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Tran Thi My Hanh
Southern Horticultural Research Institute, Vietnam

Nguyen Trinh Nhat Hang
Faculty of Agriculture and Food Technology, Tien Giang University, Vietnam

Corresponding Author:
Tran Thi My Hanh
Southern Horticultural Research Institute, Vietnam

Study on natural enemy composition and effectiveness to control pupa and adult of Oriental fruit fly (*Bactrocera dorsalis*) in star apple (*Chrysophyllum cainito*) using entomopathogenic fungi

Tran Thi My Hanh and Nguyen Trinh Nhat Hang

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Abstract
Study on natural enemy composition and effectiveness to control pupae and adult of Oriental fruit fly (*Bactrocera dorsalis*) damage on star apple using entomopathogenic fungi conducted from August 2020 to August 2021 at Southern Horticultural Research Institute and star apple orchards in Chau Thanh district, Tien Giang province. The results research on natural enemy composition of fruit flies at 10 star apple orchards recorded that there were nine natural enemy species such as *Paecilomyces lilacinus*, big-headed ant (*Pheidole megacephala*), fire ant (*Solenopsis invicta*), and parasitoid wasp (*Diachasmimorpha longicaudata*) appeared commonly with the frequency of 32.20%, 37.14%, 39.28%, and 48.00%, respectively. Followed by black earwig (*Chelisoches morio*), jumping spider (*Plexippus paykulli*), *Metarhizium* sp. and weaver ant (*Oecophylla smaragdina*) occurred less commonly with a frequency of 7.86%, 12.86%, 15.40%, and 20.00%, respectively. *Beauveria* sp. was very rarely with a frequency of 3.40%. Experimentally evaluating the ability to control *B. dorsalis* pupae and adults by entomopathogenic fungi indicated that *P. lilacinus*, *Metarhizium* sp., and TKS - BTMET (*Metarhizium* sp. + *Beauveria* sp. + *Paecilomyces* sp.) product were equally effective to control fruit fly adults with effectiveness of 79.12%, 69.22%, and 66.30%, respectively.

Keywords: *Bactrocera dorsalis*, entomopathogenic fungi, fruit fly, natural enemy, star apple

Introduction
The star apple or caimito tree *Chrysophyllum cainito* L., family Sapotaceae is a tropical fruit tree. The plant is highly desired throughout the tropics as an ornamental tree and for the production of its large edible fruits (Parker *et al*., 2010) [17]. However, this fruit is only commercially produced on a very limited scale in a few regions. In the Mekong Delta of Vietnam the area growing star apple is approximately 4,209.7 ha with an average yield of 12 tons/ha, the productivity of 39,532.6 tons/year. Tien Giang, Soc Trang and Can Tho are provinces with large and concentrated planting areas of star apple. In recent years, star apple is one of five fruits from Vietnam export to the United States market (Department of Plant Protection, 2017) [6]. Some orchards in Mekong Delta granted planting area codes and traceability. Farmers need to apply the process of fertilizing, spraying and fruit bagging according to regulations. Survey results of star apple orchards recorded that the appearance frequency of six common insect species were fruit flies *Bactrocera dorsalis* (51.8 - 59.6%), fruit borers *Nephopterix* sp. (51.2 - 55.4%), Tussock moth *Dasychira osseata* (25.4 - 37.4%), Beetle *Pachyteria equestris* (3.2 - 19.6%), Chafer Beetle *Adoretus* sp. (2.0 -7.6%), Mealy bug *Planococcus lilacinus* (22.4 - 38.2%). Fruit fly was a serious pest that directly affected the yield causing yield loss and quality degradation of fruit and they were considered pest to be quarantine for importing countries (Orankanok, 2007)[16]. Some entomopathogenic fungi had the ability to prevent pests and replacement of chemical methods (Lacey và Kaya, 2007; Mascarin và Jaronski, 2016) [12, 14]. To control fruit flies, farmers applied chemical measures, the excessive use of pesticides with high toxic groups caused undesirable effects such as environmental pollution, leaving pesticide residues on the products, affecting human health, killing natural enemies, leading to an increasing number of pests that reduce yield and product

quality. Hence, to understand the composition of natural enemies of fruit flies in the orchards and find biological solutions to reduce the population of fruit flies in the star apple orchards is very necessary.

Materials and Methods

Survey on the natural enemies composition of fruit flies in star apple orchards

Investigate natural enemies of fruit flies in 10 star apple orchards at Vinh Kim and Long Hung communes, Chau Thanh district, Tien Giang province. The survey was carried out monthly from August 2020 to August 2021 according to the method of investigation and detection of plant pests promulgated by the Ministry of Agriculture and Rural Development (MARD, 2010). The orchards use less pesticides and with an area of 2000 m^2 selected and observed to detect natural enemies, monitor their activities. Slow moving insects were collected by hand. Flying insects were collected using sweep-net. Natural enemies were also collected and preserved for identification. Samples of natural enemies (predatory, entomopathogenic fungi, and parasitic wasps) were collected and stored in plastic bags/paper bags. The samples were labeled and quickly brought to the laboratory for examination under a magnifying glass or under a microscope. The insect specimens were identified by the experts from SOFRI.

