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# A dead heat in pollination race: A comparative evaluation of the efficiency of a fly (*Chrysomya megacephala*) and a bee (*Apis florea*) in mango pollination

**V Varun Rajan and PV Rami Reddy**

**Abstract**
Mango (*Mangifera indica* L.) is an entomophilous crop and is attractive to an array of insects which forage on mango flowers for pollen, nectar or both. Hymenopterans (bees) and Dipterans (flies) constitute the major group among the different insects visiting mango blossom. Though the contribution of insect visitors as a whole in mango pollination is well established, the relative pollination efficiency of individual species remains unaddressed. We conducted studies to assess the pollination efficiency of two species viz., *Apis florea* (Hymenoptera: Apidae) and *Chrysomya megacephala* (Diptera: Calliphoridae). It was evident from the results that there was a reduction of 41.93% in fruit set when inflorescence was completely excluded from insect visitation. Among different pollination modes, fruit set was highest (13.5 ± 6.5 / panicle) in open pollinated trees followed by fly pollination (11.3 ± 5.4) and bee pollination (10.09 ± 4.7). Our studies clearly showed that the Calliphorid fly, *C. megacephala* was as good as wild bee, *A. florea* in effecting mango pollination and enhancing fruit set. Besides the fruit set, fruit traits like length, width, weight and Total Soluble Solids (TSS) were not significantly different among trees pollinated by these two species. Hence, it is worthwhile to augment the populations of *C. megacephala* in mango orchards during flowering period to reap better yields as it is relatively easy and economical compared to honey bees.

**Keywords:** Mango, pollination efficiency, *Chrysomya megacephala*, *Apis florea*, fruit set

## 1. Introduction

Pollen vectors are often instrumental in the reproductive success of several angiosperms [1]. Mango trees are andromonoecious and flowers are borne on inflorescence. Each inflorescence bear only few perfect flowers depending on the cultivar [2, 3]. Mango inflorescence attracts an array of flower foragers belonging to Orders *viz.*, Diptera, Coleoptera, Hymenoptera and Lepidoptera. However, dipterans constitute the major proportion of mango flower visitors in terms of diversity and abundance. *Chrysomya* spp. and different syrphid species were reported foraging on mango flowers across the mango growing regions during flowering season [4, 5]. Interestingly, honeybees the most widely acknowledged generalist pollinator, which are ubiquitous in every landscape involved in a diverse pollination network, were reported to forage at lower densities in mango panicles despite the gregarious flower display [6, 3]. Nevertheless, different *Apis* spp. was documented as foragers on mango inflorescence [7]. Little bee, *Apis florea* forages comparatively at higher densities than *Apis cerana* and *Apis dorsata* on mango inflorescence [8]. In general, increasing demand and escalating costs for managing pollinators like honey bees (*Apis cerana* and *Apis mellifera*) along with decline in natural wild populations add to the need for alternatives in pollination services. Pollinator's foraging decision and resource utilization pattern vary among and within a species accounting for the variations in the effectiveness of pollination services by different pollinator species [9, 10]. Insect pollination has increased fruit set and fruit quality in a variety of crops [11]. Literature on the degree of dependency of mango on insect pollinators is scarce. In mango, fruit set occurs even without pollinators to an extent but insect pollinators increase fruit set by several fold [12]. In a study insects contribution towards fruit set in mango was estimated at 53 percentage [13]. India stands first in mango production but its productivity is comparatively less compared to other mango growing countries [14]. In general one of the factors attributed to low productivity in mango is pollinator deficit [15].

Considering the literature, an experiment was designed to compare the pollination efficiency of a fly, *Chrysomya megacephala* (Diptera: Calliphoridae) and bee, *Apis florea* (Hymenoptera: Apidae) in mango cutivar *Alphoso*, which is the most widely cultivated variety in India for its taste, aroma and texture.

## 2. Materials and Method

Studies were conducted at the mango (cv. *Alphonso*) orchard at ICAR-Indian Institute of Horticultural Research, Bengaluru. Data were recorded on flower sex ratio, pollinator dependency, contribution of two pollinators on the fruit set and the resulting fruit traits. The experimental plot consisted of 25 trees of about 20 year old, planted at a spacing of 10m x10m and was maintained with uniform agronomic practices. No pesticides were sprayed on the trees to avoid any possible pollinator repellence.

