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Some Protein Supplements as Alternatives of Standard Protein Hydrolysate in Mediterranean Fruit Fly, *Ceratitis capitata* (Wied) Flies Dietary Regular Diet

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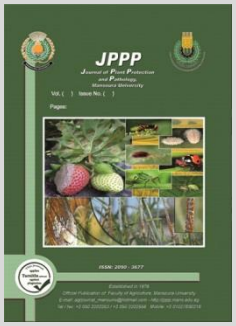

ABSTRACT

Insects require a diet containing a source of energy,a protein source,vitamins and certain mineral salts.The nutrients can influence the quality control parameters of the flies such as mating ability,fecundity,fertility,longevity,pupal weight and adult emergence.Yeast extract(YE)and three chemically composed protein supplements(FA),(FB) and (FC)were tested for their efficiency to provide *Ceratitis capitata* (Wiedemann) flies with protein as alternatives for the protein hydrolysate (PS). The shortest flies maturity period expressed in the first couple performance was related to the protein supplement (FC) 1.26, 1.01 and 1.03 days for parents, first and second generations, respectively. The highest numbers of eggs were laid by females fed on the food supplements (FB) 4.13 and (FC) 4.08 in the parents' generation,(FB)2.81 and(PS)2.79 in the first generation and (PS) 4.29 and (FB) 4.26 in the second generations. The highest and the least egg larval recovery percentages were recorded after flies' feeding on protein supplement (FC) 97.53% and (PS) 91.17% at the parents' generation. The highest and the least pupal recovery percentages were observed after flies feeding on (FC) 99.17% and (PS) 90.71% while flies emergence percentages were (FC) 99.81% and (PS) 90.92%. The least male flies mortality percentages were recorded after flies fed on (YE), 12.06%, 3.20% and 3.37% at the parents, first and second generations, respectively while female flies recorded 8.09%, 6.09% and 2.11 at the same generations. Larval measurements and pupal weight of the produced individuals of the four tested protein supplements at the three generations showed non-significant differences in comparison to the standard protein-feeding source (PS).

Keywords: Mediterranean fruit fly, *Ceratitis capitata*; Protein supplements; Standard protein; Dietary regular diet.

INTRODUCTION

Egypt is one of the most producing horticultural fruit countries. The presence of Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) represents an obstacle in front of fresh fruit export, as it is an important quarantine pest all over the world. An important step to change this situation should be to setup of an area-wide integrated pest management program (IPM) which aim to entitle the Egyptian fruit production regions the status of "low infested or pest free areas". The use of IPM programs has shown high efficacy to reach the population suppression of key pests in different crops. As an example, SIT programs that could also be used in a preventive way to the establishment of the pest in free areas or even to eradicate the species, depending on the region and objective of the program (Hendrichs *et al*., 2005). To respond to the demand of insects for research and area-wide programs, it is necessary to mass rear these insects. Among the factors affecting the insect mass rearing, the flies feeding diets that depends mainly on imported protein hydrolysate (*saccharomyces cerevisiae*) mostly imported from USA constituting high cost. Therefore, the search for cost reduction of flies' diet that keep the balance between cost and insects quality became a necessity. This detrimental effect hinders the fitness and everyday activities of insects (Andersen *et al*., 2010). This is largely due to the fact that insect body tissues require a specific amount and composition of nutrients to function adequately (Boggs, 2009). Depending on dietary sources of nutrients, particularly plant-based diets, may have low nutrient content or unbalanced nutrient composition (Simpson and Raubenheimer, 2000). Flies diet contains protein and carbohydrate sources to provide flies with essential amino acids, vitamins and minerals necessary for development and biological activities. Nutritional quality of the diet significantly affect males' reproductive success and females' fecundity and fertility (Blay and Yuval 1997; Kaspi *et al*., 2002 and Mohamed *et al*., 2016). Males with constant access to protein and carbohydrate, regardless of size, mated more often, had shorter copulations and induced longer refractory periods in females than males fed a low quality diet as adults (Aluja *et al*., 2009). The aim of this work was to evaluate some protein supplements on *C.capitata* flies' diet that could substitute the expensive imported protein hydrolysate without decreasing the quality parameters of mass reared flies.

MATERIALS AND METHODS

Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann):

Insects used in the present work were reared in Horticultural Insect Research Department laboratories, Plant

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Protection Research Institute, Agricultural Research Center. Matured flies (aged 6 days) captivated in adults' cage (40×40×50 cm) and left to lay eggs. Eggs collected and planted on larval artificial medium (Tanaka *et al*., 1969). Pupae collected one day before flies' emergence and isolated individually for initiation of parents' generation test.