Assessed parameters

Natural enemy species composition and popularity of each natural enemy species.

Appearance frequency of natural enemies followed formula:

Frequency (%) = (Number of times the species were occurred)/ Total number of observation) x 100

Popularity is divided into 4 levels: + (frequency<5%); ++ (frequency 5-<25%); +++ (frequency 25-<50%); ++++ (frequency ≥50%).

Evaluation the effectiveness of entomopathogenic fungi on fruit fly pupae *Bactrocera dorsalis* in the laboratory condition

Preparation of fruit fly pupae

Collected star apple fruits with symptoms of fruit fly damage in the orchards and brought them to the laboratory, then placed fruits in the plastic box (size 33 x 17 x 10 cm), inside the box containing sterilized sawdust, covered by cotton. After 14 days collecting the pupae to carry out the experiment. Before spraying the fungi on the pupae that were washed with a solution of 0.5% sodium hypochlorite (NaClO) then keep it dry. Sprayed fungi on pupae (the concentration of fungi had 1.06 x 10^9 of spores), covered them by dry soil, and maintained 70-85% humidity during the procedure.

The experiment was conducted in randomized complete design (RCD) with six treatments and four replications (20 pupae/ replicate). The treatments were *Metarhizium* sp.1; *Metarhizium* sp. 2; *Beauveria* sp; *Paecilomyces lilacinus*; TKS - BTMET (*Metarhizium* sp. + *Beauveria* sp. + *Paecilomyces* sp.) and control (water).

Assessed parameters

Percentage of pupa emerging (%) = (Number of pupae emerging/number of pupae observed) x 100

Percentage of fungi grown on pupae (%) = (Number of fungi grown on pupae /number of observed pupae) x100.

Evaluation the effectiveness of entomopathogenic fungi on fruit fly adult *Bactrocera dorsalis* in the laboratory condition

The experiment was arranged in a randomized complete design (RCD) with five treatments and four replicates. The treatments were *Metarhizium* sp. (T1), *Paecilomyces lilacinus* (T2), *Beauveria* sp. (T3); TKS-BTMET (T4), and control (T5).

Collected star apple fruits with symptoms of fruit fly damage in the orchards, the fruits brought to the laboratory, and put in a white plastic box of size (33 x 17x 20 cm) underneath with a layer of sterilized coco peat, plastic box covered with a white cloth. After larvae emerged, 20 selected adults were put in a plastic box covered by white cloth. Water and sugar were food for the larvae. Experiment was carried out with a fungi suspension solution containing 1.06 x 10^9 spores/mL, sprayed directly on the adults, and a control sprayed of water.

Assessed parameters

Observed and recorded the number of fruit fly adults at 3, 7, 10 and 14 days after treatment. The efficiency of entomopathogenic fungi calculated by the formula (Abbott, 1925) [1].

$H (\%) = [(C_a – T_a)/C_a] \times 100$

There in:

C_a: Number of surviving fruit fly adults in the control after treatment

T_a: Number of surviving fruit fly adults in the treatments after treatment

Data analysis

Data collected and analyzed using analysis of variance (ANOVA), and Duncan's Multiple Range Test (DMRT) used for means comparison when treatments were significant by using SPSS program.

Results and Discussions

Natural enemy composition of fruit flies in the star apple orchards in Tien Giang province

The survey results from Table 1 showed that there were three groups of natural enemies of fruit flies on star apple orchards in Tien Giang province.

(1) The group of predatory were found in the orchards such as big-headed ant (*P. megacephala*) and fire ant (*S. invicta*) appeared commonly with the frequency 37.14% and 39.28%. Black earwigs (*C. morio*), jumping spiders (*P. paykulli*), and weaver ants (*O. smaragdina*) were the next most frequent occupants, with frequencies of 7.86%, 12.86%, and 20.00%, respectively. These predators mainly attacked the larvae and pupae of fruit flies. There were 11 species of fruit fly predatory spiders (Salticidae, Araneidae, Lycosidae, and Philodromidae families) in the United States (Garcia, 2020) [9]. Ants eat fruit fly larvae when the larvae fall to the ground to pupate (Fernandes *et al*., 2012) [8]. Big-headed ants (*P. megacephala*) and fire ant (*S. saevissima*) were important predators on *Anastrepha fraterculus* larvae in Brazil. In field conditions, there were up to 42.86% of fruit fly larvae were attacked by ants (*S. saevissima*) (Abeijon, 2019) [2] and 95.00% of fruit fly larvae were eaten by ants (*S. geminate*) in Mexico (Thomas, 1995) [13]. Weaver ants, *Oecophylla* spp. (Hymenoptera: Formicidae) are dominant ants in a variety of tropical plant species (Lim, 2007) [9]. Adandonon *et al*. (2009) [3] reported that the presence of weaver ants restricted fruit fly activity, and they have been used for biological control of

insect pests in many crops (Wargui *et al*., 2022) [21].

