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# Evaluation of the Larvicidal Activity of Marigold (Tagetes erecta) Flower Extracts in Mixed Cultured Mosquitoes

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## Abstract

For decades, scientific studies related to wildlife have been little reviewed for national inventories, with birds and mammals being the only faunal groups that have official lists and specific numbers. Although it is true that species of insects and other invertebrates appear constantly, this is not an excuse for not having official lists of species reported for science. In this review, a total of 507 were found for Costa Rica, 7 of Andrenidae, 299 of Apidae, 30 of Colletidae, 106 of Halictidae and 65 of Megachilidae. Any other species outside of this list that has been found, collected in Costa Rican territory and is not on this list is a new report.

**Keywords:** Bumble Bees; Carpenter Bees; Cucu Bees; Stingless Bees; Oil Bees; Orchid Bees; Solitary Bees

## Abbreviations

DOH: Department of Health; CRD: Completely Randomized Design; LC: Lethal Concentration; ROTAVAP: Rotary Evaporator.

## Introduction

Among the most medically important vectors spreading parasites and pathogens are the mosquitoes [1]. Mosquito-borne diseases such as dengue, filariasis, malaria, and chikungunya remain to be a major public health concern. Over 300 million clinical cases and one million deaths worldwide are mosquito-related. In the Philippines, the Department of Health (DOH) has documented around 216,927 cases of dengue from January to December 2022. There are 266 malaria cases reported nationwide from January 1 to December, 2022. Nevertheless, about 106 million people worldwide are affected of another mosquito-borne disease, lymphatic filariasis as reported by the DOH Dengue Disease Surveillance [2,3]. One of the effective methods to control these diseases is targeting the vectors or interrupting the disease transmission. All stages of the life cycle of mosquitoes can be the target of control efforts, but the focus was mainly on the adult stage and larval stage [4].

Comprehensive strategies have been made in the control of mosquitoes at different stages but the control during larval stage found to have many advantages. As the population of mosquitoes is relatively immobile in the immature larval stage, it is much easier to control compared to adults. Moreover, focusing on the larval stage has the advantage of controlling the vector prior to the acquisition of the disease and interrupting the life cycle before transmission of the disease. Hence, the ideal control method would be the systematic treatment of their breeding places through larvicides [5].

The usual method in controlling mosquitoes is through the spray of insecticides which has become the primary concern of several new researches over the past decades [6]. However, the growing incidence of insect resistance and the toxicity problem is the drawback with the use of chemical insecticides [7-9]. The extensive and unbalanced usage of synthetic insecticides such as organochlorides, organophosphates, and carbamates has disrupted natural biological control systems, which led to the development of resistance as in the case of Malathion and Deltamethrin resistance in adult mosquito named *Aedes aegypti* [10]. Furthermore, it can cause harmful effects on beneficial non-target fauna inhabiting the same environment and also bring out serious harm to human health as well [11]. In recent years, the increasing information on the hazardous effect of synthetic insecticides on the plant, animal, and human health has alarmed scientists to seek some alternative ways such as insecticides that are eco-friendly [12]. Therefore, the need for alternate, more effective, and environment-friendly control agents or insecticides became urgent [13].

Biological control using extracts of plant parts with larvicidal property are the best alternatives in addressing the problem on hazardous chemicals which can destroy our ecosystems and are hazardous to human race. They are plant–driven insecticides which are either naturally occurring plant materials that contain insect toxins [14]. The Philippines is rich with indigenous herbal plants. Many of these plants have not yet been studied and some are neglected and remain underutilized (Medical Health Guide 2011). Although many plant products are used as insecticidal, repellent and oviposition deterrent properties against mosquitoes, the search for effective natural products as alternative vector control agents is continuing. Therefore, the study aimed to explore larvicidal potentials of essential oils from the flowers of *Tagetes erecta* against larval mosquitoes of different species.

Marigold (*Tagetes erecta*) belongs to the family Asteraceae. It is a common herb and is a therapeutically important medicinal plant with strong therapeutic importance in the medicinal industry [15]. This plant contains the following phytochemical components: thiophenes, alkaloids, phenols, flavonoids, carotenoids, glycosides, triterpenoids, quinines, terpenoids, coumarins, carbohydrates, tannins, steroids, terpenes and salicylic acid. Some of the extracted phytocompounds from *T. erecta* contain quercetagetin, syringic acid, methyl-3, 5-dihydroxy-4benzoate methoxy, ethyl gallate, quercetin, thienyl, piperitone, and D-limonene [16]. The plant is also used as a poison for invertebrates and in controlling plant pests. Furthermore, it has been reported to be used as an insecticide against Anopheles stephensi, Culex quinquefasciatus, and Tribolium castaneum mosquito species [17]. Based on these studies, it is noted that plants enriched with phytochemicals have particularly larvicidal and insecticidal characteristics.