Flies protein feeding sources:

Yeast protein hydrolysate and the other tested four protein supplements feeding sources composition were presented in Table (1). The standard yeast protein hydrolysate (PS) was obtained from Santa Cruz Biotechnology Inc., USA; the yeast extract powder (YE) was obtained from Nice Chemicals Pvt. Ltd., India and the new protein supplements BioGainers (FA), L-Emental (FB) and Magic formula (FC) were obtained from Prime Pharmaceutical Co., Egypt.

Table 1. Protein supplements components of flies' diet

Constituents	Natural source flies' diet		Chemical composed flies' diet		
	PS *Saccharomyces Cerevisiae* β-glucan in %	YE Yeast extract Total nitrogen %	FA	FB	FC
			Value in g/100g		
Protein	60	30	22.27	15.00	26.70
Fat	-	0.42	0.94	12.30	1.70
Carbohydrate	-	-	64.8	57.30	16.70
Fiber	-	12.18	0.65	-	10.00
Amino acids	Value %/100g	Value %/100g	Value/mg/ml	Value in µg/ml	
Aspartic acid	6.41	6.41	1017.23	0.07	
Serine	3.08	2.72	566.6	0.40	
Threonine	2.88	2.90	583.69	-	
Glutamic acid	8.78	11.05	3225.35	-	
Proline	3.08	2.34	1290.56	1.40	
Glycine	3.21	3.03	198.612	0.70	
Alanine	4.68	5.78	448.09	1.30	
Cysteine	-	0.96	32.50	0.05	
Valine	3.53	4.02	915.21	2.00	
Methionine	1.09	1.18	56.20	0.20	
Leucine	4.36	4.30	728.09	2.70	
Isoleucine	2.88	3.14	1426.50	2.20	
Phenylalanine	2.69	2.53	244.21	0.00	
Tryptophan	0.64	0.47	-	0.14	
Tyrosine	2.24	2.27	532.47	0.08	
Lysine	4.81	5.04	304.74	1.30	
Lysine acetate	0.00	0.00	-	1.82	
Arginine	4.04	5.02	299.43	1.30	
Histidine	1.60	1.30	-	-	
Vitamins			Value in µg/mg	Value in µg/mg	Value in µg/mg
Cobalamin B_{12}	-	-	-	-	17.60
Folic acid	-	-	-	-	1076
Riboflavin	-	-	2.8	-	32.34
Thiamine	-	-	272	-	39.33
Nicotinic acid	-	-	0.538	-	15.34
Pyridoxine B_6	-	-	-	-	19.60
Vitamin A	-	-	1.15	-	-
Minerals			Value in µg/g	Value in µg/g	
Selenium	-	-	0.0334	-	-
Iron	-	-	6.2	-	33.30
Potassium	-	-	7762	-	88000
Sodium	-	0.67	4768	-	125000
Cupper	-	-	8.79	-	-
Manganese	-	-	1.24	-	-
Magnesium	-	-	1158	-	-
Calcium	-	-	3151	-	6000
Zinc	-	-	35	-	-

Effect of food supplements on flies' biological activities:

Five cages contained healthy new emerged immature 25 pairs of flies each were set up for the study. Each flies cage was provided with one of the protein feeding sources separately and sugar in the ratio of 1:3, respectively in addition of water source. Each feeding protein source treatment was replicated four times. The feeding sources of all treatment were replaced weekly along the test period to avoid food deficiency. Another cage contained flies of the same age fed on sugar and water only was ran in parallel as a compensation source for dead flies in the treatments. The first five treatments were considered as the Parents' generation. New produced individuals from the parents' generation were selected for the tests of the first generation biological activities and the same steps were repeated for the study of the second generation. All treatment were kept under laboratory conditions (21 ±3ºC and 57~75% RH).

Maturity:

Five plastic transparent cages (15×10×5 cm) containing ten pairs of new emerged flies (aged 2 hours) were provided with the protein feeding sources separately. A day after flies' emergence and feeding, the cages were observed along the day for couples and mating status. The

first couple for each treatment was recorded by time from emergence and setting up the cages. The first eggs laid by females fed on the protein sources were counted and recorded by time after coupling. Each treatment was replicated five times. Egg, larval and pupal durations were calculated as the number of days necessary for transformation from a stage to another.

