# EFFECTS OF GAMMA IRRADIATION ON EGG HATCHABILITY, PUPATION, AND ADULT EMERGENCE OF THE IMMATURE STAGES OF THE ORIENTAL FRUIT FLY, *Bactrocera dorsalis* (HENDEL) (DIPTERA: TEPHRITIDAE) FROM MALAYSIA

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## ABSTRACT

Studies on the effect of irradiation on egg hatchability, pupation and adult emergence of the immature stages of the Oriental Fruit Fly, *Bactrocera dorsalis* (Hendel) was conducted in Malaysian Nuclear Agency, Dengkil, Selangor, Malaysia using a Co-60 source. Eggs, 1st instars to 3rd instars larvae and pupae stages were treated separately with dosages 50Gy to 400Gy to determine the most tolerance stage and the optimum dose for provide quarantine security. Each treatment involves a minimum of 100 individuals for each dose with four replications. The hatch from eggs to 1st instar larvae, pupation from eggs and all of three instars larvae to pupae, and eclosion from pupae to adult, were used as criteria to determine the effect of irradiation. Egg hatch was completely inhibited by 100Gy for early egg and 300Gy for late eggs. Dose of 200Gy and 250Gy were able to prevent completely pupation when treated from egg stage and 1st instar larvae, respectively. Unfortunately, those dosages did not affect pupation when applied to 2nd and 3rd instars, but it did prevent any from emerging as adults. Adult emergence was 100% prevented when eggs and all larvae were treated at 100Gy as compared to pupae was not completely prevented even by 400Gy. Among the four immature stages of *B. dorsalis*, the 3rd instar larvae (inside fruits) and pupae (outside fruits) were found to be the most tolerance stage and the early eggs are the most susceptible stage to irradiation. Our results suggest that minimum dose 100 to 150Gy is sufficient to provide a high level of quarantine security against this important pest and the dose might allow for irradiation be accepted as a quarantine treatment for most tropical fruits from Malaysia.

**Keywords**: Phytosanitary Irradiation, *Bactrocera dorsalis*, most tolerance stage

## ABSTRAK

Kajian kesan rawatan sinaran ke atas penetasan telur, pempupaan dan kemunculan dewasa bagi peringkat pra matang Lalat Buah Oriental, *Bactrocera dorsalis* (Hendel) telah dijalankan di

Agensi Nuklear Malaysia, Dengkil, Selangor, Malaysia menggunakan sumber Co-60. Peringkat telur, larva instar pertama hingga ketiga dan pupa telah dirawat secara berasingan mengunakan dos di antara 50Gy hingga 400Gy untuk menentukan peringkat hidup yang paling rintang dan dos yang paling optimum untuk keperluan sekuriti kuarantin. Setiap dos rawatan melibatkan 100 individu dengan empat replikasi. Penetasan telur ke instar pertama, pembentukan pupa daripada telur dan ketiga-tiga instar larva dan kemunculan dewasa lalat buah dari pupa merupakan kriteria yang digunakan untuk menentukan kesan bagi sinaran. Penetasan telur dapat dihalang sepenuhnya dengan dos 100Gy bagi telur muda dan 300Gy bagi telur matang. Dos 200Gy dan 250Gy boleh menghalang pempupaan sepenuhnya apabila dikenakan rawatan ke atas peringkat telur dan larva instar pertama. Walaubagaimanapun, dos tersebut tidak berkesan terhadap larva instar kedua dan ketiga, namun ia dapat menghalang daripada kemunculan ke peringkat dewasa. Kemunculan dewasa lalat dapat dihalang 100% apabila telur dan kesemua instar larvae dirawat dengan dos 100Gy berbanding peringkat pupa di mana ia tidak dapat dihalang sepenuhnya walaupun pada dos 400Gy. Di antara keempat-empat peringkat hidup *B. dorsalis*, peringkat larva instar ketiga (di dalam buah) dan pupa (di luar buah) dikenalpasti sebagai peringkat hidup yang paling rintang dan telur muda yang paling terkesan terhadap rawatan sinaran. Hasil kajian mensyorkan dos minimum 100 hingga 150Gy adalah yang paling optimum untuk keperluan aras tinggi bagi sekuriti kuarantin terhadap serangga perosak ini dan ia mungkin dibenarkan dan diterima pakai sebagai rawatan kuarantin ke atas buah-buahan tropika Malaysia.