The results of this study could add up to the marigold's reputation and importance, which will therefore invoke people to preserve and further utilize the plant species. The results could also be a great help to households in eradicating larval sites of mosquitoes with the use of a cheaper, eco-friendly, and effective larvicidal. This would further benefit consumers and the populace to eliminate mosquitoes in their locality. Along with the control of mosquitoes, there is thus the decline in health issues and epidemics such as malaria, dengue, and chikungunya which they transmit. This would also eradicate the mosquitoes before they could transmit the diseases that they carry. The study could also endorse and promote further queries and studies concerning the pest management of mosquitoes and other beneficial uses of T. erecta.

The study generally aimed to evaluate the larvicidal activity of *T. erecta* flower extracts in mixed cultured mosquitoes. Specifically, it aimed to:

1. Determine the mortality rate of 4th instar larvae when exposed to the following treatments: the positive control which is a commercial insecticide, the control, and the three different flower extracts with concentrations of 30%, 60%, and 90%; and
2. Determine which concentration of *T. erecta* flower extracts is effective as larvicide against mixed cultured mosquitoes.

## Materials and Methods

### Research Design

A Completely Randomized Design (CRD) was used. Twenty mosquito larvae were randomly assigned to 5 treatments that were replicated 9 times. There were five independent variables: the three different concentrations of Marigold flower extracts, the negative control distilled water and the positive control, Abate 1 SG Insecticide.

### Flower Collection

Flowers of *T. erecta* were collected in a farm at the municipality of Carmen, North Cotabato, after five (5) months of planting 100 hills of *T. erecta* flowers and were used for the tests. The flowers which have withered, have dust and dirt particles and have insect bites were discarded. The accumulated flowers were rinsed and washed with running water.

### Ethanolic Extraction of Marigold Flowers

The collected flower samples were air-dried for four to seven days cut into small portions and was mechanically ground and sieved to get a fine powder. Five hundred grams of the dried fine flower powders were extracted in 1,000 mL of 95% ethanol for 48 hours at a temperature not exceeding the boiling point of the solvent. The extracts obtained were filtered using Whatman filter paper (No. 1) and then concentrated using a rotary vacuum evaporator at 45˚C under low pressure for ethanol removal. After complete evaporation of the solvent, the concentrated extract was collected in sterile bottles and stored under refrigerated conditions until use. The dry weight of the flower plant extracts was obtained by ethanol solvent evaporation and used to determine the concentration in mg/ml. The extract obtained was directly used in the bioassay of mosquito larvicidal activity.

### Mosquito Larvae Collection

Mosquito larvae were acquired from containers with stagnant water. The larvae acquired and their related water was carefully transferred to 12oz sized plastic cups and allowed to acclimatize for five minutes to prevent stressing the larvae.

### Mosquito Rearing

Larvae were checked from time to time. They were fed ½ teaspoon of drained koi fish food. White pans were spat and thinned as needed since larvae should not exceed 200 per pan. Feeding of larvae was regularly done to check whether they have grown into pupae and emerged into adult mosquitoes. Pupae were collected from day 7 to 15 that was then transferred to the plastic pan and placed inside the mosquito rearing cage 30 cm × 30 cm × 30 cm (12" ×12" ×12") until they emerged. When all the adults have emerged, female mosquitoes usually must ingest a blood meal for ovarian development. Adults of both sexes require carbohydrate foods in addition. Carbohydrates are generally supplied as a sugar solution. 10% sucrose was used, by dissolving 100 g of white sugar in 1 L of water.

### Hatching of Mosquitoes' Eggs

For the egg batch, 100 mL of dechlorinated tap water was added to a rearing pan so that water just completely covers the bottom of the pan. A yeast suspension was added also to a final concentration of 0.02%. 300 mL of dechlorinated tap water and 2 mL of a 2g/100mL yeast solution were added. The yeast was swirled until it dispersed throughout the pan. It was placed on the shelf or rack where it was kept.

The pans were covered to prevent contamination that could occur from accidental splashing. Mosquitoes' eggs were waiting to hatch until they became fourth larval instars (between 3 to 4 days) that were prepared and used for larvicidal bioassay. Mosquitoes' eggs are generally hatched immediately or within 24-48 hours after placement in water.