### 2.1 Sex ratio

In order to assess the sex ratio ten panicles were randomly selected and tagged before the flowers started to bloom. From the time of the opening of the first flower, the male and hermaphrodite flowers in a given panicle were removed daily and counted until the flowering ceases in the panicle.

### 2.2 Pollinator dependency and Pollination efficiency of the test species

**Net house:** Three net houses of 12ftx12ftx12ft dimensions (Figure 3b) were erected enclosing a single mango tree in each enclosure. The net was made of nylon mesh so as to prevent entry of bees and flies inside the net enclosure unless purposefully introduced. Fifty panicles were labeled in each of the three enclosed trees and a control tree near by the enclosed trees. Insects, *A. florea* and *C. megacephala* were introduced into the net enclosure separately. The third net enclosure was maintained as negative control by completely excluding insect pollinators. One positive control tree was maintained by allowing uninterrupted pollinator visits near the net enclosures.

**Insect culture**: *Apis florea* colony was relocated from the farm adjacent to the experimental plot. Initially sugar solution was provided as a food source until the bees acclimatized and stared to forage on mango flowers in the net enclosed tree. The colony had approximately 600 worker bees. Individuals of *C. megacephala* were reared in the laboratory according to methodology by Reddy *et al* [16]. Adult flies were baited using fish and released in a cage containing artificial diet for oviposition. After oviposition the egg mass was collected and placed on the rearing media for rearing pupa. Prepupal wandering larvae were provided with soil for pupation. The soil was sieved to collect pupa which were later kept in small cages until emergence. Adult were released in the net house during the morning hours at the onset of flowering.

Fruit set in the open pollinated panicles and those excluded from insects was compared to determine the degree of pollinator dependency. When pollinators were experimentally excluded from flowers, based on the magnitude of pollinator dependency the fruit set percentage will differ in open pollinated and pollinator excluded flowers. Pollinator dependency was calculated as essential (more than 90% fruit set reduction), great (>40% – 90%), modest (10% – 40%), little (>0% - <10%) and none (there is no reduction in fruit set) [17]. Two pollinators *viz.*, *C. megacephala* and *A. florae* were evaluated for their efficiency in pollinating mongo flowers.

### 2.3 Fruit traits

The fruits harvested from the tagged panicles on the experimental trees were observed for quality traits *viz*., weight, volume, length, breadth and TSS. Fresh weight of the fruit after ripening was measured on balance sensitive to nearest 0.1 g. Length and width were measured using a vernier caliper sensitive to 0.1mm. The total soluble solid (TSS) in the ripe fruit was determined by squeezing a drop of juice from the pulp onto the hand held refracto meter surface. TSS is expressed as °Brix.

Student's t test was performed for the pollinator dependency experiment. ANOVA was performed to compare the fruit set indifferent modes of pollination and fruit trait analysis. Variables were sin-transformed when necessary to meet normality assumption. All analyses were performed using Graph Pad Prism, version 7.

## 3. Results

**Sex ratio:** The mango inflorescence on an average was in flowering for 16 days. Significant variation was observed among the panicles for total number of flowers (Mean= 2684.4) comprising on an average 2545.4 male flowers and 139.6 bisexual flowers (Figure 1) resulting in a bisexual flower proportion of 5.4 per cent.

**Pollinator dependency:** Open pollination and pollinator exclusion (net) experiments showed significant reduction in fruit set ($t= 8.022$, $df=89$, $p<0.0001$). On an average open pollinated and pollinator excluded panicles had 13.9 and 5.16 fruits set per panicle respectively by the end of the third week of flowering (Figure 2). Accordingly, there was a great dependency of mango cultivar *Alphonso* on pollinators for fruit set (62.95% decreased fruit set).

**Pollination efficiency of the test species:** In pollination by fly (n=47) and pollination by bee (n=50) the average fruit set was 12.53± 5.21 and 11.56± 4.75 per panicle respectively (Table 1). There is no statistically significant difference in fruit set between open pollination, fly pollination and bee pollination, but significant difference was found between pollinator exclusion mode and the other pollination modes. (kruskall-Wallis test, $X = 59.23$, $p < 0.0001$, Dunn's multiple comparison at $p< 0.05$).

