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Evaluation of Using Certain Sources of Protien for Feeding Mediterranean Fruit Flies, *Ceratitis capitata* Adults

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ABSTRACT

Mass rearing cost is one of the important tools for successful implementation the sterile insect technique (SIT) programme. Yeast hydrolyzed (YH) is an effective protein source for feeding the adult flies of *C. capitata* and widely used in the mass rearing facilities in spite of it is imported product in many countries and very expensive. Three local protein sources, meat bone meal (MBM), plant protein meal (PPM) and soybean protein (SP) were evaluated as adult feed instead of YH. The life span of adult flies, fecundity, fertility and the other reproductive biology were studied to explore the feasibility of these alternative protein sources for maintaining the performance of medfly colony. The results indicated that the fecundity and fertility were drastically reduced in the case of using the three protein sources as compared with the stander one (YH). While, the percentages of adult survival of male and female flies were relatively similar in the different protein sources and it was clearly higher than those fed on only sugar. The results revealed that when the tested protein sources were supplied with YH (1:1), the reproductive biology parameters were improved, the percentages of egg hatch were insignificantly increased to (84.1, 83.5 and 82.4 %) in YH/PPM, YH/MBM and YH/SP treatments, respectively as compared to YH (80.6%). Also, in the same treatments, the egg laid per female per day were higher (24.5, 23.4 and 22.6 eggs/day), respectively than YH tratment (22.2 eggs/day). Moreover, the pupation were significantly reduced and the larval durations were prolonged in PPM, MBM and SP treatments and not affected in YH/PPM, YH/MBM and YH/SP treatments as compared to YH treatment. The results indicated that the three commercial protein sources were effective dietaries when used with the yeast hydrolysate at the ratio 1:1, this procedure will significantly diminished the mass rearing cost of *C. capitata*.

INTRODUCTION

Mediterranean fruit fly, *Ceratits capitata* Wiedemann (Diptera: Tephritidae) is considered one of the most destructive fruit pests around the world causing great loses of fruit and certain vegetable productions (Thomas *et al.,* 2013). It is commonly known as the medfly and widely spread in whole Egypt country attacking most of the fruit species all the year round (Afia 2007). Sterile insect technique (SIT) is considered one of the important methods for management the Tephritidae insect pests (Calkins and Parker, 2005). The insect produce in large numbers (millions per week) and irradiated with ionizing radiation (gamma or x ray) to induce sexual sterility, and then releasing into the field to mate with the wild types, consequences released sterile insect lead to suppress the population (Hendrichs *et al.*, 2002). Mass rearing of sterile insects is one of the main tools for successful use of sterile insect technique (SIT) programs (Cladera *et al.,* 2014). The artificial diet of both larvae and adult flies could consider as one of the most important components of mass rearing facility and together with labor are constituted the main costs of SIT program (Chapman, 2013 and Nestel *et al.,* 2016). Feeding medfly adults on carbohydrates and protein are necessary dietary components for optimum development and could impact on the effectiveness of sterile male flies. Many studies highlight the complex interactions between the protein and carbohydrate ratio and adult performance (Teal *et al.* 2004 and Colarsurdo *et al.,* 2009). In the mass-reared facilities of tephritidae, protein is usually provided by yeast hydrolysed (Meats *et al*., 2004). However, yeast hydrolyzed as a protein source of adult diets is highly expensive resulting in high production costs. Searching for alternative protein sources to reduce the production costs is required. Meanwhile, the search for cost reduction of artificial diet is constant; the quality of insects should take into consideration where a balance between the costs and insect quality is very important. (Parker, 2005). Strong relationship between adult nutrition and lifespan and reproductive biology (Walker *et al.,* 2005).The effects of adult diet were commonly assessed by determining the number of eggs produced (Leather 1995), whilst its consequences for egg hatch and offspring viability were neglected (Meats *et al.,* 2004 and Geister *et al.,* 2008) and that the latter may contribute significantly to reproduction. The quality control parameters of sterile insects focused mainly on the adult insects in particular survival and mating competitiveness (Calkins & Parker 2005). Moreover, the quality control covers the biological parameters of the mass reared insects such as female fecundity and also egg hatch may indicate problems with mating in the colony, in addition larval development durations can be related to change in courtship behavior (Dowell *et al.,* 2000). Furthermore, sterile male longevity after release and competitiveness with wild males are two factors that can impact on the effectiveness of SIT program. The present study aims to reduce the mass rearing cost of *C. capitata* by using local alternative dietary protein sources for feeding medfly adults to replace the yeast hydrolyzed protein that it is expensive and to evaluate their impact on the quality of insect colony and adult performance.

