Research article

Combination of Entomopathogenic Fungi with Essential Oil for Controlling Bean Fly, *Ophiomyia phaseoli* Tryon (Agromyzidae: Diptera)

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Abstract The objective of this study was to evaluate the compatibility of entomopathogenic fungi with repellants to control bean fly, *Ophiomyia phaseoli* Tryon (Agromyzidae: Diptera). Nine isolates of entomopathogenic fungi belonging to 3 genera and 5 species were screened for pathogenicity against bean fly. The results found that *Beauveria bassiana* isolate Bff and *Metarhizium anisopliae* isolate Mff caused 100% mortality to both its larval and adult stages. The repellency of 7 essential oil was also evaluated against adult bean fly in the laboratory. The results showed significant differences in repellency among essential oil and concentrations. Essential oil extract from pomelo peeling was the strongest repellant followed by eucalyptus and sweet basil. However, essential oil extract from camphor was the weakest repellant. On the other hand, these seven essential oils (pomelo, zingiber, citronella grass, eucalyptus, camphor, kaffir lime and sweet basil) at a concentration of 0.1% had no effect on the viability of conidia, but at higher concentrations it was found that citronella grass and zingiber reduced viability of *B. bassiana*. The results on the effect of essential oils on colony growth showed the same tendency as the one on the viability of conidia.

Keywords bean fly, entomopathogenic fungi, essential oil, repellent

INTRODUCTION

Phaseolus vulgaris L., French bean is a herbaceous annual plant in the Fabaceae family, it grows to between 20-60 cm high; and twining, climbing vines up to 2-5m long. The stems of bushy types are rather slender, pubescent with many branches (Chávez-Servia et al., 2016). In twinning types, the stems are prostrate for most of their length and rise toward the end. The leaves, borne on long green petioles, are green or purple in colour and trifoliate. Leaflets are 6-15 cm long and 3-11 cm broad. The inflorescences are axillary or terminal, 15-35 cm long racemes. The flowers are arranged in pairs or solitary along the rachis, white to purple and typically papillonaceous. Once pollinated, each flower gives rise to one pod. Pods are slender, green, yellow, black or purple in colour, sometimes striped (Caproni et al., 2019) . They can be cylindrical or flat, straight or curved, 1-1.5 cm wide and up to 20 cm in length. The pods may contain 4 to 12 seeds. The seeds are 0.5-2 cm long, kidney-shaped and highly variable in colour depending on the variety: white, red, green, tan, purple, gray or black.

French bean is a major plant of Royal Project Foundation for the growers of organic vegetable system in Thailand. However, the problem of French bean plantation is bean fly, *Ophiomyia phaseoli* Tryon)Agromyzidae: Diptera) (Ojwang et al., 2010) .They lay eggs in the leaf tissue or directly on the stem. Early signs of attack are egg laying punctures on the primary leaves which tend to be concentrated around the leaf base (Letourneau, 1994). Eggs hatch into small white maggots which migrate down the stem to the root zone where they pupate into brown puparia. Severe damage is

indicated by wilting and dying of seedlings. The attack disrupts nutrient transportation, causing the tap root to die (Ssekandi et al., 2016). The plant attempts to recover by forming adventitious roots above the damaged area (Ojwang et al., 2010). Young seedlings under stress wilt and die within a short time. Older and more vigorous plants may tolerate the damage but become stunted and will have reduced yield. Control strategies for grower to use were as follow: application of botanical pesticides, natural enemies, sticky trap and also chemical seed dressing with systemic insecticides. *Plutachis* sp. Eulophid, *Biosteres* sp. *Pteromalid* sp. nematode and *Oecophylla smaragdina* Fabricius were effective against bean fly by 52% but bean fly still serious pest of French bean plantation (Jaramillo et al., 2013).

OBJECTIVES

The objectives of this study were: firstly to screen promising entomopathogenic fungi against bean fly and secondly to evaluate plant-based insect repellants and their compatibility with the entomopathogenic fungi in controlling bean fly.

MATERIALS AND METHODS

Selection of Entomopathogenic Fungi from Collection

Nine isolates of entomopathogenic fungi consisting of 3 different genera and 5 different species were selected from Agricultural Technology Research Institute, Rajamangala University of Technology Lanna, Lampang 52000, Thailand. These were screened for their efficacy against bean fly under laboratory condition.

