Nucl. Sci. and Tech., Vol.7, No. 4 (2017), pp. 42-48

Nuclear Science and Technology

Journal homepage: https://jnst.vn/index.php/nst

Influence of adult diet and exposure to methyl eugenol in the mating performance of *Bactrocera correcta*

Hien, N. T.T[1], Khanh, L.D.[1], Thanh, V.V[1], Trang V.T.T[1], Lien, H. K[1], Xuyen, L.T[1], Rui Peirera[2].

[1]*Entomology Divison, Plan Protection Research Institute;*

[2]*Insect Pest Control Section, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria.*

Email: thanhhien1456@gmail.com

Abstract: The Guava fruit fly *Bactrocera correcta* is serious pest attacking Dragon fruit (*Hylocereus undatus* and *Hylocereus polyrhizus*). The study on the impact of (i) Protein in adult diet on sexual maturity of Guava fruit fly; (ii) Methyl eugenol (ME) feeding on sterilized male of Guava fruit fly; and (iii) Longevity of sterilized males and females feeding on different diets. All those trials were conducted for both sterile males and laboratory flies reared at Plant Protection Research Institute, Hanoi, Viet Nam. The initial results showed that the first age of sexual maturation of laboratory reared females are 7 days old and 8 days old when fed on a mix diet of hydrolyzed yeast: sugar (1:4) and only sugar diet, respectively. Four days after emergence, females on hydrolyzed yeast fed diet died. The highest percentage of mating for the period of 20 first days after emergence was recorded 7.19% at 11 days old of flies fed on sugar; 40.08% at 10 days old of flies fed on hydrolyzed yeast and sugar. The longevity of sterilized males was 30.7 days, 28.1 days and 3.2 days as feeding on mix of hydrolyzed yeast: sugar, sugar only and hydrolyzed yeast alone, respectively. Likewise, the longevity of females was 32.5 days on hydrolyzed yeast: sugar diet, 30.5 days on sugar only diet and 3.7 days on hydrolyzed yeast only diet. The percentage of sterile males feeding on ME at 3 days old was only 5% and reached 40% at 8 days old with feeding times of 44 seconds and 4 minutes 27 seconds respectively.

Keywords: *Bactrocera correcta, Dragon fruit, Methyl eugenol, sterile fly sexual maturity.*

I. INTRODUCTION

The Sterile Insect Technique (SIT) is an environmentally-friendly method successfully used against many fruit fly species in many countries all over the world. The success of the SIT depends on the quality and the ability of sterile males to mate with wild females. Meanwhile, the protein diets play an important role in fostering the copulation performance of fruit flies which has been demonstrated by several studies belonging to Kaspi and Yuval (2000) [1], Shelley et. al. (2002) [2]; Yuval (2007) [3]; Pérez Staples et al. (2009) [4]; and Pereira et al. 2013 [5]. Additionally, numerous studies implemented that using male lure could increase copulation competitiveness of sterilized fruit fly males of some species belonging to *Bactrocera* genus (Shelly 1994 [6]; Shelly and Dewire, 1995 [7] ; Shelly and Villabolos, 1995 [8] ; Wee and Tan, 2000, 2005 2007 [9][15][10]. Methyl eugenol (ME) is known as a natural attractant to many *Bactrocera* species. Unfortunately, the study on the effects of male lure or protein diets to *Bactrocera correcta* is limited *B. correcta* is one of the most important insect pests on Dragon fruits (*Hylocereus undatus* and *Hylocereus polyrhizus*). To face with this problem, an Area-wide Integrated Pest

https://doi.org/10.53747/jnst.v7i4.96
Received 19 Octorber 2017, accepted 27 December 2017

Management (AW-IPM) programme has been implemented to control fruit flies on Dragon fruit production under a project coordinated by Viet Nam and supported by the Joint FAO/IAEA (Food and Agriculture Organization/International Atomic Energy Agency). The project has a plan to use the integration of SIT with other suppression techniques. Understanding the role of factors such as adult diet and exposure to ME on improving sexual performance, survival and competitiveness of Guava fruit fly need to be investigated to optimize the use of sterile flies.