(2) The group of entomopathogenic fungi. According to the survey results revealed that three species of entomopathogenic fungi were found on the larvae, pupae, and adults of fruit flies in the orchards, with *P. lilacinus* appearing most frequently (32.30%), *Metarhizium* sp. appearing less frequently, and *Beauveria* sp. appearing very infrequently. Ekesi *et al*. (2003) [7] reported that the fungus *Metarhizium anisopliae* was parasite on fruit flies. (3) The group of parasitoid wasps. The survey revealed that the parasitoid wasp *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) frequently (48.00%) occurred in star apple orchards. For augmentative applications against fruit flies, it is regarded as one of the most significant biological control agents. It has already been applied to reducing the population of *Ceratitis capitata* in mangoes (Wong *et al*., 1991; Sanchez *et al*., 2016) [22, 18]. *Bactrocera dorsalis* (Hendel) (Vargas *et al*., 2012) [20], *Anastrepha obliqua* (Macquart), and *Anastrepha ludens* (Loew) (Montoya *et al*., 2000) [15], and it proposed as a potential natural biological control (Harush *et al*., 2021) [11].

The investigation results showed that the natural enemies of fruit flies in the orchard were abundant (Fig.1). Therefore, it is necessary to protect these natural enemies. In order to improve biological management for fruit fly control in star apple orchards, it is also required to artificially produce some natural enemies, such as the parasitoid wasp *D. longicaudata* and entomopathogenic fungi.

Table 1: Natural enemies composition of fruit fly in star apple orchards

Common name	Scientific name	Attack stage	Appearance frequency (%)	Popularity
Big-headed ant	*Pheidole megacephala* (Hymenoptera: Formicidae)	Larva, pupae	37,14	+++
Fire ant	*Solenopsis invicta* (Hymenoptera: Formicidae)	Larva, pupae	39,28	+++
Weaver ants	*Oecophyllas maragdina* (Hymenoptera: Formicidae)	Larva, pupae	20,00	++
Jumping spider	*Plexippu spaykulli* (Araneae: Salticidae)	Larva, adult	12,86	++
Black earwig	*Chelisoches morio* (Dermaptera: Chelisochidae)	Larva, pupae	7,86	++
Fungus	*Metarhizium* sp. (Hypocreales: Clavicipitaceae)	Pupae, adult	15,41	++
Fungus	*Beauveria* sp. (Hypocreales: Clavicipitaceae)	Pupae, adult	3,40	+
Fungus	*Paecilomyces lilacinus* (Hypocreales: Clavicipitaceae)	Pupae, adult	32,20	+++
Parasitic wasp	*Diachasmimorpha longicaudata* (Hymenoptera: Braconidae)	Larva, pupae	48,00	+++

(+) Very less popular (Appearance frequency<5%); (++) Less popular (Appearance frequency 5-<25%); (+++) Popular (Appearance frequency 25-50%).

The effectiveness of entomopathogenic fungi on fruit fly pupa *Bactrocera dorsalis* in the laboratory condition

The results of the pupal parasitism evaluation of the entomopathogenic fungal strains recorded (Table 2). After 7 days of treatment, the pupae metamorphosis rate of the *P. lilacinus* treatment was 16% lower than in the TKS-BTMET (*Metarhizium* sp. + *Beauveria* sp. + *Paecilomyces* sp.) and control treatments (46% and 55%, respectively). At 10 days after treatment, the pupation rate of the *P. lilacinus*, *Metarhizium* sp. treatments were 23% and 30% lower than these other treatments and control. At 14 days after treatment, the *P. lilacinus, Metarhizium* sp. treatments were the pupationrate 44% significant difference compared with TKS-BTMET (*Metarhizium* sp. + *Beauveria*sp. + *Paecilomyces* sp.) and control treatments (75% and 95%, respectively). *P. lilacinus*, *Metarhizium* sp., and *Beauveria* sp. grew on pupae at a rate of 53.75%, 54.17%, and 60.09%, respectively. (Fig. 2). According to Goble (2009)[10] the fungi *Metarhizium* sp. and *Beauveria* sp. were effective in managing the fruit fly pupae *Ceratitis rosa* and *C. capitata* at a concentration of 10^7 spores, with the percentage of fungi sprouting on the pupae ranging from 1.0% to 14.0%.