### Phytochemical Analysis of Marigold Flower Extract

The procedure used in the determination of the Phytochemical Analysis was based on the procedures of Jabin, et al. [16]. This was followed for the determination of alkaloids, tannins, saponins, phenolic compounds, and flavonoids. The results were found to be positive in all tests. Air-dried flowers of marigold were used for the phytochemical analysis.

### Mosquito Larvicidal Bioassay

Tagetes erecta flower extracts were used to test the efficacy as a larvicide against the larval mosquito which was evaluated and performed under the guidelines of the World Health Organization (WHO). Batches of 20 early fourth instar larvae (5 days old and 5–6 mm in length) were transferred by plastic droppers to 12 oz (340mL) disposable plastic cups containing 50 mL of dechlorinated tap water.

Each test cup of the larvae were exposed to the following treatments: the positive control which was 50 ml of commercial insecticide, 50mL dechlorinated tap water for the negative control, and also the three different ethanolic flower extracts concentrations which is 30%(30ml of *T. erecta* + 70ml of distilled water), 60% (60ml of *T. erecta* + 40ml of distilled water), and 90% (90ml of *T. erecta* + 10ml of distilled water) that can be obtained through percentage. The test cup was netted and was held in the stockroom at room temperature. Each treatment was replicated nine times. The effects of the flower extracts were monitored by carefully counting the number of dead larvae after 24 and 48 hours of treatment, and the percentage of mortality was computed.

Another three replicates were set up for each concentration and an equal number of controls was set up simultaneously with dechlorinated tap water, to which 1 mL ethanol was added. Each test was run three times on different days having 180 larvae per treatment , 300 larvae per set up and 900 fourth instar larvae in all for three set ups. After determining the mortality of the larvae in this wide range of concentrations, a narrower range from 5 to 50 mg/L concentrations yielding between 10% and 95% mortality in 24 or 48 hours was re-evaluated to determine the lethal concentration (LC) LC50. Probit was used to calculate the

LC50 values analysis. On mixed cultured larvae, the LC50 value of *T. erecta* was 1.10 ppm.

## Statistical Analysis

The statistical tools that were utilized to ensure valid and reliable analysis and interpretation of data in this study are the following: the Arithmetic Mean to get the average number of dead mosquito of larvae, and the data from all replicates were subjected to log-probit analysis for calculating Lethal concentrations (LC) of the plant extracts on larval mosquito after 24 and 48 hours of treatment which result in 50%, 90% mortality (LC50) values using the SPSS 20.0 (Statistical Package of Social Sciences) software. Standard deviation or confidence intervals of the means of LC50 values are calculated and recorded.

The One-Way ANOVA was used for the interpretation of the data to determine and distinguish the differences that have occurred in the mortality of mosquito eggs and larvae between the five applied treatments: the 30%, 60%, and 90% ethanol flower extract concentrations, the positive control commercial insecticide, and dechlorinated tap water for the negative control.

The percentage rate of larval mortality was computed using the formula:
Percentage of Mortality = (Number of dead larva) / (Number of larva) x 100

The Average mean is computed using the formula:
Average Mean= Number of dead larva/Total number of larvae treated per treatment

## Results

In this study, Marigold (Tagetes erecta) extract was used to evaluate the larvicidal activity and its inhibitory activity on egg hatchability of mixed cultured mosquitoes. Three treatments with 30%, 60%, and 90% concentrations prepared from extracted marigold flowers revealed significant findings as potential larvicidal against cultured mosquito larvae. Results on the repellent effects of flower extracts were reflected in Tables 1 & 2. ANOVA after 12 and 24 hours for the significant differences in the average mean are reported in Tables 3-4. While the phytochemical analysis was reported in Table 5.

| Treatment | Concentration (%) | Mortality | |
|---|---|---|---|
| | | 12 hrs | 24hrs |
| 1 | 30% | 72.47% | 94.20% |
| 2 | 60% | 72.20% | 96.87% |
| 3 | 90% | 80% | 92.47% |
| 4 | Negative Control (Distilled water) | 0% | 0% |
| 5 | Positive Control (Abate 1 SG Insecticide) | 96% | 100% |

**Table 1:** Percentage mortality rates of marigold ethanolic flower extract against mosquito larvae subjected to three different concentrations after 12 and 24 hours.