Fecundity and hatchability:

All laid eggs from each treatment were collected and counted daily for 30 days. A hundred laid eggs sample randomly collected from each treatment were transferred using fine brush and placed on moistened black soft tissue in Petri dishes (9 cm). The egg samples were checked daily for four days for larval hatching. Larvae were counted and removed and the unhatched eggs were counted. Each sample treatment was replicated five times and the eggs samples continued until the end of the test.

Larval recovery:

A hundred hatched larvae were placed in cups (15×10×3cm) contained the regular larval medium (Tanaka *et al*., 1969) (30 g) and left to complete the three larval instars. The cups were placed in containers with fine sand and covered by muslin cloth and rubber bands. After six days, the containers were checked for larval popping. Full-grown larvae were counted and allowed to pupate in fine sand cups. The larval duration was considered from egg hatching until full-grown larvae popping form the larval medium. Each treatment was replicated five times. The mentioned steps ran in all the tested generations.

Pupal recovery flies emergence and sex ratio:

A hundred pupae collected from each treatment were isolated individually in perforated Eppendorf tubes after seven days from larval popping. Number of emerged males and females and unemerged pupae were recorded. No malformations were observed in pupae or in flies. Sex ratio was calculated as the number of emerged females/number of emerged flies. Each treatment was replicated five times. The same steps were repeated in all tested generations.

Flies' mortality:

Dead flies in all the treatments of the tested generations were recorded by time along the test period and replaced with other individuals at the same age from the compensation cage.

Larval and pupal measurements:

Ten randomly selected larvae from each food supplement community were measured for length and width using micrometric lens binocular. A hundred pupae produced from each treatment were collected randomly and weighed using digital balance.

Statistical analysis:

Numbers of laid eggs were transformed $\log_{x+1}$ then subjected for analysis of variance ANOVA using MaxStat Pro 3.6 software program (2015). Average of fecundity, fertility and flies' sex emergence among treatments were assessed using paired "*t*"-test.

RESULTS AND DISCUSSION

Tephritids flies nutrition studies have been conducted throughout the last decades in considerable advances about diet formulations with nutritional analysis of adult flies. Insects require a diet containing a source of energy, a protein source, vitamins and certain mineral salts. The nutrients can influence the quality control parameters of the flies such as mating ability, fecundity, fertility, longevity, pupal weight and adult emergence.

Maturity:

In parents' generation, early coupling and mating activities were observed in flies' treatments that fed on protein supplements FC, FA, FB while flies fed on YE and PS showed that, normal activities in the three tested generations. With respect to *C.capitata*, males need protein source in dietary diet to produce pheromone, accessory gland secretions and renewal of sperm supplies (Yuval *et al*., 2007). The chemically composed adult flies diet FA, FB, FC contained amino acids, vitamins, and mineral salts while the standard protein hydrolysate PS and the yeast extract YE contains amino acids only. Insects cannot synthesize vitamin B that function as coenzymes in various required enzymatic reactions (Douglas, 2017). Protein enriched diet used for feeding the Chinese citrus fruit fly, *Bactrocera minax* supported with vitamin B improved and accelerated females' ovarian maturation, mating activities and early egg production (Wang *et al*., 2018). Apart from amino acids, other substances as vitamin B complex and minerals incorporated in flies' diet play an important catalytic role in physiology of many insect species (Zhou *et al*., 2016). Hanai and Esashi (2012) found that rats that fed on diets contained folic acid, niacin; riboflavin and thiamine showed acceleration in their gonadal maturity. These results are supporting our findings and may explain the early sexual maturity and activity of males and females fed on chemically composed protein supplements FC and FA that composed of essential amino acids with vitamin B (riboflavin and niacin) and minerals (Table 2). Absence of minerals in dietary flies intake is slowing *B. tryoni* males maturity (Fanson and Taylor, 2012).