**Fruit trait:** Trait such as the length and width of the fruit showed no statistically significant variation among the treatments with a fruit length range between of 6.80 ± 0.7cm to 7.9 ± 0.42cm and width ranging between 5.07 ± 0.12cm to 6.77 ± 0.25cm (Table 1). Total soluble sugar (TSS) in the fruit pulp showed significant difference among the treatments. Statistically significant difference was observed in fruit weight and fruit volume (Table 1) among the different treatments, ($F(3, 77) = 7.393$, $P=0.0002$).

**Table 1:** Pollination mode and corresponding fruit trait summary

| Pollination mode | Fruit trait | | | | |
|---|---|---|---|---|---|
| | Length (cm) | Width (cm) | Fruit weight (g) | Fruit volume (ml) | TSS (°Brix) |
| Open pollination | 7.38 ± 0.72$^{a}$ | 6.17 ± 0.37$^{a}$ | 187.02 ± 25.46$^{ab}$ | 174.67 ± 7.06$^{ab}$ | 19.85 ± 1.10$^{a}$ |
| *Chrysomya megacephala* | 7.98 ± 0.42$^{a}$ | 6.77 ± 0.25$^{a}$ | 209 ± 24.66$^{a}$ | 196.67 ± 32.53$^{a}$ | 17.73 ± 0.42$^{b}$ |
| *Apis florea* | 7.73 ± 0.15$^{a}$ | 6.23 ± 0.45$^{a}$ | 198.67 ± 24.03$^{a}$ | 183.00 ± 7.21$^{ab}$ | 17.10 ± 0.72$^{b}$ |
| Insect exclusion | 6.80 ± 0.70$^{a}$ | 5.07 ± 0.12$^{b}$ | 163.67 ± 10.12$^{b}$ | 150.00 ± 8.19$^{b}$ | 12.70 ± 1.13$^{c}$ |
| SEm | 0.37 | 0.18 | 12.88 | 9.38 | 1.16 |
| CD (0.05) | 0.80 | 0.39 | 27.4 | 20.07 | 2.49 |

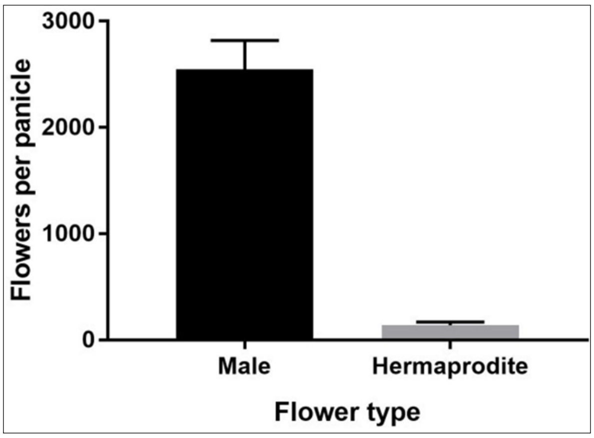


**Fig 1:** Average Number of male and hermaphrodite flowers in a panicle

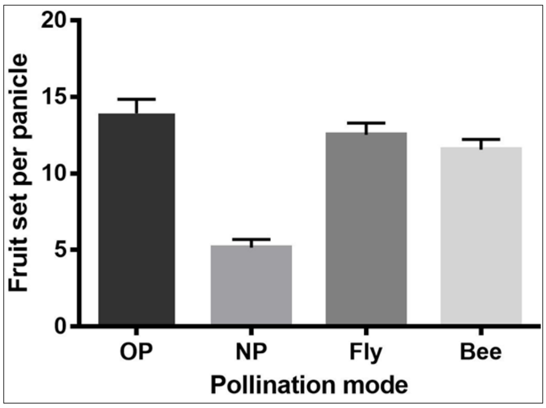


**Fig 2:** Fruit set per panicle in different pollination treatments viz., open pollination (OP), pollinator exclusion (NP), pollination by *Chrysomya megacephala* (Fly) and pollination by *Apis florea* (Bee)

**Fig 3:** a) Pollination dependency experiment, b) Net enclosure for pollinator efficiency studies, c) *Apis florea* colony installed inside the net enclosure, d) *Chrysomya megacephala* foraging on mango flowers inside the net enclosure

## 4. Discussion

Mango inflorescence produces numerous flowers. Male flowers outnumber bisexual flowers at different ratio in the panicle [18]. It appears that the investment in producing more male flowers is to ensure reproductive success. In Cultivar “Alphonso” flower sex ratio was observed to be 5.4% which means only 5.4 percent of the flowers have chance of bearing fruit in a panicle. This ratio is highly variable within and among cultivars grown in different geographic locations and age of the crop [19]. For instance, sex ratio of cultivar Anwar Rataul is 7.25% and of Langra is 79.01% [20]. However, a higher proportion of bisexual flowers not necessarily translate into a higher fruit set.

