GROWTH AND DEVELOPMENT OF ORIENTAL FRUIT FLY, *Bactrocera dorsalis* HENDEL (DIPTERA: TEPHRITIDAE) REARED ON SWEET POTATOES (*Ipomoea batatas* L.) BASED ARTIFICIAL DIET

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ABSTRACT

A study to determine the growth and development of *B. dorsalis* reared on sweet potatoes-based artificial diet (*Ipomoea batatas* L.) was conducted under laboratory conditions. Four types of diets used were; orange sweet potato (OSP), purple sweet potato (PSP), white sweet potato (WSP) and wheat germ (WG) as a standard diet. Biological parameters recorded were; number of pupae, pupae weight (mg), pupae and adult survival (%), number of adult emergences, developmental time from eggs to adults' stage (day), and adult longevity (day). Results showed that among the diet tested, the WG based diet displayed a significant difference ($P<0.05$) on the growth and development of *B. dorsalis* particularly on number of pupae, pupa and adult survival and developmental time from egg to adult. However, no significant difference ($P>0.05$) was shown on pupa weight between WG, OSP, and WSP diet. Adult longevity of *B. dorsalis* also showed no significant difference ($P>0.05$) among all the diets. Based on the results obtained, the growth and development of *B. dorsalis* reared on sweet potatoes based diet was as good as the standard wheat germ-based diet and showed a potential as a main new source of diets for *B. dorsalis* mass rearing especially in biological control program of the fruit flies.

Keywords: Artificial diet, *Bactrocera dorsalis*, fruit fly, sweet potatoes.

ABSTRAK

Kajian untuk menentukan pertumbuhan dan perkembangan *B. dorsalis* yang dipelihara menggunakan diet ubi keledek (*Ipomoea batatas* L.) dan dibandingkan dengan diet berasaskan germa gandum sebagai diet piawai telah dijalankan di bawah kawalan makmal. Empat jenis diet yang digunakan ialah; ubi keledek jingga (OSP), ubi keledek biru (PSP), ubi keledek putih (WSP) dan germa gandum (WG) sebagai diet piawai. Parameter biologi yang direkodkan adalah; bilangan pupa, berat pupa (mg), kelangsungan hidup pupa dan serangga dewasa (%), bilangan kemunculan serangga dewasa, masa perkembangan dari telur hingga dewasa (hari), dan jangka hayat dewasa (hari). Hasil kajian menunjukkan bahawa di antara diet yang diuji, diet berasaskan WG menunjukkan perbezaan yang bererti ($P<0.05$) terhadap pertumbuhan dan perkembangan *B. dorsalis* terutamanya berkaitan bilangan pupa, kelangsungan hidup pupa dan serangga dewasa dan perkembangan dari telur hingga dewasa. Walau bagaimanapun, tiada perbezaan yang bererti (P>0.05) ditunjukkan pada berat pupa antara WG, OSP, dan diet WSP. Jangka hayat dewasa *B. dorsalis* juga menunjukkan tiada perbezaan yang bererti (P>0.05) di kalangan semua diet. Berdasarkan hasil yang diperoleh, pertumbuhan dan perkembangan *B. dorsalis* yang dipelihara dengan diet ubi keledek adalah sama baik dengan diet berasaskan germa gandum dan menunjukkan potensi menjadi sumber diet baru bagi pembelaan besar-besaran *B. dorsalis* terutama dalam program kawalan biologi lalat buah.

Kata kunci: Diet tiruan, *Bactrocera dorsalis*, lalat buah, ubi keledek.

