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MODIFIED AGAR-BASED DIET FOR SMALL SCALE LABORATORY REARING OF OLIVE FRUIT FLY, *BACTROCERA OLEAE* (DIPTERA: TEPHRITIDAE)

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ABSTRACT

Five larval diets for laboratory rearing of *Bactrocera oleae* Gmelin were tested. These diets were based on soy hydrolysate, yeast, sugar, casein, wheat germ, microcellulose and agar. The quality of diets was evaluated by measuring larval and pupal survival, larval and pupal weights, and development times. The best results were obtained with an agar-based diet that was modified from the currently used cellulose-based diet for rearing olive fruit fly in mass rearing facilities. Under these conditions, 77% of the larvae reared on the new agar-based diet completed development and achieved higher pupal weight than larvae reared on the currently available cellulose diet. The average life cycle was completed in 25.2 ± 0.4 d on the agar diet, and other biological parameters were also very close to those on the cellulose diet. Olive fruit fly larvae were reared continuously and successfully for 4 generations on the new diet. The preparation of the new agar-based diet is simple, the cost is low, and it is useful for small-scale laboratory tests and rearing.

Key Words**:** agar, *Bactrocera oleae*, cellulose, larval diets, laboratory rearing, nutrition

RESUMEN

Cinco dietas de larva para criar *Bactrocera oleae* Gmelin fueron probadas en el laboratorio. Estas dietas fueron basadas sobre el hidrolĬzate de soya, levadura, azúcar, casein, germen de trigo, micro celulosa y agar. La cualidad de las dietas fue evaluada para medir la sobrevivencia de larvas y pupas, el peso de larvas y pupas y el periodo de desarrollo. Se obtuvieron los mejores resultados con la dieta de base agar que fue modificada de una dieta de base celulosa usada actualmente en las facilidades donde crĬan la mosca del fruto de olivo en masa. Bajo estas condiciones, 77% de las larvas criadas sobre la dieta nueva de base agar terminaron su desarrollo y lograron a tener pupas con mayor peso que las larvas criadas sobre la dieta de celulosa actualmente disponible. El ciclo de vida tardo un promedio de 25.2 ± 0.4 dĬas sobre la dieta de agar, y los otros parámetros biológicos también fueron similares a los de la dieta de celulosa. Se crĬa la mosca del fruto de olivo continuamente y con éxito por 4 generaciones sobre la dieta nueva. La preparación de la dieta nueva de base agar es sencilla, el costo es bajo y es útil para usar en la crianza de las moscas y en pruebas en laboratorios de una escala pequeña.

The olive fruit fly, *Bactrocera oleae* Gmelin, is the most important and widespread pest in olive growing countries in the Mediterranean basin (Economopoulos 2002). Control of this pest has been based on organophosphate insecticides for many decades, but their intensive use has lead to development of enzyme (acetylcholinesterase (AChE)) resistance due to the selection of 2 resistance mutations (I199V and G488S) (Vontas et al. 2002). The sterile insect technique (SIT) is one of the most promising control approaches for the future of fruit fly integrated management (Enkerlin & Mumford 1997; Hendrichs et al. 2002). A primary requirement for success of SIT of olive fruit flies is mass rearing of the flies on a larval diet of high efficiency and low cost.

Olive fruit flies cannot be reared on a completely defined synthetic diet because detailed nutritional information is lacking, but several artificial diets have been developed for rearing the flies. The first artificial diet developed by Hagen et al. (1963) for the olive fruit fly was based on dehydrated carrot powder and brewer's yeast. This diet was used successfully for rearing more than 3 generations. Later, several artificial diets were reported as satisfactory for continuous rearing of the olive fly (Tzanakakis et al. 1966; Tzanakakis & Economopoulos 1967; Rey 1969; Tzanakakis et al. 1970; Mittler & Tsitsipis 1973; Tsitsipis 1975; Tsitisipis & Kontos 1983).

Rate of larval development, diet consistency, distribution of nutrients in the diet, microclimate conditions during larval feeding, availability of diet ingredients, and amount of consumed diet each play an important role in evaluating a satisfactory larval diet (Pašková 2007). The diet should be dense enough for larval movement and feeding but not be too wet or larvae may drown. Olive fruit fly larvae, like other tephritid larvae, typically crawl out of the rearing containers to pupariate (Pašková 2007).

Various nutritive and bulking components used in larval diets include agar, cellulose, ground corn cobs, *Eucalyptus* pulp, soy hydrolysate, yeast hydrolysate, roasted peanuts, chickpea seedlings, and sucrose (Tzanakakis 1989). Nutritive and bulking components can be costly and sometimes difficult to obtain in some countries, and diet components often have to be imported into some countries where rearing of olive fruit flies is desirable. Replacement of imported components by local products would be advantageous.