MATERIALS AND METHODS

1. Rearing Technique of *C. capitata*

Eggs were obtained from a laboratory strain in the medfly laboratories of the Egyptian Atomic Energy Authority reared under of 23±2 ^{o}C and 65-75 % RH conditions. Male and female flies of medfly strain were transferred into the adult rearing cage (40 x 20 x 10 cm). Emerged adult were fed on sugar plus yeast hydrolyzed enzymatic 3:1, respectively (Bradford 1976). Deposited eggs were gathered through the muslin side of the adult rearing cage. Eggs were daily collected in a plastic vial with water placed below the cage, then transferred to a plastic tray containing artificial diet that developed by (Tanaka 1967).

2. Investigated Diets

Three commercial sources of protein, meat bone meal (MBM), plant protein meal (PPM) and soybean protein (SP) were evaluated for feeding medfly adults alongside the standard source of protein yeast hydrolyzate (YH). MBM and PPM were purchased from Arabian Milling and Food Industries Co., Egypt. While, SP was purchased from the

local markets in Egypt and the YH from MP. Biomedical LLC, Ohio, USA. The experiment of life span was conducted to evaluate the impact of different protein sources on the adult medfly survival, the ratio of sugar and different protein sources was kept as the standard ratio of adult diet 3:1 (wt:wt), respectively. In addition, the impact of protein sources on the reproductive biology was carried out with additional treatments where the three dietaries protein sources MBM, PPM and SP were added and mixed with YH by the ratio 1:1 (wt:wt) (Table 1).

Table 1. Ingredients of *Ceratitis capitata* adult diets based on the standard ratio 3:1(wt:wt) of sugar: protein, respectively.

Ingredients	Ratio of the ingredients in different adult diets (wt:wt)							
	YH	PPM	MBM	SP	YH/ PPM	YH/ MBM	YH/ SP	SUGAR
Yeast hydrolyzate	1.0	0.0	0.0	0.0	0.5	0.5	0.5	0.0
Plant protein meal	0.0	1.0	0.0	0.0	0.5	0.0	0.0	0.0
Animal bone meal	0.0	0.0	1.0	0.0	0.0	0.5	0.0	0.0
Soya Protein	0.0	0.0	0.0	1.0	0.0	0.0	0.5	0.0
Sugar	3.0	3.0	3.0	3.0	3.0	3.0	3.0	1.0

3. Biological Assay

Male and female flies were separated and placed into experimental cage (20 x 20 x 20 cm), five replicates were carried out per treatment (50 flies per each), the number of adults that survived for a definite time period by feeding on different treatments were determined. The dead flies were counted daily. For fecundity, 100 pairs of male and female flies were placed on the experimental cage supplied with muslin cloth to allow emerged females to lay their eggs (Five replicates per each treatment). Deposited eggs of different treatments were gathered through the muslin side of the experimental rearing cage, where a plastic trays filled with water was placed. Eggs were daily collected and counted. For fertility, five replicates (200 eggs each) of different dietaries were collected, before being transferred onto the larval diet. Sex days later, the number of hatched eggs was counted and the percentage was calculated. On the other hand, percentages of pupation were determined based on the estimated number of pupae resulted from the number of hatched eggs which calculated in each treatment and the larval duration were estimated in each treatment (Five replicates per each). The newly formed pupae were collected at the same day of formation. The pupae were placed in a plastic Petri dish covered with muslin for aeration, then pupal duration, adult emergence and sex ratio were recorded.

