A non-meat-based artificial diet and protocol for mass rearing of *Chrysomya megacephala* (Fab.) (Diptera: Calliphoridae), an important pollinator of mango

Mango (*Mangifera indica* L.) produces both male and hermaphrodite flowers and depends on insects for transfer of pollen from male to bisexual flowers or from anthers to stigma within a bisexual flower. The literature throws enough evidences of low fruit set in mango being attributed to inadequate pollination[1]. Mango flowers attract several species of insects, mainly from orders Diptera and Hymenoptera[2–4]. Among more than 30 species found foraging on mango flowers in India, only a few species like *Apis florea* Fab. (Hymenoptera: Apidae), *Chrysomya megacephala* (Fab.) (Diptera: Calliphoridae) and *Eristalinus arvorum* (Fab.) (Diptera: Syrphidae) were reported as major contributors in terms of pollination services[3,4]. Of late, decline in pollinator populations, both in natural and agro-ecosystems, has become a matter of concern. Pollinator decline is attributed to several factors like large-scale use of insecticides, clean cultivation, large tracts of monocropping, absence of off-season flora and climate change[5,6]. The surveys we undertook in major mango belts of the country, viz. the Konkan region in Maharashtra, Chittoor and Krishna districts in Andhra Pradesh and Lucknow region in Uttar Pradesh indicated that the pollinator density in commercial orchards is significantly low compared to undisturbed orchards of research institutes. Hence it is desirable to augment the pollinator numbers by artificially releasing them during the blossom period. Though the introduction of honey bee colonies to achieve pollination is a practice in vogue for some temperate fruit crops[7], it has limited scope in mango as the efficiency of hive bees, viz. *Apis cerana* and *A. mellifera* in mango pollination is often debated because of the limited quantity of pollen produced by mango[8]. The scheduled sprayings of insecticides during blossom period to manage leafhoppers and inflorescence caterpillars make introduced honey bees vulnerable leading to the desertion of colonies, thus limiting their economic viability. Besides the cost factor, the availability of adequate number of colonies within accessible distance is another crucial limitation for the large-scale use of bee colonies in mango orchards. Keeping these in view, it is worthwhile to explore other species to meet the pollination needs in commercial mango orchards. In this context, we found blow fly *C. megacephala* commonly called 'Oriental latrine fly' or 'blue bottle fly' to be a potential pollinator, provided suitable protocols are developed for its multiplication. Releasing *C. megacephala* into mango orchards for pollination was reported from Taiwan[8] and Australia[9]. However, mass rearing is feasible only when there is an artificial diet to support the larva, the main feeding stage.

Despite the problems that blow flies are known to be associated with like carriers of diseases under unsanitary conditions[10,11], and being pestiferous to drying fish[12], they play a valuable role as pollinators of horticultural crops, scavengers, fish bait, feed to fish and poultry and in forensic research[13,14]. There were attempts to rear *C. megacephala* in large numbers and the diet used elsewhere mainly consisted of meat-based constituents like beef, liver and fish[15]. Our experience of rearing it on meat brought to fore the practical difficulty in handling as well as hygiene factor owing to the bad odour emerging from the putrefied meat. If the technology of producing and releasing the insect in fields has to be popularized, there should be a medium which is easy to handle and also safe from hygiene point of view. With this objective, different combinations of ingredients were used to standardize a medium. Here we describe the development of a mass rearing system using a non-meat-based artificial diet to produce *C. megacephala* by avoiding contact with putrefied meat and the associated unpleasant odour.

First, flies were collected from the field using a fish meal-based bottle trap[16] and maintained in the laboratory for two generations on fish meat prior to their use in these studies. Adults that emerged from this stock culture were used to assess the suitability of non-meat diets. Cultures were maintained at ambient room temperature (27 ± 2°C) and 60–70% RH. Different diets of varied composition with green gram, chickpea flour, soya and milk powder as the main ingredients were tested before arriving at the successful one. It consisted of soya flour, milk powder and egg as protein