Selection of Plants as Repellants Against Bean Fly

Selection of plants that have potential to repel bean fly was conducted according to the literature reviewed that hinted on the plants that have essential oils that repel invertebrates (Showler, 2017 and Abtew *et al.*, 2015). Extraction of essential oil was done using the Steam-distilled method (Mcguinness, 2003). After that, Study on the efficacy of essential oil that repel bean fly at difference concentration was conducted. Moreover, study on effect of essential oil to germination of entomopathogenic fungi and study effect of essential oil to growth of entomopathogenic fungi was also conducted (Liu, 2012).

Efficacy of Formulation of Entomopathogenic Fungi Combine with Repellant

This was conducted with the aim to produce an efficient formulation of entomopathogenic fungi for controlling bean fly. The formulation was produced by mixing 20% of entomopathogenic fungi + 1% of Tween 80 at a concentration of 10% + 1% of Glycerin + 5% vegetable oil + 3% Propylene glycol (Malee, 2015). This was then applied on French bean leaves to assess the spore viability and efficacy of formulation of entomopathogenic fungi against bean fly. The experiments were carried out in 3 treatments: formulation 1 (*Beauveria bassiana*), formulation 2 (*Metarhizium anisopliae*) and control. Suspension was directly sprayed on to the bean fly.

RESULTS AND DISCUSSION

Efficacy of Entomopathogenic Fungi to Infected Bean Fly

Nine isolates of entomopathogenic fungi belonging to *M. anisopliae*, *B. bassiana Isaria fumosoroseus*, *Isaria tenuipes* and *Isaria farinosus* were selected for this study. The results for pathogenicities of 9 isolates of these entomopathogenic fungi showed high virulence against larval

and adult stage of bean fly. It was observed that *B. bassiana* isolate Bff and *M. anisopliae* isolate Mff caused 100% mortality to both larval and adult stages of bean fly as shown in Table 1. This was a very impressive result and is in agreement with several studies (Letourneau, 1994). The other advantage of these entomopathogenic fungi is that they offer endophytic advantages as well to the planted when they are inoculated (Mantzoukas and Eliopoulos, 2020).

Table 1 Percent mortality of entomopathogenic fungi to bean fly (larval stages)

Entomopathogenic fungi*	Isolates	Percent mortality
Metarhizium anisopliae	Ma.4849	80.00
	Ma.7965	93.33
	Mff	100.0
Beauveria bassiana	Bb.5335	80.00
	Bb.4591	80.00
	Bff	100.0
Isaria fumosoroseus	Pfu.2507	60.00
Isaria tenuipes	Pt.6073	46.67
Isaria farinosus	If 2549	40.00

**Fungus isolates from Agricultural Technology Research Institute, Rajamangala University of Technology Lanna, Lampang*

Efficacy of Plants as Repellants Against Bean Fly

The repellency of 7 essential oil was evaluated against adult bean fly in the laboratory. The results showed significant differences in the repellency among essential oils in different concentrations. Essential oil extract from peel of pomelo was the strongest repellent followed by eucalyptus and sweet basil. Essential oil extract from camphor was observed to be the weakest repellent as indicated in Fig. 1. In addition, the seven essential oils (pomelo, zingiber, citronella grass, eucalyptus, camphor, kaffir lime and sweet basil) a concentration of 0.1% had no effect on the viability of conidia, but in higher concentrations, it was observed that citronella grass and zingiber reduced viability of *B. bassiana*. The results on the effect of essential oils on colony growth showed the same tendency as shown in the viability of conidia (Fig. 2). These results did not deviate much from other studies (Liu, 2012). Compatibility of these EPFs is known to be of paramount effect on their effectiveness and hence cannot be omitted (Neves et al., 2001).

