0.25$        | $P = 0.01$           |                         |
|               |                 |            | $P < 0.05$        | NS                | $P < 0.01$           |                         |

Table 2 presents the duration of egg, larva and pupa and developmental time of *M. domestica* treated different concentrations of *M. discoidea*. Eggs hatched within 24 h in diets treated with 2%, 5%, 10% and 15% *M. discoidea* and the control. Duration of the 1st larval instar in the various diets as well as the control was also 24 h. There was significant variation in the duration of the 2nd instar larval

on the treated media which varied between  $(1.25 \pm 0.25)$  and  $(2.25 \pm 0.25)$  days ( $P < 0.02$ ). Duration of the 3rd instar larva which varied from 1.00 day to  $(1.75 \pm 0.25)$  days were not significantly different from each other. The total days of development from egg to adult emergence were not significant and varied from one concentration to the other, from  $(9.25 \pm 0.25)$  to  $(10.75 \pm 0.48)$  days with 9.00 days in the control diet.

**Table 2**

Egg, larva and pupa duration and development time of *M. domestica* treated with different concentrations of *M. discoidea*.

| Treatment (%) | Eggs | Duration (days) |                      |                   | Pupa                 | Development time (days) |
|---------------|------|-----------------|----------------------|-------------------|----------------------|-------------------------|
|               |      | Larval          |                      |                   |                      |                         |
|               |      | 1st Instar      | 2nd Instar           | 3rd Instar        |                      |                         |
| 2             | 1.00 | 1.00            | $2.25 \pm 0.25^b$    | $1.75 \pm 0.25^a$ | 5.00 <sup>b</sup>    | $10.75 \pm 0.48$        |
| 5             | 1.00 | 1.00            | 2.00 <sup>ab</sup>   | $1.50 \pm 0.29^a$ | $4.50 \pm 0.29^{ab}$ | $10.25 \pm 0.48$        |
| 10            | 1.00 | 1.00            | $1.75 \pm 0.25^{ab}$ | $1.25 \pm 0.25^a$ | $4.75 \pm 0.25^{ab}$ | $9.75 \pm 0.25$         |
| 15            | 1.00 | 1.00            | $1.25 \pm 0.25^a$    | $1.75 \pm 0.25^a$ | $4.25 \pm 0.25^{ab}$ | $9.25 \pm 0.25$         |
| Control       |      |                 | 2.00 <sup>ab</sup>   | 1.00 <sup>a</sup> | 4.00 <sup>a</sup>    | 9.00                    |
|               |      |                 | $F = 3.83$           | $F = 1.96$        | $F = 3.75$           |                         |
|               |      |                 | $P = 0.02$           | $P = 0.15$        | $P = 0.02$           |                         |
|               |      |                 | $P < 0.05$           | NS                | $P < 0.02$           |                         |

The duration of egg, larva and pupa and developmental time of *M. domestica* treated with different concentrations of *A. leptopus* is shown in Table 3. Eggs introduced into 2%, 5%, 10% and 15% *A. leptopus* treated diets together with control diet hatched within 24 h. Duration of the 1st larval instar in the various treated diets as well as the control was also 24 h. There was variation in the duration of the 2nd instar larval on the treated diet ranging from  $(1.25 \pm 0.25)$  to  $(2.25 \pm 0.25)$  days and were significantly difference from each other ( $P < 0.01$ ). There was absence of significant difference in the duration of the 3rd instar larva ( $P = 0.25$ ) with the lowest and highest number of days at 1.00 and  $(1.75 \pm 0.25)$  days, respectively. There was no significant difference in the total days of development from egg to newly emerged adult which varied from  $(9.75 \pm 0.25)$  to  $(10.00 \pm 0.58)$  days and the duration in the control was 9.00 days.

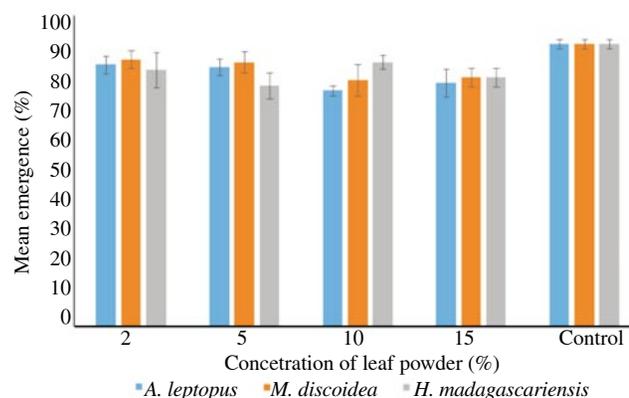
**Table 3**

Egg, larva and pupa duration and development time of *M. domestica* with different concentrations of *A. leptopus*.