Statistical Analysis

Data were analysed using the analysis of variance (ANOVA) technique and the means were analysed using Duncan's multiple range test (P= 0.05) (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

Bioassay Studies:

The current study of male and feamle flies survival were conducted using one part of different protein substrate YH, PPM, MBM and SP which were added to 3 part of sugar alongside only Sugar as feed. Survivorship could be considering one of the important parameters for evaluating the quality of adult and larval diets (Chang *et al*., 2001). The results of the impact of dietary protein sources on male flies survival were presented in Fig (1), the date showed that in the 10 days, the percentage of adult survival in the male flies fed on the dietary Sugar was lower (79%) than those for protein and sugar as feed (85, 84, 86 and 82%) at the dietaries YH, PPM, MBM and SP, respectively.

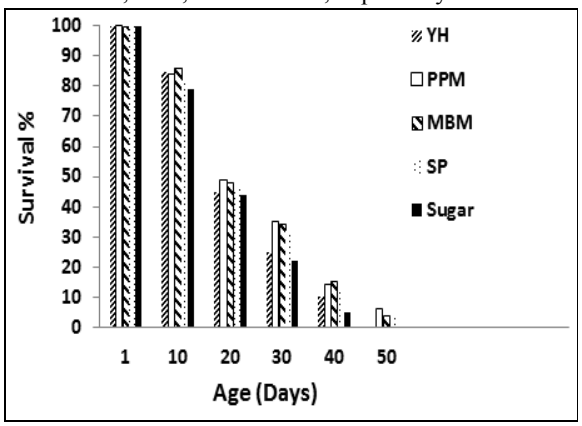

Fig. 1. Survival percentages of male flies of *C. capitata* that fed on different sources of protein and sugar

In the 20 days, the percentage of survival was insignificantly higher (49, 48, and 46%) in PPM, MBM and SP, respectively than (44 and 45%) in the dietaries YH and Sugar, respectively. While, the percentages were reduced significantly (25 and 22%) in YH and Sugar, respectively as compared to (35, 34 and 32%) in the PPM, MBM and SP treatments , respectively in the case of 30 days. The same trend was observed in the 40 days where the percentage of survival in the case of dietary Sugar was the lowest as compared to the protein plus sugar feedings. The data also, demonstrated that the percentages of survival in the dietaries PPM, MBM and SP were higher in 40 and 50 days than the stander dietaryprotein YH . In general, these results of male flies survivalship agree with Kaspi and Yuval (2000) who reported that the adult diet significantly affected on the medfly surviving where the males who had previously fed on protein are dying significantly faster than that fed on only sugar. Also, (Yee 2003) reported that when the female flies of cherry fruit fies, Rhagoletis indifferens was fed on cherries alone, they couldn't survive as long as those fed on sucrose and yeast was supported. Morever, Cangussu & Zucoloto (1997) and Placido-silva *et al*. (2006) showed that the detary proteins in the adult flies of *C. capitata* were increased the adult longevity. Meanwhile Shelly and Kennelly, 2002; Shelly and McInnis, 2003 reported that it is not strict link between the amount of protein ingestion in their adult flies and lifespan. Moreover, Barry *et al* 2007 found that in the prior to relase medfly adults in field cages, it was no difference in the longevity of the male flies which were provided with hydrolyzed yeast and sucrose form those were provided with sucrose and water, while in the post-release flies, the longevity of male flies increased when they were provided with a hydrolyzed yeast and sucrose mixture in comparison to those were provided with only sucrose and water.

The survival of female flies fed on different sources of protein was illustrated in Fig (2), in 10 days, the survival percentage of female flies in dietary YH was significantly higher (88%) than (79, 77, 76 and 78%) that recorded in the dietaries PPM, MBM, SP and Sugar, respectively. The survival percentages were drastically reduced in the 20 days and there were no differences were evident between the tested protein sources. In the 30 days, the percentage of

females survival in the Sugar feeding was signifcantly lower (20%) than (37,38, 39 and 37%) that recorded in the dietaries YH, PPM, MBM and SP, respectively.