**Kata kunci**: Rawatan fitosanitasi, *Bactrocera dorsalis*, peringkat hidup paling rintang

## INTRODUCTION

Fruit flies (Famili: Tephritidae) are considered a major quarantine pest on most fruits in Malaysia (Ithnin et al. 2008). Its infestation can cause serious damage and disrupt marketing of fresh agricultural products such as papaya, carambola, mango, rambutan and jackfruit (Hassanuzzaman & Idris 2014). Due to the egg-laying habit of the female flies, most tropical fruits are prohibited from entering countries such as Japan, Australia and the USA. Thus, phytosanitary treatment is very important before export to prevent the introduction and spreading of damaging pests to the importing countries (Bustos et al. 2015).

To overcome such barriers, low dose irradiation is being investigated as a postharvest quarantine treatment to permit exports of fruits to markets that currently restricted by fruit fly quarantine regulations (Zainon & Lebai Juri 2000). Irradiation is particularly suited to this purpose with applications over a wide range of commodities and pests (Heather 2004). It meets the current consumer requirement of freedom from chemical residues that are associated with fumigation and insecticide treatments. Irradiation is also the ideal technology for developing generic treatments because it is effective against most insects and mites at dose levels that do not affect the quality of commodities (Follet 2004), no development of insect resistance, absence of residue in treated food and no significant loss of nutrient in commodities (Hallman & Martinez 2001; Kwon et al. 2004). The effect of gamma radiation can also delay the ripening process in mango fruits (Manoto et al 1986).

Globally, the use of irradiation treatment for insect disinfestations of agricultural commodities, particularly against tephritid fruit flies is being adopted and well established. However, in Malaysia, research on irradiation of fruit fly particularly on *Bactrocera dorsalis* is still lacking. The increment in demand of tropical fruits by Australia, China and USA, it is therefore vital to develop protocols for some premium fruits by using irradiation treatment as

a tool to widen our export market in the future. This study was conducted to determine the most tolerant stage of the Oriental fruit fly, *Bactrocera dorsalis* and the optimum doses for inhibition of egg hatch, pupation and adult emergence.

## MATERIALS AND METHODS

### Laboratory Culturing of Fruit Flies

Culture of fruit flies that maintained in laboratory cages containing 2,500 flies of mixed sexes by using the rearing procedures of *B. tryoni* by Heather and Corcoran (1985). Fruit flies larvae were cultured on an artificial diet (wheat bran media) and rearing medium by following methods of Vargas, Miyashita, Nishida (1984). Eggs were collected from mature females using plastic receptacle which had perforated with many small holes. A few drops of the carambola's juice were put into the plastic receptacle to attract the flies. These were exposed for oviposition in a holding cage for 1 to 2 hours. Eggs were then collected and washed from the plastic receptacle using a stream of water and transferred onto rearing medium. All the stages were reared under 25 ± 2 °C, 70 ± 5% relative humidity (RH) with a photoperiod of 12:12 (Day: Light) h.

### Treatment of Gamma Irradiation

Gamma irradiation was conducted in MINTec Sinagama, Malaysian Nuclear Agency (NM), Dengkil, Selangor using a $^{60}$Co source. The egg, 1$^{st}$ to 3$^{rd}$ instars larvae and the pupae were treated separately with dosages 50 to 400 Gy. Each treatment (dose) involve with minimum of 100 individuals per dose and four replicates. Target doses were monitored by a Fricke dosimeter. The most tolerance stage of the fruit fly will be administered the various doses (0 to 400Gy) to determine the optimum dose or providing quarantine security.

Behavioral events between developmental stages, such as the percentage hatch from egg to first-instar larva, the number pupated from the all instar larva to pupa, and eclosion from pupae to adult, were used as criteria to determine the effect of irradiation. Developmental changes were observed daily and the numbers of larvae that hatched from the cohort of the number of egg, pupation from wandering larvae and emergence adults were counted for 7 days after treatments. Emerged adults from irradiated pupae were counted and sex ratio (male: female) was determined. Percentage adult emergence was the criterion used for selecting the most tolerant stage.

### Data analysis

Statistical analysis of data was conducted with SAS 9.3 program for Windows. The data on hatchability, pupation and adult emergence rates were analyzed by using analysis of variance (ANOVA) and the means compared using Duncan's new multiple range test (DNMRT; $P < 0.05$).

## RESULTS AND DISCUSSION

### Effects of Gamma Irradiation on Egg Hatching

The effects of gamma radiation on egg hatch are presented in Table 1. The result indicated that the radio-sensitivity of irradiated eggs of fuit flies *B. dorsalis* decreased with increasing age of eggs and dose. Results are similar with the study on gamma radiation against the Mediterranean fruit fly, *Ceratitis capitata* in guava fruits made by Doria et al (2007) reported that eggs mortality rate were increased with the dose increment. For early eggs (below 5 hours old egg) the percentage of unhatched eggs was 100% start from 50 to 400Gy. While the percentage

larval emergence for late egg (20-24 hours old egg) was 79.5% in the untreated eggs but reduced to 2.5% at a dose of 250Gy, with all irradiated eggs completely sterile (total mortality) at 300 Gy. This study showed that the early age of egg was more sensitive to radiation than late eggs. As stated by Nasroh Kuswadi et al. (2004), the eggs (≤ 24 hours old) of *B. carambolae* irradiated with dose up to 50Gy did not reduce the egg viability but the survivality of emerged larvae were decreased. From the morphological observation was noted that the non-viable eggs were darkened (dark orange in colour) due to the irradiation treatment.