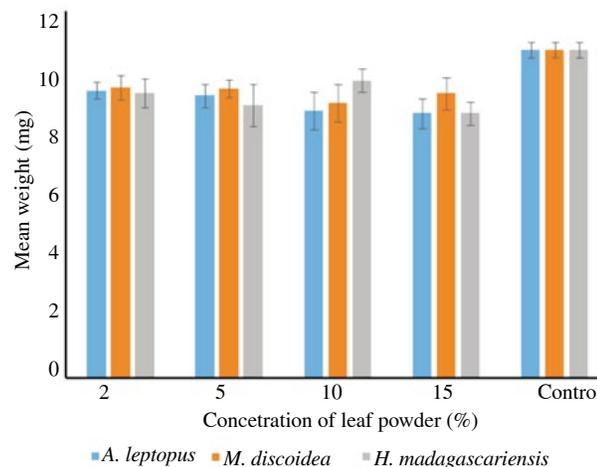
| Treatment (%) | Eggs | Duration (days) |                    |                   | Pupa              | Development time (days) |
|---------------|------|-----------------|--------------------|-------------------|-------------------|-------------------------|
|               |      | Larval          |                    |                   |                   |                         |
|               |      | 1st Instar      | 2nd Instar         | 3rd Instar        |                   |                         |
| 2             | 1.00 | 1.00            | $2.25 \pm 0.25^b$  | $1.25 \pm 0.25^a$ | 4.00 <sup>a</sup> | $9.50 \pm 0.29$         |
| 5             | 1.00 | 1.00            | $2.25 \pm 0.25^b$  | $1.50 \pm 0.29^a$ | $4.25 \pm 0.25^a$ | $10.00 \pm 0.58$        |
| 10            | 1.00 | 1.00            | 2.00 <sup>ab</sup> | $1.25 \pm 0.25^a$ | $4.50 \pm 0.29^a$ | $9.75 \pm 0.48$         |
| 15            | 1.00 | 1.00            | $1.25 \pm 0.25^a$  | $1.75 \pm 0.25^a$ | $4.75 \pm 0.25^a$ | $9.75 \pm 0.25$         |
| Control       |      |                 | 2.00 <sup>ab</sup> | 1.00 <sup>a</sup> | 4.00 <sup>a</sup> | 9.00                    |
|               |      |                 | $F = 4.50$         | $F = 1.50$        | $F = 0.26$        |                         |
|               |      |                 | $P = 0.01$         | $P = 0.25$        | $P = 0.08$        |                         |
|               |      |                 | $P < 0.05$         | NS                | NS                |                         |

Figures 1 and 2 present the mean percent emergence and adult weights respectively in different concentrations of *H. madagascariensis*, *M. discoidea* and *A. leptopus*. In *H. madagascariensis* percentage emergence varies between  $77.50\% \pm 4.17\%$  in 5% and  $85.00\% \pm 2.15\%$  in 10% but the percentage emergence in the control diet was higher at  $90.83\% \pm 1.59\%$ . With

respect to the weights of emerged adults, control group showed a slight increase ( $10.75 \pm 0.25$  mg) when compared with groups treated with plants leaf powder which vary between  $(8.67 \pm 0.38)$  and  $(9.75 \pm 0.37)$  mg, However, this difference was not statistically significant.



**Figure 1.** Mean percentage emergence of *M. domestica* treated with powdered extracts of *H. madagascariensis*, *M. discoidea*, and *A. leptopus*.



**Figure 2.** Weight at emergence of *M. domestica* treated with powdered extracts of *H. madagascariensis*, *M. discoidea*, and *A. leptopus*.

Similarly, in *M. discoidea* percent emergence varied between  $85.83\% \pm 2.85\%$  in 2% and  $79.17\% \pm 4.98\%$  in 10% but the percent emergence in the control diet was  $90.83\% \pm 1.59\%$ . There was no significant difference in the weights of the emerged adults which varied between  $(9.00 \pm 0.61)$  and  $(9.50 \pm 0.40)$  mg with the mean control adult weight at  $(10.75 \pm 0.25)$  mg.

In *A. leptopus* the lowest and highest percent emergence were  $75.83\% \pm 1.60\%$  in 10% and  $84.17\% \pm 2.85\%$  in 2% and in the control diet, percent emergence was  $90.83\% \pm 1.59\%$ . The weights of the emerged adults decreased with increase in concentration from  $(9.42 \pm 0.28)$  at 2% to  $(8.67 \pm 0.49)$  mg at 15% but mean weights of adult in the control diets was  $(10.75 \pm 0.25)$  mg. In summary, with respect to all the emergence and weight at emergence, we observed a decrease in emergence and weight at emergence as a result of treatment with plant leaf powder, but the effect was not statistically significant.