INTRODUCTION

The oriental fruit fly, *Bactrocera dorsalis* Hendel (Diptera: Tephritidae) is considered the most serious fruit fly species because it has been reported to seriously attack many species of commercial crops throughout Peninsular Malaysia such as papaya (*Carica papaya* L.), mango (*Mangifera indica* L.), guava (*Psidium guajava* L.) and star fruit (*Averrhoa carambola* L.) (Allwood et al. 1999; Wee & Tan 2005). The host plants can affect the dynamics of *B. dorsalis* populations. Therefore, it is important to fully understand the biology and ecology of this pest from the perspective of agricultural production and to formulate an intensive integrated pest management program. Due to its economic importance, various research on taxonomy, biology, morphology, physiology, and ecology had been done around the world in order to develop the best strategies to control them at an acceptable level (Am et al. 2017; Hee et al. 2015; Migani et al. 2017; Pereira et al. 2016; Salmah et al. 2017; Schutze et al. 2015; Vargas et al. 2015; Wee et al. 2016). One of the strategies is to enhance *B. dorsalis* mass-rearing techniques in laboratory particularly through larval diets provided as intended to produce high quality flies mainly for evaluation of attractants, host for parasitoids and basic biological studies (Ekesi & Mohamed 2011). Thus, a good and sufficient artificial diet is essential to produce large numbers of quality flies in the laboratory.

Artificial diets were developed for insect mass production because it is much easier to maintain in the laboratory, especially for the long term and large-scale than using a natural diet (Vanderzant 1974). The main objective of using an artificial diet is to multiply and produce selected insects in the laboratory for field release especially in biological control strategies, but it is also an important tool to study the biology, physiology and behaviour of insects (Grenier 2012). Barbehenn et al. (1999) suggested that the development of artificial diets on which insects can be reared in a laboratory has allowed researchers to determine the nutrient necessary to support the growth and reproduction of numerous species. Larvae of *Bactrocera* species usually are reared on artificial diets that well mixed which consist of protein sources, carbohydrate, lipids, sterols, bulking agent, vitamins, minerals, water, salts, microbial inhibitors and substances for adjusting pH (Ekesi & Mohamed 2011). One of the major important components in larval artificial diets is known as bulking agents which it will thickening and transform high-water content mixtures of diet into solids with a uniform distribution of the other nutritive ingredients as a larval feeding substrate. Bulking agents such as carrot, cassava, pawpaw, banana, breadfruit, sugarcane bagasse or potato are added because can absorb liquid when mixed in diets and this provides a uniform distribution of nutrients, physical consistency and texture and serving as a substrate for larval feeding and development (Ekesi & Mohamed 2011). However, a bulking agent that based on a natural diet (i.e. host fruits) is usually difficult to manage and caused higher cost as well as a limited supply of natural host. In that case, many artificial diets have been evaluated for the rearing of fruit flies due to the cost-effectiveness and easy preparation (Ekesi & Mohamed 2011). But some commercial and imported bulking agents in artificial diets such as wheat germ and carrot powder comparatively expensive. As stated by Pascacio-Villafán et al. (2017), expensive nutritional diet does not necessarily promise into a better performance of the flies than low-cost diets. Therefore, alternative sources of other cheaper bulking agents from local material is much needed and for this case sweet potatoes are one of a good potential candidate.

Sweet potatoes (*Ipomoea batatas* L.), a tuber crop under a family of Colvolvulaceae or locally known as '*ubi keledek*' is one of the crops that widely planted in East Coast of Peninsular Malaysia particularly in Terengganu and Kelantan (Tan 2015). The sweet potatoes cultivation in Terengganu and Kelantan is outstanding in its yield performance due to the BRIS (Beach Ridges Interspersed with Swales) type of soil as well as the climate which suitable conditions for growing activity of sweet potatoes. The common local varieties of sweet potatoes planted are Telong, Jalomas, Gendut, Ubi Biru and VitAto (LPP 2008). Sweet potatoes are good antioxidants food and contain high nutritional value of carbohydrates, high-quality proteins, minerals, vitamins and dietary fiber (Tan 2015). These good nutritional qualities of sweet potatoes make it a very good supplement food. For example, VitAto, an orange-fleshed sweet potato, is rich with beta carotene and Vitamin C (Mohd Hanim et al. 2014; Tan 2015) whilst Ubi Biru, a purple-fleshed sweet potato is high in anthocyanin, a powerful anti-oxidant with good bioavailability (Tan 2015). Thus, VitAto is a new variety of sweet potato introduced by the Malaysian Agricultural Research and Development Institute (MARDI) in 2007 (Mohd Hanim et al. 2014). This variety is widely cultivated in Terengganu and Kelantan which covered about 120 ha (LPP 2008). Typically, the local sweet potatoes retail price is as low as 1.50 ringgit per kilogram depends on the grade and variety. Therefore, based on the high nutritional value, abundance and cheaper price of sweet potatoes, it can be a new potential economic source of bulking agents for *B. dorsalis* artificial diets.