While, in the 40 days, the percentage of female flies survival in the dietary YH was significantly lower (15%) than the percentages (22, 25, and 23%) in the other protein treatments PPM, MBM and SP. Similarly, in the 50 and 60 days the survival of female flies fed on PPM, MBM and SP were higher than those fed on YH. The results indicated that the female flies live longer than male flies; also the both male and female flies that fed on sugar only lived shorter than those fed on protein and sugar. Furthermore the alternative protein sources PPM, MBM and SP were effective and improved the adult survival. These results are coincident with those reported by Teal *et al*. (2004) who found that the females of *Anastrepha suspensa* that were provided with a diet of sugar and protein lived longer than those were fed with only sugar. Later, (Muller *et al.* 2009), found that the mortality of female flies is much greater than in males flies in the cause of protein deprivation when they studied the impact of protien on the life expectancy of medfly. Also, Wang *et al*. (2018) found that protein intake enhanced the survival of adult *Bactrocera minax*.

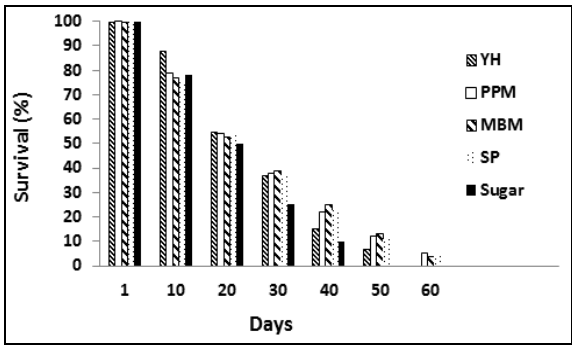

Fig. 2. Survival percentages of female flies of *C. capitata* that fed on different sources of protein and sugar

The date in Fig (3) showed that the average number of eggs laid by female per day in the case of dietary protein sources PPM, MBM and SP was drastically reduced to (8.5, 6.4 and 6.1 eggs/day), respectively as compared to (22.2 eggs/day) in the YH treatment. While the averages were insignificantly increased to (24.5, 23.4 and 22.6 eggs/day) in the YH/PPM, YH/MBM and YH/SP tratments, , respectively as compared to YH tratment . The date also presented that the female flies that fed on only sugar were laied very few eggs (1.2 eggs/day) .

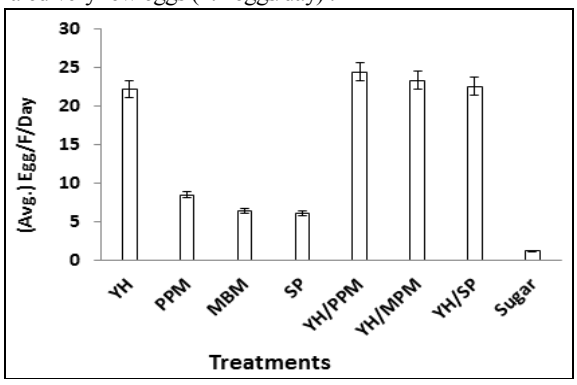

Fig. 3. The average number of eggs laid by female per day of *C. capitata* colony that fed on different sources of protein and sugar

The present results revealed that the fertility was drastically effected by the dietary protein sources that provided to male and female flies where the percentage of egg hatch in the YH treatment was significantly higher (80.6 %) than those recorded to (31.6, 35.3 and 38.5 %), in PPM, MBM and SP treatments, respectively. While, the percentages of egg hatch were insignificantly increased to (84.1, 83.5 and 82.4 %) in YH/PPM, YH/MBM and YH/SP tratments, respectively as compared to YH.