Fecundity, hatchability and larval recovery:

There was no significant difference in produced egg incubation periods, larval and pupal durations at all the protein supplements treatments (Table 2). Protein feeding is a main source for flies to produce eggs. Harwood *et al*., (2013) demonstrated that access of *C.capitata* flies at eclosion led to higher ability of egg production and laying. Data presented in Table (3) cleared that the highest number of eggs was laid by females fed on the protein source FB followed by FC, PS, FA and YE, respectively. There was a significant difference ($P<0.05$) among the total numbers of laid eggs of flies feeding on different sources of proteins, along 30 days ($F=25.99$, $P<0.0001$). The T-test and "t" values reflected significant difference between mean numbers of eggs laid by females fed on PS and YE ($t= 5.14$, $P<0.0001$), PS and FA ($t=6.22$, $P<0.0001$), PS and FB ($t=3.04$, $P=0.0050$) and showed non-significant difference between PS and FC ($t=0.71$, $P=0.4948$). Depending on the source of protein feeding, the mean number of eggs laid by females at first ten days assured that, significance ($F=22.59$, $P<0.0001$). The mean number of eggs laid by females fed on PS showed significance with those of YE ($t= 7.53$, $P<0.0001$), FA ($t=5.05$, $P=0.0007$), FC ($t=5.03$, $P=0.0005$) but showed non-significant difference when compared to FB ($t=0.36$, $P=0.7246$). The mean number of eggs laid by females fed on YE protein

source reflected significance when compared to those of females fed on FA (t=6.18, p=0.0002) and showed high significance as compared to those fed on FB (t=12.98, P<0.0001) and FC (t= 10.43, P<0.0001). The mean number of eggs laid by females fed on FA showed high significance when compared to those of FB (t=10.15, P<0.0001) and FC (t=8.53, P<0.0001). The mean number of eggs laid by females fed on FB and FC were slightly significant (t=4.79, P=0.0010). The second ten days represented the peak of egg laying for all protein treatments (Fig.1). In first generation 30 days test, eggs laid by the females that fed on the standard protein PS increased significantly (P<0.001) than those fed on the protein alternatives YE (F=3.99, P=0.0005) and FA (F=10.013, P<0.0001) (Table 3). The protein alternative FB increased egg production more than the standard protein PS and showed non-significant differences (P>0.05) (F=2.35, P=0.0296) while PS increased eggs production more than FC non-significantly (F=1.24, P=0.5733). Eggs laid by females fed on the protein alternative YE increased and presented low significance (P<0.05) when compared to FA (F=2.65, P=0.0131). The protein alternatives FB and FC increased eggs production than FA significantly (P<0.05) (F=491.78, P<0.0001). The protein alternative FB increased eggs production more than FC cleared that, no significance (P<0.05) (F=0.54, P=0.1032). In the first ten test days, the standard protein had the superiority and increased females eggs production more than the protein alternatives (Fig.1). PS increased eggs production significantly (P<0.001) when compared to the protein alternative YE (F=7.33, P<0.0001) and FA (F=14.93, P<0.0001). PS increased egg production in non-significantly (P>0.05) when compared to FB (F=1.62, P=0.1481) but it was significant (P<0.001) when compared to FC (F=5.95, P=0.0002). The protein alternative FB increased eggs production significantly (P<0.05) more than FC (F=3.42, P=0.0077). In the second ten test days, the standard protein PS continued its notability and increased females' eggs production more than the other protein alternatives (Fig. 1). Females fed on PS increased egg production significantly (P<0.05) when compared to the protein alternative YE (F=4.53, P=0.0014) and FA (F=10.98, P<0.0001). Females eggs production increase when fed on PS non-significantly (P>0.05) when compared to those fed on FB (0.84, P=0.4237) and FC (F=1.67, P=0.1287). In the third ten test days, the protein alternative FB replaced the standard protein PS and increased females eggs production but non-significantly (P>0.05) (F=0.345, P=0.0401). Moreover, eggs laid by females fed on PS were not significantly different when compared to those of YE (F=1.74, P=0.1158) while it showed significance (P<0.001) when compared to eggs laid by females fed on the protein alternative FA (F=11.68, P<0.0001). Females fed on the standard protein PS showed slight increase in eggs laying as compared to females fed on the protein alternative FC presenting non-significant difference (P>0.05) (F=1.23, P=0.7571). The protein alternatives FB and FC influenced females eggs laying non-significantly (P>0.05) (F=3.64, P=0.0710) (Fig.1). Females' eggs laying fluctuated along the whole 30 days test and differed depending on the protein feeding sources. Females fed on the standard protein PS showed significance (P<0.05) in eggs production at the three ten days periods (F=22.47, P<0.0001). Eggs laying increased with low significance (P<0.05) at the first and second ten days (F=4.55, P=0.0253) but decreased significantly (P>0.05) at the last ten days (F=8.96, P<0.0001). Females fed on the protein alternative YE showed more or less stability in egg production along the test period presenting non-significance (F=3.03, P=0.0652). Females fed on the protein alternative FA showed significance (P<0.05) in eggs laying (F=5.94, P=0.0073) at the whole test period but low significance on comparing their activity at the first and second ten days (F=2.72, P=0.0234) and at the last ten days (F=3.23, P=0.0102). Females fed on the protein alternative FB approached the eggs laid numbers of females fed on the standard protein PS especially at first and second ten days and showed increase at the last ten days. Eggs produced from females fed on FB fluctuated significantly (P<0.001) along the whole test period (F=16.48, P<0.0001). The same was repeated with females fed on the protein alternative FC that increased and decreased significantly (P<0.001) along the test period (F=29.25, P<0.0001). Summing up the data in Fig. (1), it is cleared that the peak of females' eggs production was at the second ten days of the whole test period regardless the protein-feeding source with superiority to the standard protein PS and the alternative FB. The second-generation test, females fed on the standard protein laid the highest eggs number followed by females fed on the protein alternative FB and FC, YE and FA, respectively (Table 3). Following up the second progeny, there was an increase in laid eggs number by all females fed on the standard and alternative proteins more than the parents' generation and the first progeny. This variation in eggs production may be due to photoperiod that was 12 D :12 L while it was in the parents and first generation 14 D:10 L.. Females fed on the standard protein kept its superiority and produced the highest eggs number while the lowest was related to the protein alternative FA (Table 3). Eggs laid by females fed on the standard protein PS increased significantly (P<0.05) when compared with those laid by females fed on the protein alternative YE (F=9.20, P=0.0008), FA (F=35.29, P<0.0001). It showed non-significant difference with those of FB (F=1.98, P=0.5501) and increased in low significance ((P<0.01) with FC (F=4.77, P=0.0089) but. Females fed on the protein YE showed significant increase (P<0.001) as compared to those fed on the alternative FA (F=21.98, P<0.0001) but there was no significant difference (P>0.05) with those fed on FB (F=1.73, P+0.6121) and FC (F+ 0.18, P=0.1271). It was notable that females fed on the standard protein achieved its highest egg laying activity at the first 10 days of the test while those fed on YE, FB and FC protein alternatives showed its peak activity during the second 10 days. Females fed on the protein alternative FA presented their highest eggs laying activity at the last ten days (Fig.1). Manrakhan and Lux (2006) found that rich protein diet affected females' oviposition frequency higher more than poor protein diets. Chang (2009b) found the yeast type has a direct effect on egg production and laying. Amino acids are essential for ovarian maturation and egg production but vitamins and minerals play a key role in increasing insect production. Zou *et al*., (2017) reported the effect of riboflavin in increasing egg production and larval

