# Effect of Marigold Extract in Controlling Cowpea Aphid, *Aphis craccivora* Koch (Homoptera: Aphididae)

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## Abstract

The objective of this study was tested as the repellent, insecticidal and anti-feedant activity of ethanol extracts from marigold extract, *Tagetes erecta* L. on cowpea aphid, *Aphis craccivora* Koch. The leaf dipping method at various concentrations of marigold extract 0, 0.5, 1, 2, 4 and 8% (w/v) were applied. The results found that the repellent, insecticidal and anti-feedant activity of marigold extract on cowpea aphid were significantly effective high ($p<0.05$). The concentration at 8% of marigold extract, the percent repellent was the highest value with 60% at 24 hr and 100% at 48 hr. The anti-feedant activity, the number of probing was high when the concentration was high. The concentration at 8% of marigold extract, the number of probing was the highest with 8.20 + 0.74 at 15 min when compared with the control 1.40 + 0.48. Time of penetration was low when the concentration was high. The concentration at 8% of marigold extract, time of penetration was the least with 0.17 + 0.03 min when compared with the control 8.74 + 1.03 min. The percent mortality was the highest 100% and the $LC_{50}$ at 24 and 48 hr value with 2.38 and 1.64, respectively. Therefore, the use of marigold extract can be protect the cowpea aphid as the repellent, insecticidal and anti-feedant activity and reduce the use synthetic chemicals, save the human health and environment.

**Keywords:** Marigold Extract, Cowpea Aphid (*Aphis craccivora* Koch)

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## Introduction

Cowpea aphid (*Aphis craccivora* Koch) is an important piercing-sucking insect pest of yardlong bean and cowpea (Emden & Harrington, 2007). The cowpea aphids cause significant economic damage directly by sucking sap from leaves, pods, terminal shoots, flowers and other aerial tissues (Blackman & Eastop, 2000). Indirectly, the cowpea aphids through transmit of major viruses like the cowpea aphid-borne mosaic virus (CAMV) and disturbs the photosynthesis process by the presence of fungus on the leaves. Several cowpea aphid infestations can cause leaf distortion, stunning and reduce pod set in the plant (Damiri et al., 2013).

Farmers are generally depending on chemical insecticides for controlling insect pests (Aktar et al., 2009). However, the problems associated with its usage such as; insect resistance, pest resurgence, health hazards, residual toxicity, increasing costs of application and widespread environmental hazards have directed the need for effective, biodegradable pesticides (Moreby et al., 1997; Han & Li, 2004).

Many natural botanical insecticides have been considered research interest because of their lower toxicity to mammals, less environmental residue, inability of pests to develop resistance (Isman, 2008; Prakash et al., 2008). The botanical insecticides contain many secondary metabolites that act as repellents, anti-feedant, toxicity and oviposition deterrent which have a role in defense against insect pests (Isman, 2006). The marigold plant contained essential oils that have strong aromatic components and that give distinctive odour, flavor to cowpea aphid. The marigold, which were plant extracts as active components, are safe as well as environmentally friendlier than synthetic insecticides. Extracts of marigold are known to be effective repellents against aphids and other insects (Azad, 2012). Therefore, this research was conducted to assess the effects of marigold extract on cowpea aphid, *Aphis craccivora* Koch as repellents, anti-feedant and insecticide in laboratory test.

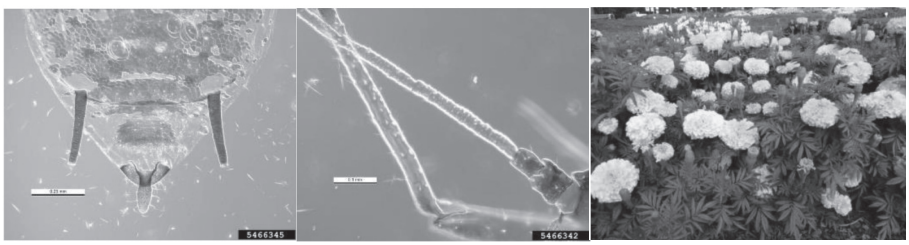
## Objectives

To study the repellent, anti-feedant and insecticidal activities of marigold crude extract on cowpea aphid in laboratory.