The objective of the present work was to develop a suitable and economic artificial diet based on alternative sources of protein and bulking agent for laboratory rearing of olive fruit flies in Turkey.

Materials and Methods

Olive Fruit Fly Colony

Olive flies were collected from infested olive fruits in 2006 in Canakkale province, Turkey. The diet developed by Tsitsipis & Kontos (1983), based on soy hydrolysate as the protein source, was used to maintain a laboratory colony. A detailed description of the rearing conditions can be found in Tzanakakis (1989). Green olive fruits for a natural diet were obtained from the local market. Colony flies were kept at 26 ± 1°C with 18:6 (L: D) photoperiod and 65% RH. Adult flies (100♀ : 70♂) were maintained (20 × 20 × 20 cm in dimension) on a diet of yeast hydrolysate: sucrose (1:3). Paraffin domes (6 cm in length and 3.5 cm in width), used as oviposition devices, were placed in the rearing cages for 2 h to obtain eggs. Eggs were washed off domes with 0.05% propionic acid solution and transferred to sterile black filter paper previously moistened with water. Eggs were incubated at 27°C until hatching.

Test and Control Diets

Five artificial diet formulations were experimentally tested. Two control diets, one developed by Tsitsipis & Kontos (1983), and the natural larval host of fresh green olives were included in all tests. The composition of the Tsitsipis & Kontos (1983) diet and experimental diets is shown in Table 1. Experimental and control diets were freshly prepared before each experiment, and fresh green olives were obtained from local markets in Canakkale, Turkey. The diet mixture was formulated by weighing all ingredients and mixing them in a 500-mL beaker with 206.3 mL of distilled water. Stirring continued until the diet ingredients appeared to be homogeneously mixed. The pH of all diets was adjusted to 3.8-4.0 with 2N HCl. Fifteen grams of diets were added to 6-cm disposable Petri dishes, with 5 Petri dishes used for each test diet and control, including 5 dishes with green olives. The 5 dishes of each diet were considered as 5 replicates. Olive fruit fly eggs were collected from several cages of colony flies, incubated at 27°C until hatching, and newly hatched larvae were collected with a camel-hair brush, and 100 larvae were transferred to each experimental and control diet within 2 h of hatching. Larval development was monitored daily under an Olympus SZX9 stereozoom microscope. When larvae began crawling out of a diet, the uncovered Petri dishes were transferred to sterile sand for pupation. Pupae were sifted from the sand after 5 d. For each Petri dish of diet, the following biological parameters were reported: percentage of mature larvae (total number of larvae produced from the number of 100 neonates), percentage of pupal recovery (total number of pupae produced from the number of original larvae), percentage of adult emergence, larval weight, pupal weight (at day 5 after pupariation), larval development time (from hatching until first larvae started to crawl out of diet to pupariate), pupal development time (from the onset of pupariation of the mature larvae until the emergence of the first adults), adult fecundity and longevity. Fecundity was based on daily egg collection from 10 pairs of flies held after a preoviposition period of 4 d. Diets were evaluated against the control diet (Tsitsipis and Kontos, 1983) and the natural diet of olives.

When an effective diet (diet C in Table 1) was identified from among the 5 experimental formulations tested, diet C, the Tsitsipis & Kontos (1983) control, and green olives were used to continuously rear 4 successive generations of olive fruit flies, with 5 replicates of diet in each generation. All experiments were conducted at 26 ± 1°C with 18:6 (L: D) photoperiod and 65% RH.

Statistical Analyses

Descriptive statistics as mean values ± standard error (SE) were calculated. The differences among the diets quality control parameters were determined by analysis of variance (ANOVA).The LSD test at $P = 0.05$ level of significance was used to determine separation and significance of means (SAS 1999).

Results

Diet components, their quantity in a diet mixture, cost per kg, and cost of one batch of mixed diet (391 mL) are shown in Table 1. Diet evaluations based upon mature larvae reared (%), pupal recovery (%), adult emergence (%), weight of mature larvae and pupae, duration in larval and pupal stages, generation time, adult fecundity, and adult longevity are given in Table 2. Table 2 also includes data from rearing of flies on their natural diet of fresh olives. The

TABLE 1. COMPOSITION OF ARTIFICIAL DIETS USED TO REAR *BACTROCERA OLEAE* LARVAE IN THIS STUDY.

