



## Safety of an oil based formulation of entomopathogen, *Metarhizium anisopliae* to pollinators of mango

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**ABSTRACT:** An oil based formulation of *Metarhizium anisopliae* developed by ICAR- Indian Institute of Horticultural Research, Bengaluru, for leafhopper management in mango, was evaluated for its safety to mango pollinators. The effect on pollinators was compared with a botanical (azadirachtin 1%) and a chemical (imidacloprid 17.8 SL) insecticide. Four species of pollinators, viz., *Apis florea*, *Apis cerana*, *Eristalis arvorum* and *Chrysomya megacephala* were recorded as major foragers on mango. Results indicated that, after spraying the pollinator activity was significantly higher on trees treated with entomopathogen (biological control) compared to those on botanical and insecticide treated ones. There was no significant reduction in pollinator activity on *M. anisopliae* treated plants compared to untreated control. The frequency of visitation by *C. megacephala*, was also highest in biological control treatment than in chemical and botanical respectively. This indicates that application of the oil based formulation of *M. anisopliae* developed by ICAR-IIHR to manage leafhoppers and thrips on mango does not affect the pollinator activity and is safe to pollinators.

**Keywords:** Entomopathogen, *Metarhizium anisopliae*, mango, pollinators, safety

### INTRODUCTION

Biological control using entomopathogens is an environment friendly approach and could be a viable component of Integrated Pest Management (IPM). Entomopathogens have an advantage of being amenable for mass multiplication in desired formulations with a shelf life of up to a year unlike parasitoids and predators. Fungi are known to be pathogenic to several sucking pests like hoppers and thrips under natural conditions and hence they have a greater scope as tools of pest management (Butt *et al.*, 1997; Reddy *et al.*, 2019). Over the years, there has been a considerable progress in formulation and use of entomopathogenic fungi as biopesticides (Lacey and Goettel, 1995). Several species of entomopathogenic fungi like *Metarhizium anisopliae*, *Beauveria bassiana*, *Verticillium lecanii* etc. were evaluated against different groups of pests and varying degrees of success were reported. *Metarhizium anisopliae*, a soil borne fungus is reported to infect more than 200 species of insects and is one of the first fungi used as biocontrol agent (Contreras *et al.*, 2014). Research on isolation, evaluation and formulation of entomofungi, at ICAR- Indian Institute of Horticultural Research Bengaluru resulted in standardizing an oil based formulation of *M. anisopliae* which was proved to be effective against leafhoppers (*Idioscopus* spp.) and thrips (*Scirtothrips dorsalis* Hood), two economically important pests of mango (Ganga Visalakshy, 2009; Reddy and Ganga Visalakshy, 2018; Reddy *et al.*, 2019). Since these

two pests occur on mango during flowering period, it is very much desirable to have management options which are safer to pollinators, as mango is predominantly an entomophilic crop. Various insects such as flies, bees, wasps, ants, butterflies, beetles etc. are reported to forage on flowers of mango (Singh, 1988). The role of the insect pollinators in mango production has been well studied and documented in various mango producing countries including India (Sung *et al.*, 2006; Balachandra *et al.*; 2014, Reddy *et al.*, 2018). Presently neonicotinoids like imidacloprid, thiomethoxam or synthetic pyrethroid like lambda-cyhalothrin, are widely used to control hoppers on mango which are highly detrimental to bees and other pollinators as well as environment. Several studies have established the essentiality of insect pollinators for fruit set in mango. Of late there are growing concerns on the decline of pollinators due to agrochemicals as well as climate change (Reddy *et al.*, 2012). In order to take forward the use of the formulation of *M. anisopliae* as an IPM component of mango, there is a need to assess its safety to the pollinators visiting mango blossom. In this background, the present study was carried out to determine the safety of the oil based formulation of *M. anisopliae* to the pollinators of mango.

### MATERIALS AND METHODS

Field studies were conducted during 2014-16 in the mango orchards (cv. Alphonso) of ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru, India.

The trees were of about 15 year old. There were four treatments including the oil formulation of *M. anisopliae* (0.5 ml/L with a spore count of  $10^8$ ), an insecticide (imidacloprid 17.8 SL @ 0.3 ml/L), a botanical pesticide (azadirachtin 1 % @ 3 ml/L) and an untreated control. Each treatment was replicated five times with one tree per one replication.