Since there is no intensive study in Malaysia has been conducted on the effect of local sweet potatoes as bulking agents on *B. dorsalis* mass rearing, therefore, this study aimed to determine the growth and development of *B. dorsalis* reared on sweet potatoes-based diet (*Ipomoea batatas* L.) in the laboratory conditions. The nutrient contents of each diet tested were also evaluated.

MATERIALS AND METHODS

Cultures and Rearing of *B. dorsalis*

The experiment and insect culture were conducted at the Laboratory of Entomology, Faculty of Bioresources and Food Industry, University Sultan Zainal Abidin, Besut Campus, 22200 Besut, Terengganu. The laboratory cultures and rearing techniques of *B. dorsalis* were modified and adopted from Ekesi et al. (2007), Ekesi and Mohamed (2011), Mohd Noor et al. (2011) and Walker et al. (1997). The adults of *B. dorsalis* used in this experiment were obtained from the fruit fly colonies cultured in the rearing cage (30 x 30 x 30 cm) and maintained in the laboratory (28±2ºC, 70-80% RH, 12:12 h L:D)for five generations. The adults were fed with water soaked on sponge and sugar cubes with a mixture of yeast extract and sugar at ratio 3:1.

Larval Diet Preparation

Four types of diets used were; wheat germ as standard diet (WG), orange sweet potato (OSP), purple sweet potato (PSP) and white sweet potato (WSP). The organic wheat germ was purchased from the supermarket whilst sweet potatoes were brought from the local market. The composition of each diet was shown in Table 1. The composition of wheat germ and sweet potatoes based-diet were modified and based on Chang (2009). All ingredients were thoroughly mixed by using a blender (PANASONIC MS 801s) for 2-3 minutes. Each diet (200 g) were placed in a plastic container (15 x 12 x 5 cm) and stored in the chiller at 4°C for 24 hours. All pH diets were adjusted to value range between pH 4.5-5.0 in order to prevent any bacterial and fungal contamination. The pH value can be adjusted by using concentrated hydrochloric acid (HCl) (0.5-3.55%) or citric acid to increase the acidity (Ekesi & Mohamed 2011).

Table 1. Composition of different diets and their percentages of ingredients

Ingredients %	**Wheat Germ (WG)**	**Orange Sweet Potato (OSP)**	**Purple Sweet Potato (PSP**	**White Sweet Potato (WSP)**
Wheat germ	25.0	-	-	-
Sweet potato	-	58.5	58.5	58.5
Torula yeast	8.1	8.1	8.1	8.1
Nipagin	0.2	0.2	0.2	0.2
Sodium benzoate	0.2	0.2	0.2	0.2
Sugar	8.0	8.0	8.0	8.0
Water	58.5	25.0	25.0	25.0
Total (%)	**100.0**	**100.0**	**100.0**	**100.0**

The above formula of the larvae diet is the best diet for rearing *B. dorsalis* as it consists of protein sources, carbohydrate, water and microbial inhibitors (Ekesi & Mohamed 2011). For example, yeast products such as Torula yeast is a standard source of protein in larval diets. Whilst, nipagin and sodium benzoate are normally added in the diet as to stop bacterial and fungal development which this can cause high mortality to fruit fly larvae. Besides that, water also an important component in *Bactrocera* larval diet as it can minimize the effects of metabolic heat build-up during the final stages of larval development as well as for easier nutrient access by the larvae. The diet should be dense enough for larval movement and feeding but not too watery to avoid the larvae from drowning.