Ingredients	Control (Tsitsipis & Kontos, 1983)	Diet A	Diet B	Diet C	Diet D	Diet E	Cost in US dollars for 1 kg of ingredient
Water	206.3 mL	206.3 mL	206.3 mL	206.3 mL	206.3 mL	206.3 mL	—
Soy hydrolysate	11.25 g	—	11.25 g	—	—	—	225
Brewer's Yeast	28 g	28 g	28 g	28 g	—	28 g	85
Sugar	7.5 g	7.5 g	7.5 g	7.5 g	7.5 g	7.5 g	5
Cellulose powder	115.5 g	115.5 g	—	—	—	—	34
Agar	—	—	2.52 g	2.52 g	2.52 g	2.52 g	93.45
Casein	—	7.5 g	—	7.5 g	9 g	9 g	19.88
Wheat germ	—	3.75 g	—	3.75 g	5 g	—	6.10
Torula yeast	—	—	—	—	28 g	—	14.50
Olive oil	7.5 mL	7.5 mL	7.5 mL	7.5 mL	7.5 mL	7.5 mL	15
Tween 80	2.81 mL	2.81 mL	2.81 mL	2.81 mL	2.81 mL	2.81 mL	450
Nipagin	0.75 g	0.75 g	0.75 g	0.75 g	0.75 g	0.75 g	200
Potassium sorbate	0.18 g	0.18 g	0.18 g	0.18 g	0.18 g	0.18 g	1300
HCl 2N	11.25 mL	11.25 mL	11.25 mL	11.25 mL	11.25 mL	11.25 mL	25
Cost of 391 mL of formulated diet ($)	10.89	8.52	7.20	4.83	2.85	4.81	

TABLE 2. BIOLOGICAL PARAMETERS (MEAN ± SE) OF OLIVE FRUIT FLIES REARED ON ARTIFICIAL DIETS AND NATURAL DIET OF FRESH OLIVES.

	DIETS (Mean ± SE)							
PARAMETERS	A	B	C	D	E	Control	Olive	LSD*
Mature larvae (%)	79.20 ± 1.77 b	78.60 ± 1.54 b	81.40 ± 1.72 ab	30.00 ± 2.55 c	21.40 ± 1.36 d	85.20 ± 2.74 a	85.13 ± 5.32 a	5.696
Pupal recovery (%)	75.20 ± 2.22 c	76.60 ± 1.36 bc	78.40 ± 1.33 abc	19.40 ± 1.03 d	11.00 ± 0.70 e	79.80 ± 2.27 ab	81.82 ± 2.27 a	4.173
Adult emergence (%)	61.00 ± 2.30 c	65.80 ± 1.28 b	77.40 ± 1.33 a	15.00 ± 1.14 d	6.800 ± 0.86 e	77.20 ± 2.34 a	78.20 ± 5.83 a	4.234
Mature larva (mg)	6.470 ± 0.07 b	6.210 ± 0.37 c	6.65 ± 0.14ab	4.780 ± 0.15 d	4.450 ± 0.09 e	6.68 ± 0.13 ab	6.78 ± 0.43 a	0.254
Pupal weight (mg)	6.050 ± 0.06 b	5.510 ± 0.17 c	6.38 ± 0.15 b	4.140 ± 0.18 d	3.950 ± 0.11 d	6.36 ± 0.10 b	7.33 ± 0.78 a	0.411
Larval duration (d)	21.8 ± 0.2 c	19.2 ± 0.4 d	15.6 ± 0.2 e	24.6 ± 0.4 b	33.4 ± 1.6 a	14.2 ± 0.2 ef	13.0 ± 0.7 f	2.017
Pupal duration (d)	11.0 ± 0.3 c	10.8 ± 0.4 c	8.6 ± 0.2 d	17.4 ± 0.4 b	19.4 ± 0.5 a	7.0 ± 0.01 e	6.5 ± 0.2 e	1.017
Generation time (d)	32.8 ± 0.2 c	30.0 ± 0.3 d	24.2 ± 0.4 e	41.8 ± 0.2 b	52.8 ± 1.3 a	21.2 ± 0.2 f	19.5 ± 0.5 f	2.140
Adult fecundity	191.2 ± 71.3 c	163.6 ± 59.4 d	241.2 ± 39.1 b	36.13 ± 8.9 e	32.94 ± 9.3 e	244.6 ± 51.7 b	278.9 ± 61.4 a	20.921
Adult longevity (days)	39.13 ± 7.9 c	35.42 ± 8.4 dc	47.81 ± 7.6 ab	32.67 ± 9.2 d	36.41 ± 11.3 dc	44.11 ± 6.3 b	51.17 ± 8.3 a	4.788

*LSD = Fisher's Least Significant Difference between any 2 means. The means in a row followed by a different letter are significantly different from each other ($P \leq 0.05$).