II. CONTENT

A. Subject and methods

- Fruit fly: The Guava fruit fly namely *Bactrocera correcta* Bezzi is used for this study.

- Material:

Wild flies were recovered from infested Dragon fruits (*H. undatus)* and Guava *(Psidium guajava)* in Binh Thuan province and had been under artificial rearing for 8 generations at Entomology Division of Plant Protection Research Institute (PPRI), Hanoi, Viet Nam. Sweet potato larval diet (60% sweet potato + 5.3% torula + 1.3% nippagin + 33.4% water.) was used (Le Duc Khanh et al. 2015) [11]. Following emergence, the flies were provided with different of adult diets, depending on the treatments. All these flies used in given experiment were the same batch of pupae. All trials were conducted in laboratory room condition at Plant Protection Research Institute, Ha Noi Viet Nam (T^o ~ 26 ± 2°C, RH ~ 60-70%, and photoperiod of 10 – 14 hrs per day).

Methyl eugenol (Indonesia) and yeast hydrolysate enzymatic (MP Biomedicals, LLC – California, USA) are imported into Viet Nam, and sugar locally available (from sugarcane).

- Methodology:

Experiment 1: Effect of protein in adult diet on sexual maturity of Guava fruit fly

Thirty pairs of newly emerged *B. correcta* (not sterilized) were released into each of 3 cages (30 cm x 30 cm x 40 cm) covered by cloth mesh. Water and adult diet were supplied and refreshed every 2 days. The flies were provided with the diet at three different treatments, which were: (i) Sugar only, (ii) Hydrolyzed yeast only; (iii) a mix of hydrolyzed yeast: sugar (1:4 by proportion). The food was placed in petri dish at the corner of the cage before introduction of the adult. Every afternoon (17:00 - 22:00 h), the number of mating was observed until the age of 20 days (Fig. 1). Mating pairs were removed from the cages and recorded. They were not returned to their cages even after the end of mating. The whole procedure was repeated three times for each treatment, thus, 270 flies were used in total for this trial.

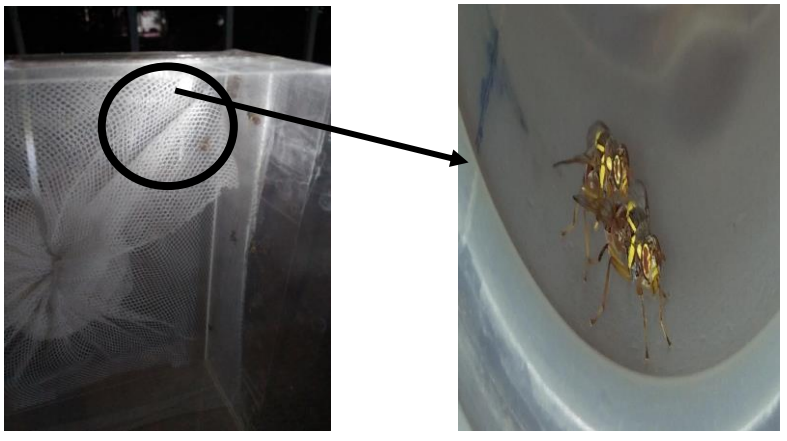

Fig 1. Mating of *Bactrocera correcta*

Experiment 2: *Effect of Methyl eugenol feeding on sterile male of Guava fruit flies*

The *B. correcta* pupae were irradiated at 85 Gy using a Co-60 gamma cell, 48 hours before emergence (age of pupae was determined by changing the colour of eye). The test was conducted as soon as male flies were emerged by releasing individual flies into cylindrical cages (30 cm height and radius 8 cm) having both openings covered with cloth mesh. The diet mixture of hydrolyzed yeast: sugar (1:4 by proportion) was placed on plastic petri dishes inside the cylindrical cages and replaced every 2 days. Water was supplied by a wet sponge placed on the top of cylindrical cages. This study included 13 adult aging from 0 (newly emerged) to 12 days old. Sterile males of each of the treatment age were separated into two groups, one exposed to Methyl eugenol (ME) and second not exposed to ME. For each age, 20 males with three replications were performed. Methyl eugenol was dropped (15 µl) with diet and exposure for 2 hours in the cages (9a.m – 12 a.m). The amount of flies feeding on the ME during the 2 hours and the length of time feeding on ME was recorded. After those 2 hours exposition, flies were again provided with the standard adult diet (hydrolyzed yeast: sugar at ratio 1:4). A control treatment without ME was conducted in other room different from room used for ME-feeding at 13 adult ages.