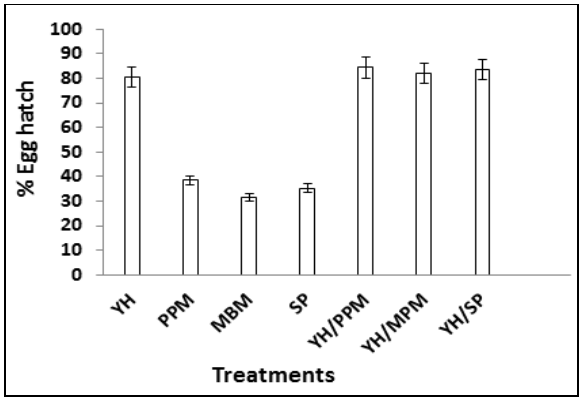

Fig. 4. The percentages of egg hatch of C. capitata colony that fed on different sources of protein and sugar

The results indicated that the different protein sources were drastically reduced the female fecundity as compared to the yeast hydrolyzate, however these dietary proteins were improved the female fecundity when added to the yeast hydrolyzate. The protein quality- based intake is very important for female flies to optimize their reproductive traits. Our results go in line with Papanastasiou *et al.* (2013) and Harwood 2013 who mentioned that when the protein sources were delayed in larval and adult diets, the rate of reproductive senescence decreased in the medfly. Moreover, Jacome *et al.* (1999) reported that *A. serpentina* female required access to protein-enriched diets in order to produce significant number of eggs and increased the egg viability.

Data presented in Table (2) indicated that, the reproductive biology of medfly colony that fed on different sources of protein as flies, the averages of larval duration of PPM, MBM and SP treatments were significantly shorter than YH/PPM, YH/MBM and YH/SP, also, the percentages of pupation in YH/PPM, YH/MBM and YH/SP were signifcantly higher than PPM, MBM and SP treatments. While no signifecantly fifferent were evident in the duration of puae and the adult emergence. The date of the same table presented that the sex ratio of male was higher in PPM, MBM and SP treatments and lower in YH/PPM, YH/MBM and YH/SP treatments than the ratio of female.

These results indicated that the insect fitness parameters (Egg production, hatching eggs, pupation, emergence , larval and pupal production) were clearly reduced in the case of the dataries of adult flies PPM, MBM and SP as compared with the stander diets YH, While, these parameters was improved when the YH was supplied with the same quantity of the three commercial protein sources YH/PPM, YH/MBM and YH/SP.

Our results is agree with Drew *et al.* (2000) who found that the groups of fruit flies fed just sucrose and water have very low fecundity, low fertility, and short lifespan, for

improvement in each of these measures can be achieved through protein-rich supplements. Also, protein intake is an important consideration for release sterile flies in the sterile insect technique (SIT) programmes. It is worth mentioning that, releasing the sterile flies with insufficient protein in nature may be resulted shortened lifespan, diminished sexual performance and delayed sexual maturation. The program could achieve high successful by releasing insect with reliably intake adequate nutrition and also may prerelease provisioning of protein can improve the sterile insect performance, (Kaspi & Yuval, 2000; Hendrichs *et al*. 2002; Barry *et al.* , 2007 and Wang *et al.* 2018).

Table 2. Effect of different of sources of protein as adult diets on certain biological parameters of *C. capitata*

Treatments	(Avg.) Larval duration (days)	(%) Pupation	(Avg.) Pupal duration (days)	(%) Adult emergence	(Avg.) Sex ratio Male : Female	
YH	7.0± 0.5a	78.2a	12.5± 0.6	69.2	0.47	0.53
PPM	7.7± 0.4b	68.2b	12.4± 0.8	68.3	0.56	0.44
MBM	7.9± 0.3b	65.0b	12.2± 1.0	67.9	0.53	0.47
SP	7.8± 0.5b	67.3b	12.3± 0.8	66.8	0.55	0.45
YH/PPM	6.9±0.4a	78.4a	12.6± 0.6	68.2	0.45	0.55
YH/MPM	7.2±0.7a	76.9a	12.4±0 .3	67.3	0.41	0.49
YH/SP	7.1± 0.6a	77.8a	12.5± 1.1	69.0	0.44	0.56

Means designated with the same letter in the same column are not significantly different ($P \geq 0.05$)

Economic Study

The economic visibility study based on the current market prices presented that the YH feed is currently costs approximately 90.00 US$ per kg (MPbio 2018). While the three commercial products PPM, MBM, SP were purchased approximately 1.0, 0.5 and 1.00 US$ /kg, respectively. These products are much lower cost than YH, which represents a huge savings the mass rearing cost of *C. capitata* when used with the YH at the percentage 1:1.