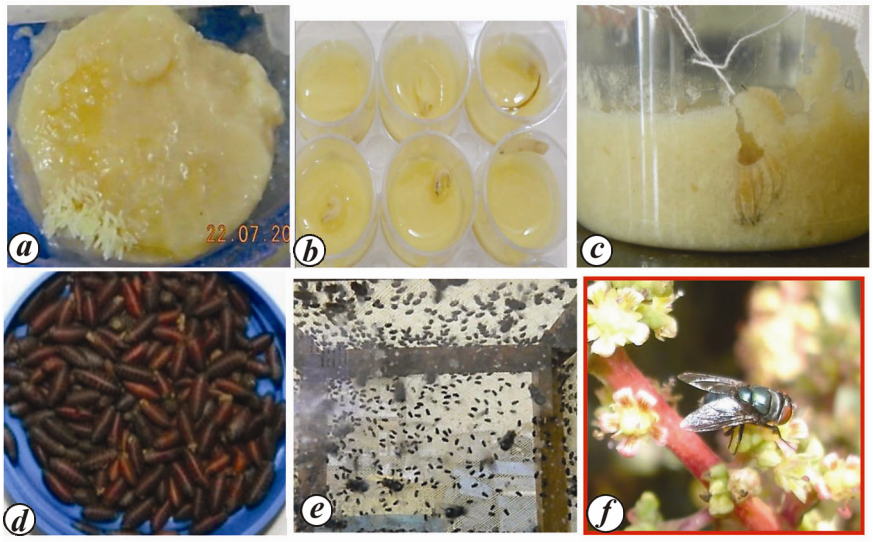

Figure 1. Different steps of rearing *Chrysomya megacephala* on artificial diet. ***a***, Cluster of eggs on the medium. ***b***, Maggots feeding on diet. ***c***, Maggots tunnelling down the medium. ***d***, Pupae. ***e***, Adults that emerged from pupae. ***f***, *C. megacephala* foraging on mango flowers.

Table 1. Morphometrics of different generations of *Chrysomya megacephala* reared on artificial diet in comparison with natural food

Generation	Weight of third instar larva (g)	Weight of pupa (g)	Head width (mm)		Wing span (mm)		Tibia length (mm)	
			Male	Female	Male	Female	Male	Female
Generation-I	0.10 ± 0.01	0.12 ± 0.01	4.08 ± 0.17	3.71 ± 0.17	8.40 ± 0.27	8.16 ± 0.27	2.88 ± 0.14	2.68 ± 0.06
Generation-III	0.11 ± 0.12	0.12 ± 0.01	4.12 ± 0.47	3.65 ± 0.19	8.41 ± 0.11	8.13 ± 0.24	2.76 ± 0.03	2.32 ± 0.16
Generation-VIII	0.11 ± 0.02	0.12 ± 0.02	4.11 ± 0.17	3.73 ± 0.13	8.40 ± 0.62	8.17 ± 1.02	2.85 ± 0.28	2.33 ± 0.12
Natural food (fish)	0.15 ± 0.01	0.14 ± 0.01	4.25 ± 0.06	4.04 ± 0.12	8.54 ± 0.16	8.38 ± 0.32	2.81 ± 0.09	2.82 ± 0.09
CD ($P = 0.05$)	NS	NS	NS	NS	NS	NS	NS	NS

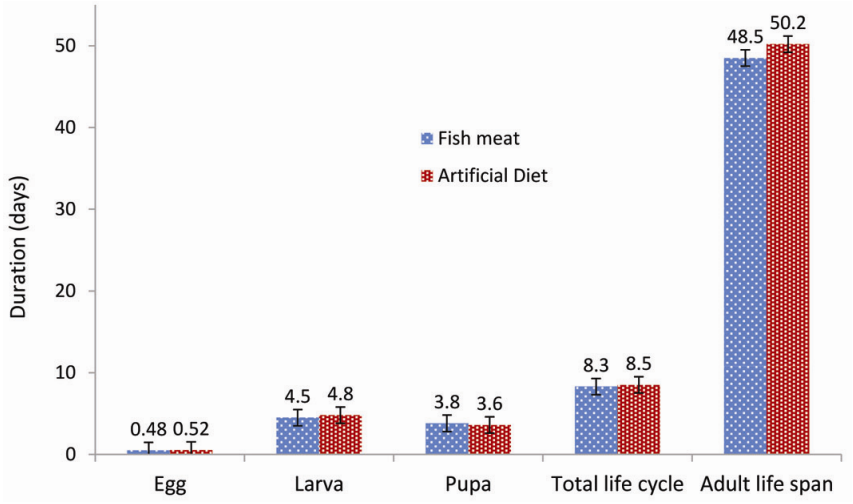

Figure 2. Comparative duration of different life stages of *C. megacephala* reared on artificial diet and fish meat. (Differences between means were non-significant according to Student's *t*-test at $P = 0.05$. At $t_{(df)}$ 24 and 38, P values were 0.38, 0.42, 0.50, 0.42 and 0.46 for egg, larva, pupa, total life cycle duration and adult life span respectively.)

sources with sodium benzoate as preservative and a gelling agent.

The protocol of medium preparation involved the following steps. Soya chunk of 50 g was soaked in water for about 30 min and made into paste using a blender. While blending, milk powder (50 g), one egg and sodium benzoate (3 mg) were added and the volume was made up to 1000 ml by adding water. This is considered as master diet from which the working diet was prepared which could be stored for a week in a refrigerator. To prepare the working diet, equal volumes of master diet and water were taken into a container to which sodium benzoate (0.3 mg/100 ml diet) and agar (1.5 g/100 ml) were added. The mixture was heated in a microwave oven for 2 min to dissolve agar gel. The contents were poured into plastic containers and allowed to cool. This diet is suitable for larval development. In order to facilitate egg-laying by adult flies, broken pieces (radius 1–1.5 cm) of soya chunks were placed on the surface of solidified jelly medium and gravid female flies @10/1000 ml diet, were released for egg-laying. The containers were covered with muslin cloth. After oviposition on soya chunk, adult flies were removed. The maggots hatched from eggs and moved to the medium and started feeding (Figure 1). The nature of their feeding was such that maggots moved downwards making tunnels through the diet. The larval period lasted for 4–5 days. The diet containers with maggots were maintained under ambient conditions with temperature and RH ranging from 27°C to 29°C and 60% to 70% respectively. In order to facilitate pupation, the muslin cloth was removed and the container was placed over a layer of fine sand. The larvae made way into the soil for pupation. Later, the pupae were collected by sieving the sand into petri plates and kept in a cage for emergence. Adults emerging out of the pupae were maintained in cages and provided with 1% sugar solution. The insect was able to complete its life cycle on the medium in 8–9 days.