#### 4. Discussion

In the study, four major observations were made. Firstly, the eggs of *M. domestica* (housefly) placed on diets treated with leaf powders of *H. madagascariensis*, *M. discoidea* and *A. leptopus* at varying concentrations of 2%, 5%, 10%, 15% hatched within 24 h. Secondly, there was delay in the larval and pupal instars which resulted in an increase in the total time of development except in the control experiment. Thirdly, an increase in the concentration of the leaf powders increased with the duration of time indicating that increasing the concentration of the leaf powders above 15% may be toxic to housefly. Finally, the mean percentage emergence and mean weight for the control were higher than any of the concentrations of the various plants because some of the larvae died at larval-pupal transition stages, while some pupa did not emerge as adults.

Our observation that *H. madagascariensis*, *M. discoidea* and *A. leptopus* increased the development time in the second and third larval instars is supported by different studies using other plants. For example, plants extract of ginger (*Zingiber officinale*), holy basil (*Ocimum sanctum*), jatropha (*Jatropha podagrica*) and turmeric (*Curcuma longa*) except *Nicotiana tabacum* (tobacco) and *Azadirachta indica* (neem) extended the duration of the various larval instars and pupation of housefly[18-20]. Moreover, *Artemisia monosperma*, *Conyza dioscoridis*, *Eichhornia crassipes*, *Clerodendrum inerme*, *Colocasia antiquorum*, and *Farestia aegyptia* lengthened the pupa duration of *M. domestica*[21,22]. The extension of the pupal and larval instar, results in an increase in the total development time from egg to adult. A delay in reproductive development appears to subsequently result in a decrease in fertility of the females and male to female contact. It is also possible that the delayed development could be as a result of delayed molting process[23] since the total number of days of development is shorter in the control diet.

The powder extracts from *H. madagascariensis*, *M. discoidea* and *A. leptopus* on the life cycle of *M. domestica* was more effective at a specific concentration. Precisely, at 15% concentration, there was an increase in development time from larva to pupa. The elongation of the larval and pupal instars resulted in the increase in total development time from egg to adult. This finding suggests that above 15% concentration, the plant extracts of *H. madagascariensis*, *M. discoidea* and *A. leptopus* may be toxic thus preventing adult emergence and normal development. Although the use of powdered leaf extracts did not significantly influence mean percent emergence and the mean weight of the plant extract at different concentrations, we did observe a decrease in these variables when compared with the control experiment. These results indicate that treatment with plant products led to more death at the larval, pupal stage while some failed to emerge as adults. This finding also suggests a direct effect of the powder extracts in shortening the life span especially at the larval-pupal intermediates.

Since different 15% concentrations of leaf powders lengthened

the duration of the larval and pupal instar, increased the total development time from egg to adult stage and decreased the mean emergence of adult including the mean weight of *M. domestica*, it is possible that the 15% concentration delayed the reproductive growth resulting in a decrease in total fertility of the females and male to female. In this context, a 15% concentration or above of leaf extracts of *H. madagascariensis*, *M. discoidea* and *A. leptopus* can be used to control *M. domestica* by hindering the developmental process.

In general, *H. madagascariensis*, *M. discoidea* and *A. leptopus* powder contain a suitable amount of pharmacological composition that prevents the metamorphosis of housefly. For example, the stem, bark of *H. madagascariensis* contains phytochemicals like alkaloids, tannin, saponins and phenols which have inhibitory effect on disease vectors[24,25]. Moreover, the alkaloid is toxic and shows significant cytotoxicity properties[26-28]. *A. leptopus* has phytochemicals like alkaloids, flavonoids, sterols, glycosides, tannins and saponins that affect the life cycle of house flies[29-31]. A study by Lawal *et al.*[15] indicates that *M. discoidea* essential oils like phytol, geranyl acetone, eremophilene has toxic and insecticidal effect on *Sitophilus zeamais*, thereby decreasing the mortality rate of *Sitophilus zeamais*. Essential oils of *M. discoidea* containing a sizable proportion of geranyl acetone with larvicidal activity against mosquito, toxicity to housefly and repellants to *Aedes aegypti*[15,32].

The current study investigated the effect *H. madagascariensis*, *M. discoidea* and *A. leptopus* including the potential of using these extracted powder materials as larvicides, or pupacides to eliminate *M. domestica* by a direct contact with the developmental stages in its breeding places. We observed that a direct contact of the powdered extracts disrupted the normal development of the different developmental stages of *M. domestica*. A major finding in this study is that the integration of the different components of *H. madagascariensis*, *M. discoidea* and *A. leptopus* may show great potentials in disrupting the developmental stages of *M. domestica*. In this context, the search for a single plant drug isolation by pharmacognostic and ethnobotanical studies may require more integrative studies since one drug from one plant may not be responsible for all plant biological activity especially in disrupting the developmental stage of *M. domestica*.

#### Conflict of interest statement

We declare that we have no conflict of interest.

#### Acknowledgments

We thank Dr. Wendy Ingiamo for reviewing the previous versions of this manuscript.

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