## Methods

### 1. Cowpea aphid rearing

Cowpea aphids, *Aphis craccivora* Koch were collected from several home gardens. Identification of cowpea aphid was performed by verifying that the siphunculi and cauda had 4 hairs and 4 segmental antennae under the stereo microscope (Islam et al., 2015). The mass planted in the flowerpots rearing of the cowpea aphids was started on yardlong bean *(Vigna unguiculata)* planted in flowerpots in a greenhouse. Colonies of nymph and adult cowpea aphids were established colony for following experiments.



(A) (B)

**Fig. 1** Identification of cowpea aphid (Poole & Gentili, 1996) (A) and Marigold *Tagestes erecta* (Petrov, 2009) (B)

### 2. Preparation of marigold extract

The marigold (*Tagetes erecta* L.) specimens were collected from farmer's field in Pathum Thani Province. The fresh leaves and stems of marigold were washed with distilled water and dried at room temperature. The dried leaves and stems were cut by using knife. Extracts were produced by combining 100 g of fresh leaves and stems of marigold material with 800 ml of ethanol and placing the mixture a soxlet for three days. The crude extracts were passed through a Whatman #1 filter paper and concentrated by a rotary vacuum evaporator at 42 °C and under low pressure. The crude extracts were contained in brown bottle and kept inside the refigerator for the following experiment.

**3. Repellent activity test**

The repellency of marigold extract was determined in glass Petri dishes (9 cm in diameter). Whatman #1 filter paper of 9 cm diameter was divided into two equal parts. Each concentration of marigold crude extracts was placed in one side and a control ones on the opposite one. The concentrations of 0, 0.5, 1, 2, 4 and 8% of marigold crude extract were used for experimental condition. The control was treated with the ethanol. Both treated and control parts were allowed to dry completely at room temperature of 25 ± 1 °C, 70 ± 10% RH and a photoperiod of 16:8 (L:D). Ten adults (wingless) cowpea aphid were placed at the center of the filter paper. The experiment for each concentration was replicated five times. The petri dish was covered and sealed to prevent the cowpea aphids' escape and was placed at room temperatures. The percent repellency activity of plant extracts were counted at 12 and 24 h exposure period.

**4. Anti-feedant activity test**

The concentration of marigold extract at 0.5% was used for the anti-feedant activity test. The probing experiment procedure for the probing experiment was conducted according to the method of Traicevski & Ward (2002). The leaves of yardlong bean were dipped in the 0.5% concentration for approximately 10 seconds and air-dried at room temperature. Leaves of yardlong beans were dippedin distilled water to serve as the control. The petioles were wrapped with wet cotton and covered by aluminum foil to maintain leaf moisture. The leaves of yardlong bean were placed into plastic cups of 5.5 cm height and 6.5 cm diameter at the top. The age of cowpea aphids (wingless) was 4 days for this experiment. The adults of cowpea aphid were allowed to consume food for approximately 30 seconds pre-treatment. Then, one adult cowpea aphid was released on the yardlong bean. For each individual cowpea aphid, the following parameters was recorded: time to first probe (defined as the time it takes for the cowpea aphid to first penetrate the surface) and duration of each probe (defined as stylet penetrating the tissue surface with the labium extended perpendicular to the body plane). The total time for each replication was 15 min.

**5. Insecticidal activity test**

The young leaves of yardlong bean were dipped into the concentrations of 0, 0.5, 1, 2, 4, and 8% of marigold crude extract. The leaves of yardlong bean were dipped in ethanol for use as control. The petioles were wrapped with wet cotton and covered with aluminum foil to maintain leaf moisture. A quantity of tween 20 (1%) was added to assist good emulsification of the solution. Both treated and control parts were allowed to dry completely at room temperature of 25 ± 1 ℃, 70 ± 10% RH and a photoperiod of 16:8 (L:D). The concentration schedule followed completely randomized design (CRD) with three replications. The leaves of yardlong bean were placed in the petri dish. Ten adults (wingless) cowpea aphid were released on each leaf with varying marigold concentrations by means of a camel-hair brush. The petri dish was sealed to prevent the cowpea aphid escape and was placed at room temperature. The mortality of cowpea aphids was recorded in each petri dish at 24 h and 48 h after releasing the cowpea aphids.