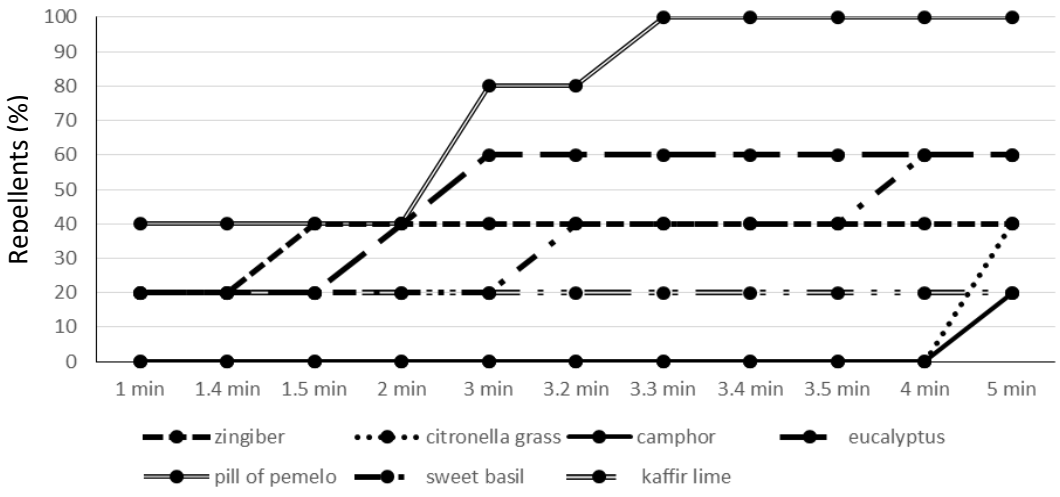

Fig. 1 Percent efficacy of plants as repellents against bean fly

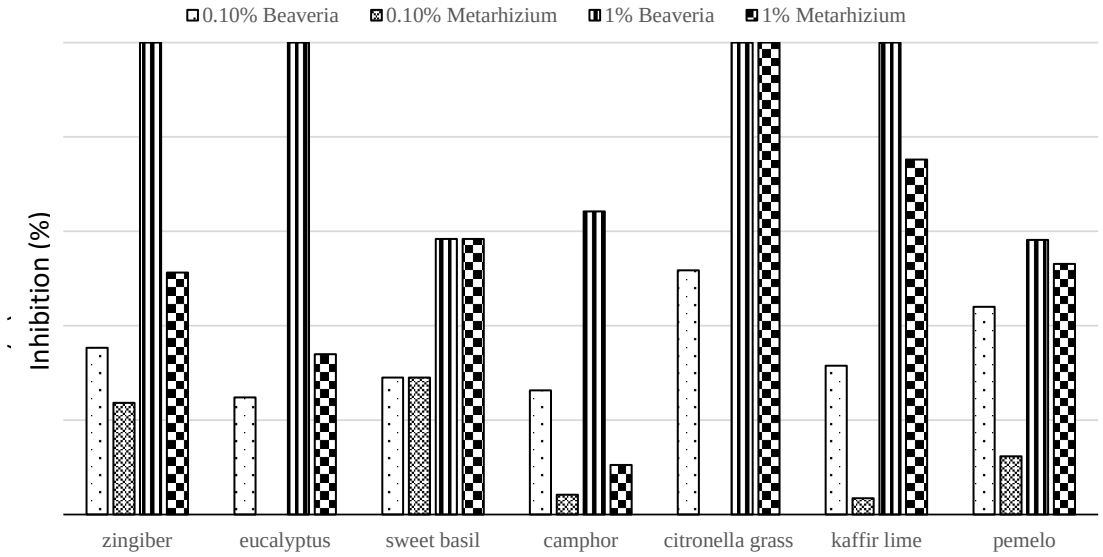

Fig. 2 Percent inhibition of plant repellent to *Beauveria* spp. and *Metarhizium* spp.

Efficacy of Formulation of Entomopathogenic Fungi Combine with Repellent

Concerning the potentiality of formulation of entomopathogenic fungi in combination with essential oils against bean fly, it was observed that when entomopathogenic fungi combine with essential oil there was increased effectiveness in controlling bean fly. This is an interesting finding as they would complement without adverse effects. Ali et al (2018) also worked with eucalyptus and entomopathogenic fungi observed remarkable potential in their compatibility and the resultant efficacy.

CONCLUSIONS

Out of 9 isolates, 2 isolates i.e., *B. bassiana* isolate Bff and *M. anisopliae* isolate Mff showed the highest mortality against both larval and adult stages of bean fly. Formulation of entomopathogenic fungi took time to ablate from French bean leave which was very impressive. Essential oil extract from peel of pomelo was a stronger repellent followed by eucalyptus and sweet basil. However, essential oil extract from camphor was a weaker repellent. It was also observed that a concentration of 0.1% had no effect on the viability of conidia, but at higher concentrations it was found that citronella grass and zingiber reduced viability of *B. bassiana*. The results on the effect of essential oils on colony growth showed the same tendency as the one on the viability of conidia. Entomopathogenic fungi combined with essential oil had high potential in controlling bean fly.

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