Determination of Nutrient Contents of Diets

All type of diets was tested for nutrient content analysis. Proximate composition analysis of the sample for moisture, ash, crude protein, crude fat, crude fiber and total carbohydrate were carried out in triplicates according to standard methods of analysis, as described by the Association of Official Analytical Chemists (AOAC 1995) using dry samples. The moisture content was determined by oven method, whilst ash content was determined using the method stated by Eze and Agbo (2011). According to Soxhlet (1987), the crude fat was determined using Soxhlet method and then modified by Buddhi (2009), which shortened the extraction time and extraction efficacy. Crude fiber was analyzed using Gerhardt fiberbag technology and crude protein reading was analyzed using the method developed by Kjeldahl (1883). Carbohydrate content will be estimated by difference [Carbohydrate (%) = 100%-% (moisture + ash + crude protein + crude fat)]. All the readings were done in triplicate.

Eggs Collection of *B. dorsalis*

The eggs of *B. dorsalis* were collected when the age of adult flies from the rearing cage reached three weeks old or around 21-22 days old. According to Walker et al. (1997), at this age the females of *B. dorsalis* lay eggs at the optimum level. To collect the eggs, papaya fruit domes was used as an egg collection device. Egg collection device refers to the tool for the fruit flies to oviposit eggs and makes it easier to collect eggs in the laboratory (in this case is a papaya fruit dome). Fruit dome is an excellent egg collection device as stated by Ekesi and Mohamed (2011).The papaya was cut into half and the flesh of papaya was scooped out and leaving as little flesh as possible on the skin before placed inside the rearing cage to allow the female flies to oviposit eggs. The fruit domes were left for 24 hours and then the fresh fly eggs were collected using a fine brush. Then, the eggs were put on the artificial diet surface.

Effects of Diet on Growth and Development of *B. dorsalis*

Approximately 400 of *B. dorsalis* newly viable eggs were collected from the fifth generation of *B. dorsalis* culture as mentioned above. For each type of diet, 100 eggs were distributed evenly over the diet surface that placed in the petri dish (15cm diameter) for the larval development. Each petri dish with diet and eggs were placed separately in a small aquarium which layered at the bottom with fine vermiculite as pupation medium with the screened tight-fitting lid sealed with parafilm and labelled to ensure the eggs remain moist. After eggs hatching, the larval developmental time was measured as time in days within each stage. Larvae reached the third instar would 'jump' out from the diet into vermiculite to pupate. After five days, the pupae were sieved from vermiculite and placed individually in small plastic cups (4 x 7cm). Then the plastic cups and pupae were transferred to adult rearing cage until the emergence of the adults. The survival and mortality of eggs, larvae, pupae and adults were observed and recorded daily. The experiment for each type of diets was repeated for five times.

Data Collection

The number of pupae collected and the number of adult emergences from each type of diets were recorded. Other biological parameters recorded were; pupae weights, number of pupae, pupae and adult survival (%), developmental time from eggs to adults' stage, and adult longevity. The parameter data of nutrients for each type of diets recorded were; moisture, ash, crude protein, crude fiber, crude fat, and carbohydrate.

Data Analysis

The experiment design was based on a Completely Randomized Design (CRD) with five replications per treatment. Collected data were analyzed using One-way Analysis of Variance (ANOVA) whereas means were separated with Tukey's Range (HSD) Test at 0.05 level of significance. All the analysis was done using MINITAB 18 software.

RESULTS

Determination of Nutrient Contents of Diets

The proximate analysis of wheat germ (WG), orange sweet potato (OSP), purple sweet potato (PSP) and white sweet potato (WSP) diet was shown in Table 2. The nutrient contents of each diet analyzed were ash, moisture, fat, fiber, protein and carbohydrate.

In detail, WG diet was significantly ($P<0.05$) recorded the highest percentage of ash content compared to OSP and PSP but no significant difference ($P>0.05$) were observed with the WSP diet. Meanwhile, the OSP diet displayed the highest moisture content (68.10±0.40%) compared to other diets. While the lowest moisture content was recorded in WG diet (12.40±0.17%).