| Treatments | No. of Hours | |
|---|---|---|
| | 12 hrs. | 24 hrs. |
| T1 (30%) | $10.87^{c}$ | $14.13^{bc}$ |
| T2 (60%) | $11.13^{c}$ | $14.53^{ab}$ |
| T3 (90%) | $12.0^{b}$ | $13.87^{c}$ |
| T4 (Negative Control) | $0.00^{d}$ | $0.00^{d}$ |
| T5 (Positive Control) | $14.4^{a}$ | $15.0^{a}$ |

**Table 2:** Average Means of the Mortality Rate of the Mosquito Larvae after 12 and 24 hrs of exposure to various concentrations of Marigold ethanolic flower extract.

| Source of variations | Degrees of freedom (df) | Sum of squares(SS) | Mean sum of squares (MS) | Observed (F) F value | Pr (>F) |
|---|---|---|---|---|---|
| **Treatments** | 4 | 374.6507 | 93.6627 | 2508.82 | 0 |
| **Errors** | 10 | 0.3733 | 0.0373 | | |
| **Total** | 14 | 375.024 | | | |
| CV = 2.00% | | | | | |
| **Significative at a level of 1% probability (p<.01) | | | | | |
| *Significative at a level of 5% probability (.01=<p<.05) | | | | | |
| Ns Non-significant (p>=.05) | | | | | |

**Table 3:** Analysis of Variance (ANOVA) of the average means after 12 hrs.

| Source of variations | Degrees of freedom (df) | Sum of squares(SS) | Mean sum of squares (MS) | Observed (F) F value | Pr (>F) |
|---|---|---|---|---|---|
| Treatments | 4 | 498.7093 | 124.6773 | 1298.72 | **0** |
| Errors | 10 | 0.96 | 0.096 | | |
| Total | 14 | 499.6693 | | | |
| CV = 2.69% | | | | | |
| **Significative at a level of 1% probability (p<.01) | | | | | |
| *Significative at a level of 5% probability (.01=<p<.05) | | | | | |
| Ns Non-significant (p>=.05) | | | | | |

**Table 4:** Analysis of Variance (ANOVA) of the average means after 24 hrs.

| Components | Tests | Indication | RESULTS |
|---|---|---|---|
| Alkaloids | • Wagner's test | · dark reddish brown precipitate | + |
| | ● Mayer's test | · Off white precipitate | + |
| Tannins | ● Lead Acetate test | · yellow precipitate | + |
| | ● Ferric Chloride test | · Change of color from blue to black | + |
| Saponins | Leibergman-bouchard test | · Honey comb froth formation | + |
| Phenolic Compounds | Aqueous ferric solution | · Dark blue coloration | + |
| Flavonoids | Bates-Smith & Metcalf | · light yellow color | + |

**Table 5:** Phytochemical Analysis Result.

## Discussion

Larvicidal Activity and its Efficacy Per Treatment. Three treatments (T1-30%, T2-60%, and T3-90% concentrations) were tested against cultured mosquito larvae. Each treatment has nine replicates with 20 early fourth instar larvae for the evaluation of larvicidal activity of marigold flower ethanolic extract.

Table 1 shows the percentage mortality rates of marigold flower extract against mosquito larvae subjected to three different concentrations. After 12 hours, data revealed that T3 with 90% concentration got the highest mortality rate with 80% efficacy, followed by 30% and 60% concentrations with 72.47% and 72.21%, respectively. The results show that as the concentration increases, the mortality rate of the larvae also increases. However, the trend otherwise was changed under 24 hours treatment. T2 with 60% concentration got the highest mortality rate with 96.87% efficacy, followed by 30% and 90% concentrations with 94.20% and 92.47%, respectively. The terpene, ocimenone, which is a part of the whole oil of Marigold (Tagetes minuta) was found to be larvicidal only at a higher concentration and its efficacy decreases within 24 hours after dispersal in water. Hence, it is observed that after 24 hours, 60% concentration of Marigold flower extract is already an effective larvicide

compared to 90% concentration. It is evident that *T. erecta* has a comparable trend of effect with that of T. minuta which is effective at 30% and 60% concentrations and that the efficacy declined at 90% concentration. The efficacy then decreases as the concentration increases after 24 hours of treatment or exposure of larvae to Marigold ethanolic flower extracts [18].