Table 2: Percentage of metamorphosis pupae and fungi growing on fruit fly pupae

Treatment	Percentage of metamorphosis pupae (%)			Percentage of fungus growing on pupae (%)
	7 DAT	10 DAT	14 AT	
Metarihizium sp. 1	24^{bc}	30^{c}	44^{c}	53.75
Metarihizium sp. 2	28^{bc}	39^{c}	65^{bc}	0
Beauveria sp.	29^{abc}	40^{ab}	59^{bc}	54.17
P. lilacinus	16^{c}	23^{c}	44^{c}	60.09
TKS–BTMET product	46^{ab}	60^{ab}	75^{b}	18.75
Control	55^{a}	76^{a}	95^{a}	0
Level of significance	*	**	**	

In a column, means followed by same letters are not significantly different at 5% probability level by Duncan's Multiple Range Test (DMRT), ns = not significant. Data converted to arcsin(x) ½, DAT = Day after treatment.

The effectiveness of entomopathogenic fungi on fruit fly adult *Bactrocera dorsalis* in laboratory condition

Table 3 indicated that at 3, 7 days after treatment the parasitic efficiency of fungi on fruit fly adults were not statistically significant differences. However, at 10 days after treatment the parasitic efficacy of the *Metarhizium* sp., TKS–BTMET product, and the *P. lilacinus* were 55.13% and 49.93%, respectively, with statistically significant differences compared to the *Beauveria* sp. (35.86%). At 14 days after treatment the parasitic efficiency of fungus *P. Lilacinus* on fruit fly adults were statistically significant (79.12%) compared to the *Beauveria* sp. (50.47%). The percentage of parasitic fungi growing on fruit fly adults in the treatment *P. Lilacinus* (Fig. 3) had the highest rate of fungal growth at 65.26%, followed by *Metarhizium* sp. (53.12%), TKS-BTMET and *Beauveria* sp. with growth rates of 48.32% and 39.70%, respectively. Yee and Lacey (2005)[23] reported that sprayed the fungus *Metarhizium brunneum* (4.6 x 10^8 spores/mL) on *R. indifferens* fruit flies in the laboratory and found that 100% of the adults were infected with the fungus, and the infection rate for pupae ranged from 15% to 68% when treated with fungi in the soil. *B. bassiana* spores (10^7 spores/mL) caused *Rhagoletis cerasi* fruit fly adults to die at a rate of 90-100%, but pupation had a lower mortality rate of 25%. Naturalis-L, a fungus *B. bassiana* applied every 7 days to control *R. cerasi*, resulted in a 65% reduction in the number of fruit infested with fruit flies at harvest (Daniel and Wyss 2009, 2010) [4, 5].

Table 3: The efficiency of entomopathogenic fungi on fruit fly adults and percentage of fungus grown fungus on adults

Treatment	Days after treatment				Percentage of fungus growing on adults (%)
	3	7	10	14	
Metarhizium sp.	6.25	20.00	55.13^{a}	69.22^{ab}	53.12
P. lilacinus	5.00	23.75	49.93^{a}	79.12^{a}	65.26
Beauveria sp.	10.00	25.00	35.86^{b}	50.74^{b}	39.70
TKS–BTMET product	10.00	27.50	55.13^{a}	66.30^{ab}	48.32
Level of significance	ns	ns	*	*	

In a column, means followed by same letters are not significantly different at 5% probability level by Duncan's Multiple Range Test (DMRT), ns = not significant. Data converted to arcsin(x) ½.

Fig 1: Natural enemies composition of fruit fly in star apple orchards a) *Solenopsis invicta;* b) *Pheidole megacephala*,, c) *Chelisoches morio*, d) *Diachasmimorpha longicaudata* adult.

Fig 2: a) Normal pupae *B. dorsalis*, b) *Metarhizium* sp. parasitizing on *B. dorsalis* pupae, c) *Beauveria* sp. parasitizing on *B. dorsalis* pupae, d) *Paecilomyces lilacinus* parasitizing on *B. dorsalis* adult

Conclusion

The survey results of the star apple orchards in Tien Giang province recorded that the natural enemies of fruit fly were big-headed ant (*P. megacephala*), fire ant (*S. invicta*), and parasitoid wasp (*D. longicaudata*) which appeared commonly with the frequency of 32.20%, 37.14%, 39.28% and 48.00%, respectively. The black earwig (*C. morio*), jumping spider (*P. paykulli*), *Metarhizium* sp., and weaver ant (*O. smaragdina*) were less common, occurring at a frequency of 7.86%, 12.86%, 15.40%, and 20.0%, respectively. *Beauveria* sp. found only 3.40%. *Paecilomyces lilacinus*, *Metarhizium* sp. And TKS-BTMET were effective in controlling fruit fly larvae and adults at 14 days after treatment. Entomopathogenic fungi are regarded as an environmentally friendly and natural pest control agent for star apple fruits.

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