**6. Statistical analysis**

The repellency test was evaluated using the following formula: Percentage repellency (PR) = (Nc/(Nc+Nt)) × 100

where Nc was the number of cowpea aphid on the control side and Nt was the number of cowpea aphid on the treated side. Whereas the percentage mortality was evaluated by using Abbott's (1925) formula: (Nd/Nt) ×100

where Nd was the number of dead aphids and Nt was the total number of aphids introduced. The $LC_{50}$ was obtained using probit analysis (Finney, 1971). Comparison of means was performed using Duncan's Multiple Range Test (DMRT).

## Results and Discussion

**1. Effect of repellent activities of marigold extract on cowpea aphid**

The marigold extract was evaluated for repellent activity effect against the cowpea aphids at concentration of 0, 0.5, 1, 2, 4 and 8 %. The result showed that the percent of repelled cowpea aphids was significantly lower in the control treatment compared to experimental treatments ($p<0.05$). The result clearly demonstrate that at higher concentration and exposure period produce increased percent repellent activity. An exposure period of 24 hr with 8% marigold extract resulted in a maximum repellent

activity of 60%; this was further increased to 100% at 48 hr under the same conditions (Table 1). The study of Promsattha et al. (2000) found that the leaves and stems of marigold contained high amounts of monoterpenes compounds, namely limonene, ocimene, linalool, t-caryophyllene. Some of these compounds have insect repellent properties. Besides, the research of Wubie et al. (2014) which found that Mentha piperita plant leaves contain alkaloids, flavonoids, steroids, tannin and phenols. In general, phenolic compound may interfer with the insect feeding. Similar to the research of Morallo-Rejesus & Decena (1982) found that 95% ethanol extracts from leaf of marigold was repellent against cowpea aphids.

**Table 1** Effect of repellent activities of marigold extract on cowpea aphid after 12 and 24 hr

| Concentration of marigold extract (%) (w/v) | Number of Cowpea aphid | | | |
|---|---|---|---|---|
| | 12 hr | (%) repellent | 24 hr | (%) repellent |
| 0 | $0.00 \pm 0.00^{c}$ | 0.0 | $0.00 \pm 0.00^{c}$ | 0.0 |
| 0.5 | $5.10 \pm 0.74^{ab}$ | 2.0 | $5.40 \pm 0.48^{b}$ | 8.0 |
| 1 | $5.30 \pm 0.48^{ab}$ | 6.0 | $5.80 \pm 0.48^{b}$ | 16.0 |
| 2 | $6.00 \pm 0.48^{a}$ | 20.0 | $6.60 \pm 0.48^{b}$ | 32.0 |
| 4 | $6.80 \pm 0.48^{a}$ | 36.0 | $8.60 \pm 0.48^{a}$ | 72.0 |
| 8 | $8.00 \pm 0.48^{a}$ | 60.0 | $10.00 \pm 0.00^{a}$ | 100.0 |

**Remark:** * Mean values in the same column with the same letter do differ significantly ($P<0.05$ according to DMRT)

**2. Effect of anti-feedant activities of marigold extract on Cowpea aphid**

The marigold extract was evaluated for anti-feedant activity effect against the cowpea aphids at 0.5, 1, 2, 4 and 8 %. The results showed that the mean number of probing and mean time spent of probing were significantly lower in the control treatment relative to experimental treatments ($p<0.05$). The concentration of 8% of marigold extract, resulted in the highest average number of probings and feedings (8.20 + 0.74), as well as the least time spent probing (0.17+ 0.03 min). Conversely the concentration of 0% (control) resulted in the lowest average number of probing and feeding (1.40 + 0.48), as well as the time spent of probing (8.74 + 1.03 min). The study of Nhuall (2010) showed the crude extracts from Tacca chantrieri Andre and Linostoma pauciflorum Griff exibibited

exhibited anti-feedant activity on the Cowpea aphid, as evidenced by decreased number of stylet injections and feeding times. This is in accordance with Dela et al. (2014) that showed a neem extract reduced duration of probing, delayed phloem access, and reduced salivation/ ingestion phases. Dancewicz et al. (2011) suggested that insect feeding can be inhibited at three level: preingestional (immediate effect associated with host finding and host selection processed involving gustatory receptors), ingestional (related to food transport and production, release, and digestion by salivary enzymes) and postingestional (long term effects involving various aspects of digestion and absorption of food.