Table 2. Percentage (%) of nutrient content in potatoes-based and wheat germs diet

Diet	**Parameter (%)**					
	Ash (Mean±SE)	**Moisture (Mean±SE)**	**Fat (Mean±SE)**	**Fiber (Mean±SE)**	**Protein (Mean±SE)**	**Carbohydrate (Mean±SE)**
WG	3.01±0.01a	12.40±0.17d	8.86±0.03a	27.29±0.34a	9.63±0.15a	51.89±0.14a
OSP	1.85±0.12b	68.10±0.40a	1.67±0.22a	22.67±0.14b	6.80±0.09a	21.63±0.04c
PSP	2.049±0.03b	62.78±0.23b	1.20±0.15a	22.52±0.18b	3.51±0.16c	30.46±0.37b
WSP	2.73±0.04a	50.05±0.53c	0.44±0.05b	23.82 ±0.02a	5.16±0.22b	41.63±0.52a

Means with the same letters in different rows are not significantly different (P>0.05)

Wheat germ-based diet significantly recorded the highest fat content (8.86±0.03%) compared to WSP which showed significantly the lowest content (0.44±0.05%). However, no significant difference (P>0.05) of fat content was observed between WG, OSP and PSP diet. While crude fiber content in WG and WSP diet were significantly slightly higher than OSP and PSP diet.

For protein content, WG diet displayed a significantly the highest of protein content (9.63±0.15%) compared to PSP and WSP diet but it was not different from OSP diet (6.80±0.09%). Also, WG diet showed significantly the highest of carbohydrate content (51.89±0.14%) compared to the OSP diet (21.63±0.04%) and PSP diet (30.46±0.37%) but did not differ significantly with WSP diet (51.89±0.14%).

Determination of Growth and Development of *B. dorsalis* on Each Diets.

Table 3 showed the growth and development parameters of *B. dorsalis* reared on wheat germ (WG), orange sweet potatoes (OSP), purple sweet potatoes (PSP) and white sweet potatoes (WSP) based diet.

Table 3. Mean comparison of *B. dorsalis* based on potatoes-based and wheat germ diets

Diet	**Parameter**				
	No. of pupa	Weight of pupa (mg)	Pupa and adult survival (%)	Developmental time from egg to adult (days)	Adult longevity (days)
WG	17.33±0.33a	14.72±0.25a	16.67±0.00a	14.00±0.58a	7.33±1.33a
OSP	9.00±0.58b	13.30±0.17ab	8.00±0.01b	21.33±0.67b	5.67±0.33a
PSP	8.00±0.58b	11.42±0.10c	7.67±0.01b	23.00±1.00b	4.33±0.67a
WSP	9.00±0.58b	13.44±0.29ab	8.00±0.02b	20.33±0.33b	5.67±0.67a

Means with the same letters in different rows are not significantly different (P>0.05)

The highest number of pupal was significantly recorded on a WG diet (17.33±0.33) compared to all sweet potatoes' diets. However, the number of pupae was not significantly different (P>0.05) among the sweet potatoes-based diets. The weight of pupae was significantly the highest (P<0.05) in WG diet compared to pupae weight in PSP diet although no significant difference (P>0.05) was recorded with OSP and WSP based diet. The pupae survival (%) and developmental time from egg to adult were significantly different (P<0.05) between WG and other sweet potato-based diets. Nonetheless, there was no significant difference (P>0.05) of pupae survival and developmental time among the three sweet potatoes diets. The duration from egg to adult was significantly the shortest time in WG diet (14.00±0.58 day) compared to sweet potatoes diet whilst adult longevity showed no significant difference (P>0.05) among all the diets tested (Table 3).

DISCUSSION

In term of nutrient contents, our results showed that although ash content was highest in wheat germ diet but there was no difference with white sweet potatoes diet which similarly with Gopalan et al (2007). According to Fay (1989), ash is an inorganic residue remaining after the organic matters have been burnt away. However, there was an obvious difference of moisture content between sweet potatoes based diet with the wheat germ based diet which the result was in line with Abubakar et al (2010). This might be due to that the low moisture content of wheat germ remains an asset in the storage and preservation of nutrients in the larval diet (Mahmoud et al. 2015). Whilst the fat content result was similar with Vasconcelos et al. (2013) and Ramadan et al. (2008) which they reported that the crude fat content of good diet normally ranges between 7% to 10%. For crude fiber, the high fiber content is very important in insect mass rearing for permits an optimum medium for larval development (Domínguez 1999) and the suitable crude fiber content is range between 5 to 26% (Fay 1989). In this study, the fiber of sweet potatoes diets was in line with a suitable range.