Table 1 Egg Hatchability after Irradiation of *Bactrocera dorsalis.*

| Dose(Gray) | Egg Hatchability (%, Mean) | |
|---|---|---|
| | Early Egg (below 5 hours old egg) | Late Egg (20-24 hours old egg) |
| 0 | 84.0 a | 84.3 a |
| 50 | 0.0 b | 79.5 a |
| 100 | 0.0 b | 43.0 b |
| 150 | 0.0 b | 24.8 c |
| 200 | 0.0 b | 13.0 d |
| 250 | 0.0 b | 2.5 e |
| 300 | 0.0 b | 0.0 e |
| 400 | 0.0 b | 0.0 e |

Mean values (n=100) with same letter within each column are not significantly different ($p \leq 0.05$), as determined by DNMRT

**Effects of gamma on pupation**

The effects of gamma radiation of various immature stages on the pupation of *B. dorsalis* are summarized in Table 2. With increasing of the radiation dose caused linear decrease in pupation. At 200Gy and 250Gy prevented the development to pupate for survived egg stage and 1$^{st}$ instar larvae, respectively. However, at 400Gy some pupation was still obtained from the irradiated 2$^{nd}$ instar and 3$^{rd}$ instar larvae. The data revealed that the younger larvae stages were more sensitive to irradiation damage as compared to matured larvae. Applying gamma irradiation as quarantine treatment against fruit flies obviously inhibited the growth and development of immature stages to access the next stage of development. According to Thomas and Hallman (2011), irradiated insect larvae usually show reduction effects on growth performance by irradiation, during a major development transition through ecdysis. This effects also can be seen from this study when the younger age of fruit flies fails to develop into next instar stage or pupa.

Table 2 Pupation of *B. dorsalis* after irradiation of the various stages.

| Dose (Gray) | Percentage Pupation (Mean) | | | |
|---|---|---|---|---|
| | Egg | 1st instar | 2nd instar | 3rd instar |
| Untreated | 45.3 a | 59.3 a | 60.3 a | 67.8 a |
| 50 | 28.5 b | 53 a | 62.5 a | 67.3 a |
| 100 | 2.5 c | 22.25 b | 28.25 b | 66.0 a |
| 150 | 1.8 c | 12.75 b | 26.75 b | 55.25 a b |
| 200 | 0.0 c | 12.5 b | 21.5 b | 54.25 a b c |
| 250 | 0.0 c | 0.0 c | 17.25 b | 46.0 b c |
| 300 | 0.0 c | 0.0 c | 16.5 b | 40.75 c |
| 350 | 0.0 c | 0.0 c | 12.5b | 32.0 cd |
| 400 | 0.0 c | 0.0 c | 10.50 b | 20.5d |

Mean values with same letter within each column are not significantly different ($p \leq 0.05$), as determined by DNMRT

**Effects of Gamma on the Percentage of Adult Emergence**

The effects of gamma irradiation on adult emergence of various immature stages of *B. dorsalis* are presented in Table 3. At 100Gy, all immature stages including1st, 2nd and 3rd instar larvae failed to develop into adult and were killed completely (100% mortality) as compared to pupae (83%). Similar to the studies on irradiation of mixed age larvae of *Anastrepha suspensa* (Loew) in grapefruit done by Burdit et al. (1981) showed that although minimum dose of 300Gy was required to prevent pupation but at 100Gy was able to prevent emergence of adults from survived immature stages. These results implied that the pupae was the most tolerant stage of all immature stages to irradiation. Previous research on phytosanitary irradiation of tephritid fruit flies had revealed that the 3rd instar larvae was most tolerant stage inside their host fruits (Hallman 1999, 2000) and pupae stage would be more tolerant stage by radiation than third instars larvae (Hallman & Worley 1999). These findings also meet the requirement for fruit flies quarantine regulation where the efficacy of the irradiation treatment had been measured based on the emergence of adults from the treated third instars (Follett & Armstrong 2004). There has been some variation in the precise definition of efficacy of irradiation as a quarantine treatment against fruit flies. Efficacy with irradiation treatment has been defined as prevention of adult emergence (Ohta et al. 1985), prevention of emergence of flies capable of flight (APHIS 1987), and prevention of flies capable of reproducing (APHIS 1989). Although the pupal stage of the fly is less concern and rarely found in fruits during shipment, however this species can still cause export risks if there are possibility of the event a larva emerged from a fruit and pupated in a package or shipping container.