Nevertheless, the efficacy of commercial insecticide (Abate $1_{Sg}$) as the positive control is highly comparable to that of different concentrations of ethanolic extracts of marigold flower as shown by the mean after 12 hours and 24 hours. Nevertheless, sixty percent (60%) concentration of marigold flower ethanolic extract gave the highest mortality among concentrations. Lethal Concentrations (LC50) of Marigold Ethanolic Extracts on Mixed Culture of Larval Mosquitoes. The mortality of the larvae was measured at 12, and 24 hours after treatment. Both Non-existent larvae were defined as those that were dead or moribund. Three replicates of each trial were carried out. The death was noticed and recorded. The 50 percent (LC50 lethal concentrations were estimated as the concentrations (ppm) required to kill 50% of the larval population, respectively.On mixed cultured larvae, the LC50 value of *T. erecta* was 1.10 ppm.

Evaluation of the Larvicidal and Inhibitory Activity on the egg Hatchability per Treatment after 12 hours and 24 hours of Test. Table 2 shows that the average means of mortality of the early fourth instar larvae of mosquitoes after 12 hours as affected by the different concentrations of Marigold ethanolic flower extract, are significantly different with increasing results as concentration increases. Moreover, the 90% concentration got the highest average mean of 12.0 followed by 60% and 30% with an average means of 11.3 and 10.57 respectively. The effectivity of marigold ethanolic flower extracts differ greatly from each concentration. Evidently 60% concentration got the highest average mean after 24 hours with an average mean of 14.53 followed by 30% (14.3) and (13.67). T1 and T2 are not significantly different while T3 differ significantly from T1 and T2 which implies that at 90% the flower extract of marigold increases its larvicidal property. However, after 24 hours of treatment, differences in the average means did not vary significantly in T5, T1, and T2 but significantly vary in T3 which only implies that when time is prolonged 30% and 60% concentrations are already effective in mosquito larvae eradication. However, when the concentration is increased to 90% the efficacy will decrease due to water dilution effect. The results conform to the findings of Marques, et al. [19] which reported that the larvicidal activity of essential oils from marigold (*T. erecta*) was highly active against the larvae of Aedes aegypti. Moreover, piperitone, d-limonene, and piperitenone which are the main components of its essential oil when demonstrated by high-performance liquid chromatography analysis. It is also reported that the mosquitocidal activity in ethanolic, chloroform and petroleum in higher concentration showed highest toxicity [20].

Meanwhile, the efficacy of commercial insecticide as the positive control is comparable to that of the different concentrations of ethanolic extracts of marigold as evident by its average means of 14.40 and 15.00 after 12 and 24 hours of test, respectively [21-30]. Nevertheless, ninety percent (90%) concentration of marigold extract gave the strongest form of larvicidal toxicity. The results imply that Marigold flower extracts have strong larvicidal properties as it contain phytochemicals (alkaloids, tannins, saponin, flavonoid, and phenolic compounds) shown in Table 5 that could be used for massive eradication of mosquitoes of different species.

- **Analysis of Variance after 12 and 24 Hours**

ANOVA show that after 12 hours of treatment, effect of marigold flower extracts gave a significant difference in T5 and T3 but average means do not vary significantly in T1 and T2.as seen in the results of ANOVA for the treatments. Although all the treatments found to be effective as larvicide for mosquito species, hence 90% concentration significantly show great efficacy as larvicide to mosquito larvae. On the other hand ,after twenty four hours, ANOVA reflects that average means of the treatments do not vary significantly vary significantly ((p>=.05) from in each other in T5 , T2 and T1 but vary significantly((.01=<p<.05) in T3. Hence after 24 hours, as the concentration increases, effectivity as larvicide declines. Water dilution is the factor contributory for the decline in efficacy of marigold flower extract. The larvicidal properties of Marigold against different species of mosquitoes are evidently reflected in Table 5. Its larvicidal potential to eradicate larvae of mosquitoes are attributed to the positive results on the phytochemical analysis that it contain alkaloids, tannins, saponins, phenolic compounds and flavonoids [31-40].

## Conclusion

The mortality rate increases as the concentration also increase after 12 hours with 80% mortality at 90% concentration of flower extracts of Marigold. It is significantly higher than 30% and 60% concentration. However, after 24 hrs, the efficacy of 90% flower extracts decline as the active chemical components diffuses and dispersed in water in longer period of time.

The Marigold (*T. erecta*) flower extract exhibited larvicidal property and is considered highly lethal to larval mixed cultured mosquitoes. Marigold therefore can be a biological control for mosquitoes or any insect of economic importance [40-50].

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## Statement on Conflict of Interest

We declare that we have no conflict of interest.

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