Eight generations of *C. megacephala* were continuously reared by following this method on artificial diet and the biology and morphometrics were compared with those reared on fish meat. The mean egg, larval, pupal periods, total life cycle and adult life span of those insects grown on artificial diet were 0.52, 4.8, 3.6, 8.5 and 50.2 days respectively, with 92.5% adult emergence while the corresponding values for the insects reared on fish meat were 0.48, 4.5, 3.8, 8.3 and 48.5 days and 94.5% respectively. The independent *t*-test was performed to test the significance of differences and it was found that the developmental periods of insects fed with artificial diet were at par with those of fish-bred flies (Figure 2). This shows that artificial diet has supported the nutritional requirements for the growth and development of all stages. Besides the biology, the morphometrics of larvae and adults of *C. megacephala* over generations were compared (Table 1). The morphological parameters like weights of third instar larva and pupa, head width, wing span and tibia length of adult flies did not differ significantly (ANOVA, $P = 0.05$) between diets and generations. These observations clearly established that the artificial diet formulation developed is an effective alternative to natural meat-based food for *C. megacephala*, thus facilitating large-scale production of the insect for pollination purpose. In a wooden cage (30 × 30 × 30 cm) fitted with a wire mesh, 500–600 flies can be produced in about 10 days. The artificial diet is proved to be as effective as the fish meat and is also economical. The cost of production of 1000 flies was estimated to be about Rs 30. With this technology, it is possible to mass produce *C. megacephala* to supply farmers with either pupae or adults for field releases during the mango

blossom period. Considering the economic impact of poor pollination of commercial crops like mango, the technology developed is a step forward in the direction of augmenting pollinator populations and enhancing the fruit set in farmers' fields. Since mango orchards are located away from human habitats and are subjected to pesticide sprayings during post-flowering period, the probability of released flies posing health problems could be ruled out. However, follow-up studies after release need to be conducted before continuing the releases in the next cropping season. Other crops of its use include vegetables under protected conditions where pollination is a limiting factor. Producing sterile adults through irradiation of pupae is an option in the future to avoid further breeding of the released flies. This technology also has applicability in medical as well as forensic research where laboratory rearing of *C. megacephala* is involved.

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Prevention of malaria through forecast-based precision vector sanitation and exposure advisory: a conceptual and feasibility analysis

Response to outbreaks of malaria is still mostly reactive, based on general schedule or post-outbreak decision. However, abundance of mosquito vector that leads to outbreaks of malaria can vary significantly depending upon the environmental conditions, making a general schedule less effective. Further, exposure of the [incidental] human host to bites also determines the intensity of the epidemic. Identification of potential sites and time of vector genesis can, therefore, enable proactive vector sanitation and reduction of encounters between mosquito and human through exposure advisories. Validated quantitative relations between weather variables and malaria vector, along with recent advances in meteorological monitoring and mesoscale weather forecasting, integrating other critical components like GIS and communication now make such a platform feasible. Such an approach and methodology would lead to a paradigm shift through ensuring wellness rather than treatment; the applicability of the approach to some other diseases is discussed.

Malaria still presents a major health risk and a considerable burden to the healthcare system worldwide[1,2], with greater challenge for areas remote from healthcare infrastructure[3]. The challenges are getting bigger due to enhanced resistance of many mosquito species[3–5]. A shift of paradigm from treatment to ensuring wellness through proactive vector sanitation and exposure advisory is possible through an integration of recent developments in modelling, weather forecasting and communication technologies. The dependence of malaria vector on weather variables has been known for a long time[4–7]. Several studies have demonstrated the conceptual basis for and the feasibility of estimating malaria vector load based on weather variables[8–10]. The first part of the proposed methodology is to use very high resolution (<1 km) forecasts of variables like temperature, humidity and rainfall at daily or shorter timescales and combine them with weather-based vector genesis model to identify potential areas of vector genesis to enable proactive vector sanitation. Severity of malaria also depends on the number of bites and thus host–vector encounter. The second aspect of proactive mitigation is to reduce the host–vector encounter cross-sections through exposure advisories, ensuring avoidance rather than medication after manifestation. This can be achieved using forecasts of vector genesis coupled with data