Protein content showed a significant difference which the highest of protein content was in wheat germ diet but not different with orange sweet potatoes diet indicated that orange sweet potatoes diet is as good as wheat germ diet. Protein is one of the most important contents in insect rearing because it is essential for the construction and regeneration of cells and organs of insect (Fay 1989). Also, protein is needed as it could promote better egg production and hatchability (Sobrinho et al. 2006). The female flies fed with protein source attained high fecundity level and reducing the amount of protein source in the diet negatively affected fecundity and ovary maturation (Goane et al. 2019).

Carbohydrate was basically obtained from granulated sugar, which has a stimulatory effect on the insect (Domínguez 1999). Based on the result in Table 2, a wheat germ diet showed significantly the highest of carbohydrate content. However, the carbohydrate content in white sweet potatoes diet showed no different with wheat germ diet. Carbohydrate is necessary to contribute the function and structure of insect and can be found in the cytoplasm, nucleus and cellular membrane, as well as extracellular hemolymph as support for the tissue (Fay 1989). Hence, carbohydrates are the main energy sources for most insects because they needed for optimal growth in most larval herbivorous insects and are essential nutrients for some species (Barbehenn et al. 1999).

Meanwhile, our results for growth and development of *B. dorsalis* showed that diets based on sweet potatoes had supported the *B. dorsalis* growth and development which similar to wheat germ-based diet results particularly on the weight of pupa and adult longevity (Table 3). In fruit flies mass rearing, high pupal weight is considered a desirable trait in the production process, as it is a good indicator of flies' body size and flies' size also a determinant factor of insect fertility and fecundity (Ekesi & Mohamed 2011). David et al. (2004) stated that pupal weight was affected by the presence of amino acid in the diet. Jaleel et al. (2018) reported that pupal development period and weight are the vital key factors for the survival of insect pest, especially in fruit flies where the high pupal body weight is positively related to high fecundity.

However, wheat germ-based diet significantly increased the number of *B. dorsalis* pupa and survival of pupa and adult and decreased the developmental time from egg to adult stage. In practical artificial rearing, pupation, emerged adults and survival rate were important parameters that minor differences in size between adults reared on different constituents of artificial diets (Sarah Najihah et al. 2019). Basically, wheat germ is the best diet for fruit flies growth and development as it contains all the nutrients required by insects. Wheat germ is high in carbohydrate, protein, fatty acids and sterols, minerals and also a source of several essential nutrients including Vitamin E and it is a good source of fiber (Sarah Najihah et al. 2019). Other than that, wheat germ also contains substances that stimulate a feeding response in insects and lack of toxins (Vanderzant 1974). However, from the results in nutrient analysis contents (Table 2), sweet potatoes also showed similar high nutrient contents with wheat germ, particularly in fat, protein and carbohydrate.

Although the protein content of wheat germ diet is not significantly different with orange sweet potatoes (Table 2), but the wheat germ diet showed significantly the shorter developmental time and higher pupal number of *B. dorsalis* compared to other diets (Table 3). Chaudhuri and Senapati (2017) reported that the shorter development period is a desirable characteristic in the mass production of any insect while Etman et al (2009) stated that the faster development of insect shows the efficiency of the rearing medium. Nonetheless, the weight of pupa reared from wheat germ-based diet was not significantly different from the weight of pupa reared from orange and white sweet potatoes-based diet.

CONCLUSIONS

Based on the results obtained, the growth and development of *B. dorsalis* reared on sweet potatoes was similar as standard wheat germ-based diet and this will give the added value to local varieties of sweet potatoes as main new sources of diets for *B. dorsalis* mass rearing program that cheaper and affordable. Further research should be done on the effect of sweet potatoes-based diet on the life history of *B. dorsalis* so that the comprehensive data will be obtained.

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