Observations on the number of pollinators visiting panicles were recorded from four randomly selected spots (each spot covering 10 panicles) in each direction of canopy of each tree. Since the panicles were close together, the area that could be covered by a single straight vision was taken for observation on the foraging activity of pollinators at each spot. Treatments were imposed during flowering period when the pollinator activity was maximum. Since the objective was to know the immediate effect of spray on pollinators, only one spray was given and number of different species of pollinators was recorded a day before and at one, two and seven days post treatment. Unlike insecticides, entomopathogens are slow acting and do not have quick knock down effect. Hence, observations were recorded one week after spray to rule out the mortality effect of entomofungus on insect pollinators. Sprays were given in the morning hours (between 8 and 10 am) when pollinator activity was high. All the insects that visited and foraged on the mango blossom were presumed to

be pollinators of mango in the present study. The insects visiting mango flowers were collected manually using an insect net before start of the experiment and identified. Data were recorded species wise, total number of foraging insects and frequency of visitation of major pollinator species. The data were subjected to statistical analysis (ANOVA) and mean differences were compared at 5 per cent level of significance ( $p = 0.05$ ).

## RESULTS AND DISCUSSION

Observations on species composition and frequency of visitation revealed that four species of insects viz., *Chrysomya megacephala* (Diptera: Calliphoridae), *Eristalis arvorum* (Diptera: Syrphidae), *Apis florea* (Hymenoptera: Apidae), and *Apis cerana* (Hymenoptera: Apidae) were recorded as dominant pollinators of mango in both the years of study. Among them, *C. megacephala* was the most dominant one (3.62/ 10 panicles) followed by *A. florea* (1.87), *A. cerana* (1.25) and *E. arvorum* (0.87). This corroborates with the report of Reddy *et al.* (2018) who also found these four species to be economically important pollinators of mango. Besides these four species, there were populations of stingless bee (*Tetragonula iridipennis*), unidentified Syrphids, wasps and moths at very low levels.

**Table 1. Effect of different treatments on pollinators of mango (2014-15)**

Treatment	No. of foragers/10 panicles/minute			
	Before spray	1 DAS	2 DAS	7 DAS
Oil formulation of <i>M. anisopliae</i> @ 0.5 ml/L	7.75	6.80	7.40	8.40
Azadirachtin 1% @ 3 ml/L	7.75	2.30	3.62	6.52
Imidacloprid 17.8 SL @ 0.3 ml/L	8.00	0.40	0.52	3.25
Control (Untreated)	7.50	7.75	7.50	8.60
CD ( $p = 0.05$ )	NS	1.24	1.87	2.35

**Table 2. Effect of different treatments on pollinators of mango (2015-16)**

Treatment	No. of foragers/10 panicles/minute			
	Before spray	1 DAS	2 DAS	7 DAS
Oil formulation of <i>M. anisopliae</i> @ 0.5 ml/L	9.50	7.40	8.50	9.60
Azadirachtin 1% @ 3 ml/L	8.25	4.50	3.54	5.20
Imidacloprid 17.8 SL @ 0.3 ml/L	9.60	1.20	0.60	2.25
Control (Untreated)	8.75	8.75	9.50	9.60
CD ( $p = 0.05$ )	NS	1.42	1.34	2.85

During the first year of study, there was no significant difference in the density of pollinators across treatments before application of treatments. Their numbers ranged from 7.5- 8.0 per 10 panicles. A day after spraying, the number of pollinators in different treatments varied from the lowest 0.40/panicle group in imidacloprid treatment to highest 6.80 in *M. anisopliae* treatment which was at par with untreated control (7.75). There was 95% reduction in pollinator density due to imidacloprid treatment followed by 70.33% with azadirachtin. The slight reduction in pollinators in entomopathogen treated trees could be attributed to the wash out effect of spray fluid. In case of azadirachtin, the significant decline pollinators might be due to the repellent effect of neem product (Table 1). The trend was almost similar in the subsequent year. The pollinator density has significantly declined in chemical and azadirachtin treated trees while there was no significant reduction in pollinator activity in *Metarhizium* treated panicles (Table 2). In both the years, though there was a slight decline in pollinator activity with biopesticide treatment after 24 hours of spray, there

was a rise in pollinator numbers at 48 hours and seven days after treatment. At all intervals, the numbers were not significantly different from control indicating the safety of biopesticide formulation.