Studies by Huda *et al* [13], Amin *et al* [21], Kumar *et al* [22], sung *et al* [7], Dag and Gazet [5], has reported important pollinators of mango in different countries. All the above studies reported dipterans as the major pollinator considering the visitation rate and number of pollen grains attached to the body surface. Honey bees were found to pollinate mango in Brazil [23], but the attractiveness of honey bees, especially that of *Apis cerana* and *Apis mellifera* to mango flowers is ambiguous because of contradicting reports. Davenport [24] and Amin *et al*. [21] reported honeybees as occasional visitor to mango infloresence. The unmanaged bee species *Apis florea* was observed more often than Indian Honey bee *Apis cerana* in mango flowers. The foraging pattern and effect of environmental factors on *A. florea* was reported in our earlier study [25]. Hence, Only *C. megacephala* and *A. florea* was selected for pollinator efficiency experiments as they are already reported to be pollinating mango and the availability of mass rearing protocol for rearing *C. megacephala* [17].

In the pollinator dependency experiment the number of fruit set in net covered panicles are in corroboration with findings of Free and Williams [26] and Sousa *et al* [27] but is in contrary to the reports of Galan-sauco *et al* [28], Singh [29], Bhatia *et al* [30] wherein, no fruit set was recorded in the bagged panicles. The possibility of mango pollination by wind and gravity was reported by early workers [31, 32] but till now no clear picture on this mode of pollination could be deciphered. However, self incompatibility is reported across mango cultivars at varying frequency Desai *et al.* [33]

The first challenge to the bees and flies inside the constructed net enclosure is to face the confinement. Behavioural changes were observed in bees after introduction into the enclosure in different studies, but studies in this respect in flies were scarce [34, 35, 36]. *Chrysomya megacephala* and *A. florea* were initially very erratic upon release into the enclosure. The flies, when introduced in their net enclosure tried to cling to the roof and aggregate in corners avoiding flowers. The bees on the other hand avoided foraging and remained in the brood. These behaviors were exhibited during the initial few days after which the bees and flies stared foraging on mango flowers.

Dag and Gazit [5] have assessed the relative effectiveness of most common mango flower visitors in their study. Efficiency was calculated based on the number of times the insect comes in contact with the reproductive structures. Two species of blow flies (*Lucilia sericata* and *C. albiceps*) were found effective when compared with honey bee (*Apis mellifera*). A recent study has reported the effect of blow flies in mango pollination [37]. The initial fruit set and quality of resulting marble size mango fruits had significant difference when compared with pollinator exclusion experiment. In an highly controlled poly house condition pollination by honey bees increased fruit set, fruit weight in mango [38]. The above results are in corroboration with our findings.

Statistically significant difference could not be drawn with respect to fruit traits such as the length and width, but traits with respect to TSS, weight and volume showed significant variations in different modes of pollination. Among the group high TSS (19.85) was found in open pollinated fruits. TSS in fruits pollinated by *C. megacephala* and *A. florea* were on par with each other (17.73 and 17.10 respectively) and fruits obtained from pollinator exclusion mode had least TSS (12.70). Fruit weight and fruit volume of open pollinated, pollination by bees and flies were on par with each other. In pollinator exclusion experiment fruits had least weight and volume comparatively (Table 1). The fruit is a complex system and many environmental factors influence its growth, often it is difficult to cover all the aspects or have control over all factors in one experiment. Accumulation of water, carbohydrate, dry matter and source sink relationship [39] influence fruit quality.

## 5. Conclusion

There seems to be a dead heat in the pollination efficiency between the calliphorid fly (*C. megacephala*) and wild bee (*A. florea*) as both the pollinators are equally efficient in pollinating mango flowers. Domestication of *Apis florea* is difficult as they abscond and avoid confinement in a box. They are also sensitive to fluctuations in temperature. On the other hand, managing flies is easier as they are non social and their foraging is less affected by environmental fluctuations. Further, the low cost artificial diet availability and short duration involved in mass production of *C. megacephala* could plausibly make it as a viable candidate for use in pollination services in mango orchards.

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