natural diet of fresh olives is clearly the best diet in the tests, but the diet of Tsitsipis & Kontos (1983) is nearly as good as the olive diet, with only minor, but significant, variations in evaluated parameters. A significant problem with use of fresh olives is that they are available in Turkey and many other countries only from Sep through Jan. All measured parameters for the experimental diets A, B, C, D, and E were significantly different from each other for each of the 5 diets. Diets A, B, D, and E are not acceptable diets based on one or more measured parameters. Measured parameters for experimental diet C are comparable to the Tsitsipis & Kontos (1983) diet, with only minor, but statistically significant, differences in duration of pupal stage, and total generation time. Percent mature larvae reared, percent pupal recovery, percent adult emergence, larval and pupal weights, duration of larval stages, adult fecundity and longevity for flies on diet C were not different from values for the Tsitsipis & Kontos (1983) diet. Both diet C and the Tsitsipis & Kontos (1983) diet have a consistency that allows olive fruit fly larvae to move and feed easily, and to crawl out of the diet prior to pupariation. The fresh olive diet allowed slightly greater pupal recovery than diet C or the diet of Tsitsipis & Kontos (1983). There is no significant difference in pupal weight for the three diets. Except for the second generation, the percent adult emergence was not different for the 3 diets.

Data for pupal recovery, pupal weight, and adult emergence for each of 4 generations reared on diet C, the Tsitsipis & Kontos (1983) control, and green olives are shown in Table 3.

DISCUSSION

Good survival and growth, mating behavior, ability to fly, and egg production are important criteria for insects reared on artificial diets (Chang et al. 2004; Chaudhury & Skoda 2007). The olive fruit fly has been mass reared for SIT programs for many years. Results of this study show that the new agar-based diet (diet C) has the potential for use as an olive fruit fly larval diet. Diet formula C was approximately equal to the cellulose control diet currently in use. Diet formulas D and E produced significantly smaller larvae, possibly indicating that insufficient nutrients for normal larval weight gain or imbalanced nutrients reduced larval growth. Diets A and B produced larvae that were just about as large as larvae produced by diet C, suggesting that these diets provided adequate nutrients, although development time was greater on diets A and B. The nutritional content of a diet can considerably affect development time, growth and survival of fruit fly larvae (Krainacker et al. 1987, Vargas et al. 1994).

TABLE 3. COMPARISON OF PERCENT PUPAL RECOVERY, PUPAL WEIGHT AND PERCENT ADULT EMERGENCE (MEAN ± SE) OF OLIVE FRUIT FLY REARED ON 2 ARTIFICIAL DIETS (TSITSIPIS & KONTOS 1983 AND DIET C) AND ON OLIVE FRUITS.

Generation	Pupal recovery (%)				Pupal weight (mg)				Adult emergence (%)			
	Diet C	Control	Olives	LSD*	Diet C	Control	Olives	LSD*	Diet C	Control	Olives	LSD*
1	76.4 ± 5.3 b	77.8 ± 6.8 b	85.3 ± 5.2 a	5.867	6.4 ± 0.2 a	6.4 ± 0.1 a	6.8 ± 0.6 a	0.513	75.2 ± 7.3 a	76.3 ± 5.3 a	78.2 ± 6.3 a	5.701
2	71.3 ± 6.3 b	70.7 ± 7.4 b	80.6 ± 4.1 a	8.754	6.4 ± 0.9 a	6.6 ± 0.2 a	6.5 ± 0.9 a	0.813	72.3 ± 8.2 b	75.7 ± 4.2 b	90.1 ± 4.5 a	5.495
3	72.7 ± 6.6 a	72.4 ± 8.4 a	77.9 ± 7.3 a	9.351	6.3 ± 0.7 a	6.7 ± 0.4 a	6.7 ± 0.9 a	0.522	79.2 ± 9.2 a	82.2 ± 7.3 a	83.6 ± 3.7 a	6.443
4	73.1 ± 9.4 b	75.5 ± 6.3 b	82.6 ± 6.6 a	6.007	6.5 ± 0.9 a	6.4 ± 0.8 a	6.7 ± 0.8 a	0.621	74.6 ± 9.6 a	75.8 ± 4.4 a	76.7 ± 7.1 a	6.587

*LSD = Fisher's Least Significant Difference between any 2 means. The means in a row followed by a different letter are significantly different from each other ($P \leq 0.05$).

Diet C components were easier to mix to homogeneous consistency than the control diet with its large amount of cellulose as a bulking agent. Although agar is more expensive (US $93.45/kg) than cellulose (US $34/kg), the amount of agar used in diet C is very low (2.52 g) and costs only $0.24 while the cellulose used in the control diet costs $3.92. Thus, the new formulation of diet C with agar reduces the cost of a batch of mixed diet to less than half that of the cellulose-based control diet (Table 1). Diet C is suitable for use in additional laboratory tests and evaluation as a rearing medium for olive fruit flies.

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