Experiment 3: Longevity of sterilized males and females on protein and sugar diets

Bactrocera correcta pupae were irradiated at 85 Gy and 48 hours before emergence (age of pupae was determined by changing the colour of eye). Each pair of newly emerged sterilized flies was released in cylindrical cage (30: 8 cm height and radius) and supplied with only water by a wet sponge placed on the top of cylindrical cages. The adult diet was provided in three different treatments, which were (i) Sugar only, (ii) Hydrolyzed yeast only; (iii) a mix of hydrolyzed yeast: sugar (1:4 by proportion). 100 couples per treatment were tested. Daily, observation and collection of the data on dead adults (both male and female) were performed until all flies were dead and then removed during daily checks.

For the 3 experiments, data was analyzed based on biostatistics described by Vo Huy Van et al. (1997) [12].

B. Results

Experiment1: Effect of protein in adult diet on sexual maturity of Guava fruit fly

Fruit flies fed with a mixture of hydrolyzed yeast: sugar had performed mating at 7 days old, one day shorter than sugar-fed flies (8 days old). No data had been recorded from flies fed only hydrolyzed yeast diet because all flies were dead by day four.

While the mating rate of flies reared by hydrolyzed yeast plus sugar was highest, reaching 40.08% at 10 days old contrary to 7.19% on sugar-fed flies at 11 days old (Fig 2).

Experiment 2: Effect of Methyl eugenol feeding on sterile male of Guava fruit fly

Sterile males *B. correcta* start to be fed on ME when they were 3 days old. The peak of this activity was established at 8 days old (Fig. 3). The time that flies started being fed on Methyl eugenol was highest at 267 seconds (at 8 days old) and lowest at 35 seconds (at 12 days old) (Fig. 4).

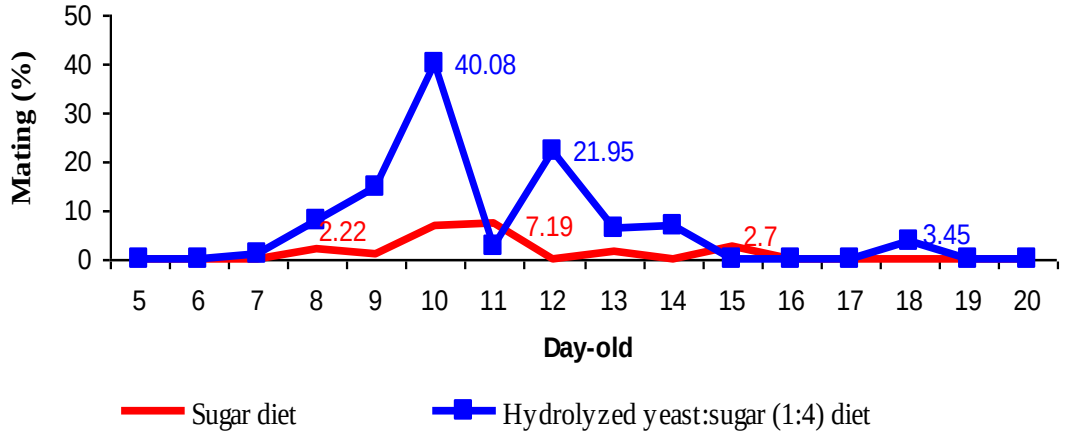

Fig.2. Mating perfomance of *B. correcta* fed on two different diets (PPRI, 2016)