CONCLUSION

This study aims to reduce the cost of mass rearing of medfly by replacing the expensive component of adult diet yeast hydrolysate by commercial protein sources. This research was elaborated the use of three different sources of protein as feed for the adult flies of *C. capitata*. The results indicated that the three commercial protein sources were effective dietaries when used with the yeast hydrolysate at the ratio (1:1) (wt:wt), this procedure will significantly diminished the mass rearing cost of *C. capitata* for improving the programme of SIT.

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تقييم إستخدام بعض المصادر البروتينيه في تربية الحشرات الكامله لذبابة فاكهة البحر المتوسط

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تعد التكلفة للتربية الموسعة إحدى الأدوات الهامة للنجاح في تنفيذ برنامج تقنية اطلاق الحشرات العقيمة . تعتبر الخميره المتحلله انزيميا احد مصادرالبروتين الفعالة في تغذية الحشرات الكاملة لذبابة فاكهة البحر المتوسط والتي تستخدم علي نطاق واسع في مصانع التربية الموسعة . تم في هذه الدراسة تقييم ثلاثة مصادر بروتينية محلية هي مسحوق العظم واللحم ومسحوق بروتين نباتي وبروتين فول الصويا كمصدر للبروتين في غذاء الحشرات الكاملة بدلاً من الخميرة المحللة انزيميا . تم دراسة طول عمر الحشرات الكاملة وكفاءة الاناث في وضع وخصوبة البيض وأيضا الصفات الحياتية الأخرى الخاصة بنمو وتطور ذبابة فاكهة البحر المتوسط . وتهدف الدراسة إلي استكشاف جدوى استخدام هذه المصادر البروتينه البديلة في الحفاظ على أداء المستعمره المرباة من ذباب فاكهة البحر المتوسط . وقد أشارت النتائج إلى أن كفاءة وضع البيض والخصوبة انخفضت بشكل كبير في حالة إستخدام مصادر البروتين الثلاثة مقارنة بالمصدر القياسي (الخميره المحلله انزيميا). في حين كانت النسب المئوية لطول فترة الحياه للذكور والإناث البالغين التي تم تغذيتها علي هذه المصادر أعلى من تلك التي غذيت على السكر فقط . واظهرت النتائج أيضا انه في حال اضافة هذه المصادر البروتينيه إلي الخميره المحلله إنزيميا بنسب متساويه (١:١) قد أدي إلي تحسين في الصفات الحياتية المختلفه ، كما حدث زيادة معنويه في نسب فقس البيض إلى (٨٢.٤ و ٨٤.١ و ٨٣.٥ %) في المعاملات التي تم فيها إضافة الخميره المحلله إنزيميا إلي كلا من مسحوق العظم واللحم ومسحوق بروتين نباتي وبروتين فول الصويا علي الترتيب بالمقارنة مع (٨٠.٦ ٪) في حالة الخميره المحلله انزيميا بدون أي إضافات ، كما ارتفع متوسط وضع البيض يوميا لكل انثي في نفس هذه المعاملات إلي (٢٢.٦ و ٢٣.٤ و ٢٤.٥ بيضة / يوم) ، على الترتيب ، مقارنة بـ (٢٢.٢ بيضة / يوم) للخميره المحلله إنزيميا فقط . بالإضافه إلى إنه حدث إنخفاض واضح في نسب العذاري الناتجه وزيادة في طول فترة نمو اليرقات في معاملات المصادر البروتينيه الثلاثه بالمقارنه بالمعامله القياسية (الخميره المحلله إنزيميا) في حين لم تتاثر هذه النسب في حالة إضافة هذه المصادر إلي الخميره المحلله إنزيميا . وقد أوضحت النتائج أنه يمكن استخدام المصادر البروتينيه الثلاثه كمادة غذائيه مناسبه في حالة ما إذا أضيفت بكميه متساويه مع الخميره المحلله إنزيميا وهذا الإجراء سيؤدي إلي تقليص تكلفة التربية الموسعه لذبابة فاكهة البحر المتوسط إلى حد كبير .