Table 3 Mean percentage of adult emergence after irradiation of immature *Bactrocera dorsalis* on various stages

| **Dose** | **Mean percentage of adult emergence** | | | |
|---|---|---|---|---|
| | 1st instar | 2nd instar | 3rd instar | Pupae |
| 0 Gray | 73.5 ±3.0 a | 75.2 ± 3.8 a | 78.5 ± 3.3a | 90.0 ± 3.0 a |
| 50 Gray | 27.5 ± 2.0 b | 12.4 ± 2.2 b | 17.5 ± 8.0b | 87.5 ± 2.0 a |
| 100 Gray | 0.0 | 0.0 | 0.0 | 83.0 ± 6.2 a |
| 150 Gray | 0.0 | 0.0 | 0.0 | 69.2 ± 3.0 b |
| 200 Gray | 0.0 | 0.0 | 0.0 | 53.0 ± 2.5 c |
| 250 Gray | 0.0 | 0.0 | 0.0 | 31.0 ± 1.5 d |
| 300 Gray | 0.0 | 0.0 | 0.0 | 12.6 ± 2.0 e |
| 350 Gray | 0.0 | 0.0 | 0.0 | 9.1 ± 5.0 e |
| 400 Gray | 0.0 | 0.0 | 0.0 | 7.3 ± 1.2 e |

Mean values with same letter within each column are not significantly different ($p \leq 0.05$).

**Sex Ratio**

Table 4 shows the effect of gamma radiation on the sex ratio of emerging adults from irradiated pupae of fruit flies. Means percentage of sex ratio were not affected by radiation at dose between 50Gy to 400Gy (with 1: 1 for normal ratio) except to flies irradiated with 250Gy (2: 1). Low dose gamma ray also shows same result with previous study by Zahran et al. (2013) which had stated that no radiation effects on the sex ration of emerged adult from irradiated pupae of *B. zonata* fruit flies but it shows significantly affect with unirradiated flies. Similar findings by Mahmoud and Barta (2011) which mentioned that the sex ratio of adult *B. zonata* from irradiated pupae, also not affected by lower dose (< 30 Gy) of gamma radiation.

Table 4 Mean percentage of sex ratio of emerging adults from irradiated pupae of *Bactrocera dorsalis.*

| Dose (Gray) | Emerging Adults | | |
|---|---|---|---|
| | Male | Female | Ratio ( ♂: ♀) |
| Control | 46.0 ± 5.86 a | 43.0 ± 6.55 a | 1 : 1 |
| 50 | 44.0 ± 8.54 a | 43.5 ± 6.00 a | 1 : 1 |
| 100 | 40.0 ± 2.52 a | 43.0 ± 5.86 a | 1 : 1 |
| 150 | 31.0 ± 2.64 b | 38.2 ± 3.78 b | 1 : 1 |
| 200 | 28.0 ± 1.73 c | 25.0 ± 2.00 c | 1 : 1 |
| 250 | 16.0 ± 1.15 d | 15.0 ± 3.51 d | 2 : 1 |
| 300 | 6.60 ± 0.58 e | 6.00 ± 1.00 e | 1 : 1 |
| 350 | 3.33 ± 1.15 e | 4.60 ± 5.67 e | 1 : 1 |
| 400 | 3.33 ± 1.53 e | 4.00 ± 0.00 e | 1 : 1 |

Mean values with same letter within each column are not significantly different ($p \leq 0.05$), as determined by DNMRT

## CONCLUSION

Early eggs are the most sensitive and susceptible stage to irradiation. Eggs irradiated with a dose of 100Gy to 400Gy were not viable. Among the four immature stages of fruit fly *Bactrocera dorsalis*, the pupae were found to be the most resistant to irradiation. At 100Gy, eggs and all larvae failed to develop into adult as compared to pupae. Generally, the percentage of sex ratio of fruit flies' adults emerged from irradiated pupae was not affected by radiation at all treated doses except to flies irradiated with 250Gy. From this study we are found that the effect of irradiation on fruit flies would not be observed immediately after treatment as complete inhibition may take longer than three weeks. Our results suggest that irradiation at minimum dose 100 to 150Gray is sufficient to provide a high level of quarantine security against this important pest and the dose might allow for irradiation be accepted as a quarantine treatment for most tropical fruits from Malaysia.

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