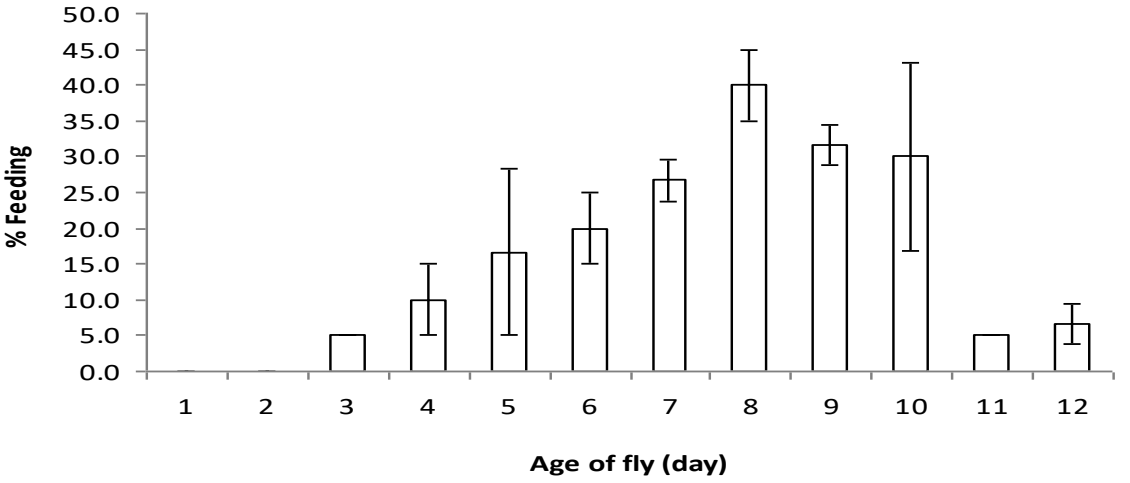

Fig. 3. The time when sterile male *Bactrocera correcta* started being fed on Methyl eugenol at different ages

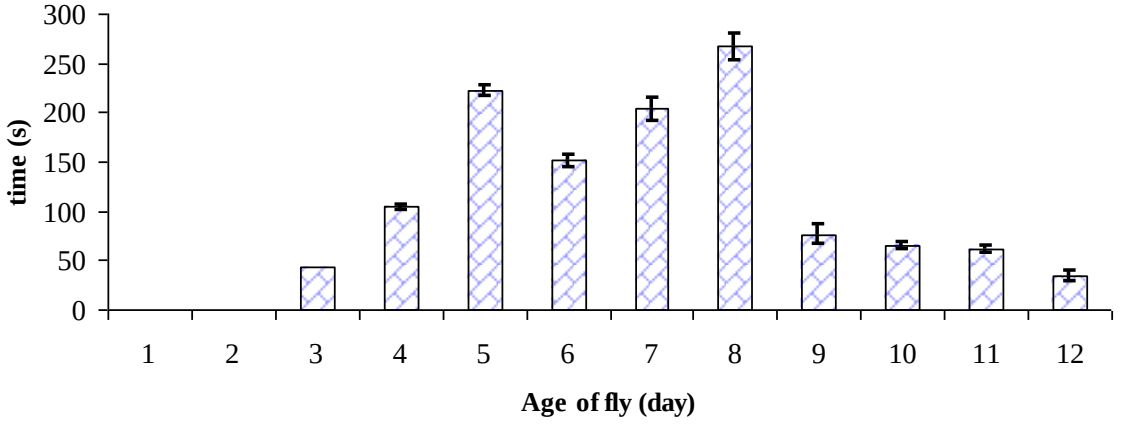

Fig. 4. Eating time of Sterile male *Bactrocera correcta* on Methyl eugenol at different ages

Experiment 3: Longevity of sterilized males and females on different diets.

The mean longevity of sterile *B. correcta* fed a mix included hydrolyzed yeast: sugar (1:4) was the longest both for females and males with 32.5 days and 30.7 days respectively. Lifetime of flies fed sugar alone was 30.5 days (female) and 28.1 days (males). However, this difference is not significant (P≤ 0.05). The data recorded of flies fed only hydrolyzed yeast was 3.7 days for females and 3.2 days for males (Fig. 5)