**Table 2** Effect of anti-feedant activities of marigold extract on cowpea aphid after 15 min

| Concentration of marigold extract (%) (w/v) | Mean number of probing | Mean time spent of probing (min) |
|---|---|---|
| 0 | $1.40 \pm 0.48^{b}$ | $8.74 \pm 1.03^{a}$ |
| 0.5 | $2.20 \pm 0.48^{b}$ | $1.44 \pm 0.33^{b}$ |
| 1 | $4.40 \pm 0.48^{ab}$ | $1.11 \pm 0.34^{b}$ |
| 2 | $6.00 \pm 0.63^{a}$ | $0.29 \pm 0.13^{b}$ |
| 4 | $7.20 \pm 0.74^{a}$ | $0.23 \pm 0.04^{b}$ |
| 8 | $8.20 \pm 0.74^{a}$ | $0.17 \pm 0.03^{b}$ |

**Remark:** * Mean values in the same column with the same letter do differ significantly ($P<0.05$ according to DMRT)

### 3. Effect of insecticidal activities of marigold extract on cowpea aphid

The marigold extract was evaluated for insecticidal activity effect against the cowpea aphids at 0.5, 1, 2, 4 and 8 %. The result showed that the mortality rate of cowpea aphids in the control treatment was significantly lower than experimental treatments ($p<0.05$). The mortality rate of cowpea aphids increased with increasing concentrations. At 24 hr, the marigold extract was effective and caused mortality values of 3.33%, 23.3%, 43.3%, 56.6% and 100%, respectively, for increasing concentrations of extract. At 48 hr, the marigold extracts were effective and caused mortality values with 23.3%, 36.6%, 63.3%, 73.3% and 100%, respectively. The $LC_{50}$ was 2.38 at 24 hr whereas the $LC_{50}$ was 1.64 at 48 hr. (Table 3).

The percent mortality was mainly associated with the volatile nature of the essential oil present in marigold leaves. The study of Wubie et al. (2014) explained that the odour of the plant extract may enter through the spiracle and block respiratory activity, increasing mortality rate by arresting metabolic activity. According to Phoofolo et al. (2013) crude extracts enter into aphid bodies because of the piercing-sucking feeding habits of aphids. Piercing sucking insect like aphids do not get poisoned by insecticide that work as stomach poisons unless the insecticide is systematic and flows the plant's vascular system after being absorbed form soil.

**Table 3** Effect of insecticidal activities of marigold extract on Cowpea aphid after 24 and 48 hr

| Concentration of marigold extract (%) (w/v) | Percent mortality of cowpea aphid | | | |
|---|---|---|---|---|
| | 24 hr | (%) mortality | 48 hr | (%) mortality |
| 0 | $0.00 \pm 0.00^{cd}$ | 0.0 | $0.00 \pm 0.00^{c}$ | 0.0 |
| 0.5 | $0.33 \pm 0.47^{bc}$ | 3.33 | $2.33 \pm 0.47^{b}$ | 23.3 |
| 1 | $2.33 \pm 0.47^{b}$ | 23.3 | $3.66 \pm 0.47^{b}$ | 3.66 |
| 2 | $4.33 \pm 0.47^{b}$ | 43.3 | $6.33 \pm 0.47^{b}$ | 63.3 |
| 4 | $5.66 \pm 0.47^{b}$ | 56.6 | $7.33 \pm 0.47^{b}$ | 73.3 |
| 8 | $10.00 \pm 0.00^{a}$ | 100.0 | $10.00 \pm 0.00^{a}$ | 100.0 |

**Remark:*** Mean values in the same column with the same letter do differ significantly ($P<0.05$ according to DMRT)

## Conclusion

The study of effect of marigold extract in controlling Cowpea aphid was effective as repellent, anti-feedant and insecticidal activities. The marigold extract showed high effective repellant on the Cowpea aphid and affected the probing and anti-feedant of cowpea aphids. The chemical constituents and physical factors of marigold were interfered the inhibition to Cowpea aphid, behavior during probing and feeding, aphid stylet activities, the total time of probing, number of probes duration of phloem sap ingestion. The study should be useful in development of marigold extract to reduce the use synthetic chemicals in preventing cowpea aphids. Therefore the use of marigold extract was in order to minimize the negative side effects of chemical insecticides to human health and to save the environment.

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