Besides recording the density of all pollinator species in toto, observations were also recorded on the species wise response to treatments at 24h and seven days after treatment, pertaining to four major species. As mentioned earlier, *C. megacephala* was the most dominant forager followed by *A. florea*, *A. cerana* and *E. arvorum*. In the oil based formulation of *M. anisopliae*, activity of all species of pollinators was found to be at par with untreated control. A mean of 3.0 visits by *C. megacephala* were recorded as against 3.5 in control. Similarly *A. florea*, *A. cerana* and *E. arvorum* were also not significantly different from those recorded from untreated trees. Their numbers were 2.80, 0.80 and 0.40 respectively in *Metarhizium* treatment compared to 3.20, 1.0 and 0.40 in control. There was hundred per cent reduction in the activity of two bee species viz., *A. florea*

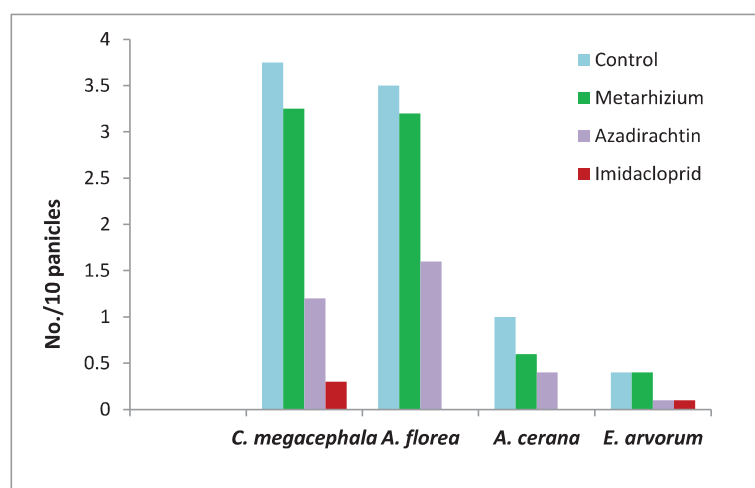


Fig 1. Density of four major species of pollinators 24h after spray in different treatments (mean of two years)

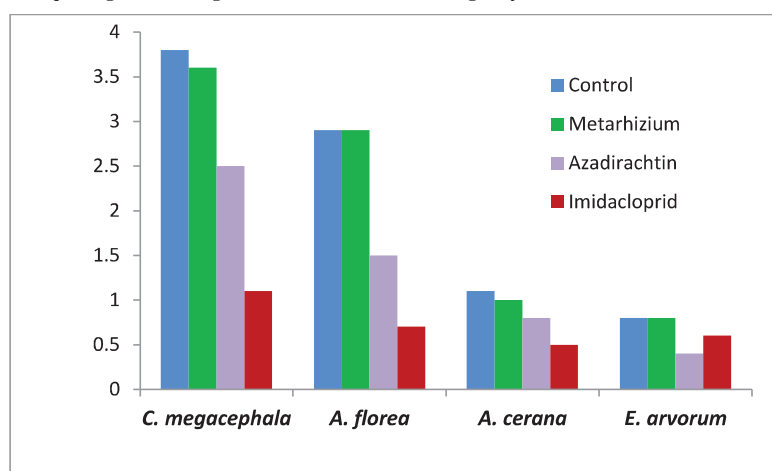


Fig 2. Density of four major species of pollinators seven days after spray in different treatments (mean of two years)

and *A. cerana* in imidacloprid treatment (Fig.1). The adverse impact of neonicotinoids (Blacqui re *et al.*, 2012), especially imidacloprid (Zhu *et al.*, 2017) on bees and other pollinators is well documented and our present observations confirm the toxic effect of this group of synthetic chemicals on pollinators.

An increase in the activity of pollinators at seven days after treatment over their numbers at 24h after treatment was observed in all the treatments. This is because of the influx of pollinators from other part of the field as well as the degradation of treatment effect. However even at this stage the number of pollinators in chemical and botanical treatments were significantly lower than control while biopesticide treatment recorded all species of pollinators in numbers comparable to untreated control (Fig. 2). Aliakbarpour *et al.*, (2011) reported the adverse effect of neem oil (3%) on mango pollinators at 24 and 96 h after the second application. An insight into the findings of the study clearly indicate that the oil based formulation of *M. anisopliae* tested is safe to pollinators of mango and its use to manage leafhoppers and other sucking pests coinciding with flowering stage does not hinder pollinator visitation. Thus the entomopathogen could be an ideal component of integrated pest management in mango.

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