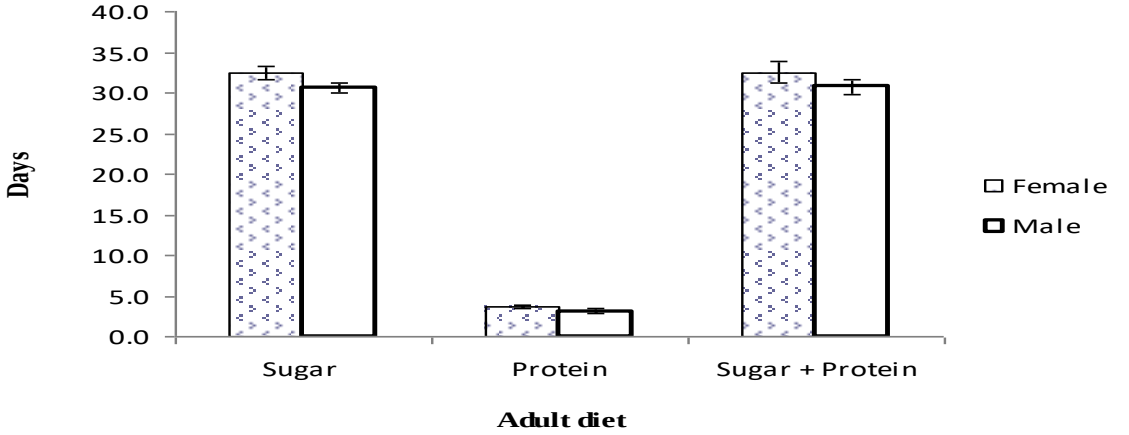

Fig. 5. Longevity of sterilized *Bactrocera correcta* males and females at different adult diets

III. DISCUSSION

In laboratory condition, *B. correcta* reared by the mixture of sugar and hydrolyzed yeast had sexual maturity earlier than flies fed with only sugar. The mating rate of fed with a mixture was also higher than fed with sugar or hydrolyzed yeast. This result suggested that protein may impact the sexual maturation and also action of mating. However, this still need further study in field cages. In the literature, similarly on small-cage study conducted by Obra and Resilva (2013) [13] on *B. dorsalis (*known at the time as *B. philipinensis*), fed sugar-hydrolyzed yeast wild flies and sterile flies recorded that there were 89% of sterile flies had mated within 7 days post-ecolosion in compare with 48% of wild flies at 14 days of age. The sterile males of *B. dorsalis* and *B. correcta* provided with only water-agar as a pre-release diet during this critical initial period obtained very low mating success, achieving 50% less mating pairs than young sterile males exposed to an improved pre-release diet (Orankanok et al., 2013) [14]. Regarding to mating age, Obra and Resilva (2013) [13] mentioned that the mass-reared sterile flies have a median mating age of about 3.85 days after emergence and wild flies about 10.5 days.

Previous work demonstrated that ME is a strong attractant to fruit flies, specially *Bactrocera* genus (Hee and Tan, 1998, 2006 [16][17]; Wee and Tan, 2000 [10]; Shelly and Dewire, 1994 [7]). The exposure to ME for both wild flies and sterile flies play an important role in performance mating and competitiveness. Shelly et. al. (1994) [7], mentioned that sterile flies exposed to ME had increased the number of mating with wild females over wild males. Orankanok et. al. (2013) [14] recorded a significant mating advantage of ME- exposed over non- exposed

sterile males, even though feeding at a younger age. In our result, sterile flies only come to be fed ME after 3 days old and at peak at 8 days old, which suggest that the sterile fly release can be done earlier. The next question is that what age of *B. correcta* is suitable for feeding ME in order to improve the sexual performance with wild flies?

IV. CONCLUSIONS

The results of the research recorded still a primary study and it may be too early to conclude the impact of ingredient diet and Methyl eugenol on *B. correcta* species in Viet Nam. Research of some factors affecting ecology of wild flies and irradiated *B. correcta* flies are therefore needed in order to develop an effective and sustainable Area-wide Integrated Pest Management by integrating the Sterile Insect Technique with the male annihilation technique to control this fly species in Viet Nam.

ACKNOWLEDGEMENTS

We are grateful to the IAEA funded to the Research Contract Number 20394R0: Influence of adult diet and exposure to Methyl eugenol in the mating performance of *Bactrocera correcta* in Viet Nam.

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