recovery of *Drosophila melanogaster*. Riboflavin is one of the components of the protein supplements; FA, FB and FC that provide a reason for the higher egg production more than the standard protein hydrolysate and the yeast extract powders. Percentages of hatched larvae were calculated on 100 eggs collected daily from each protein supplement treatment for 30 successive days in the three tested generations (Table 3). Data in Table (3) showed significance among number of hatched larvae produced from eggs laid by females fed on the tested protein sources (F=4.32, P=0.0025) (*P*<0.05). Percentages of hatched larvae produced from flies fed on PS (60% total protein) showed significance (*P*<0.05) more than those fed on YE (30% total protein) (F=2.10, P=0.0091). Hatchability increased by using FC, FB and FA protein sources, respectively when compared to PS and reflected high significance with FC (F=46.91, P<0.0001) , FB (F=34.63, P<0.0001) and significance with FA (F=2.96, P=0.0047). In addition, eggs hatchability decreased significantly on the use of YE as compared with FA (F=8.01, P<0.0001), FB (F= 83.85, P<0.0001) and FC (F=127.77, P<0.0001). FB and FC increased eggs hatchability and showed insignificant difference within treatments (F=0.825, P=0.4417). Eggs hatchability due to flies fed on PS increased insignificantly along the 30 test day (F=0.641, P=0.5044) and YE (F=3.26, P=0.0618). Moreover, hatchability due to flies fed on the chemically composed proteins increased and decreased insignificantly along the 30 test days, FA (F=0.81, P=0.4615), FB (F= 1.55, P=0.2390) and FC (F=2.83, P=0.5931). From the previous results, it was obvious that eggs hatchability increased when flies fed on the chemically composed protein supplements. We suppose that the quality of sperm is affected by the daily intake adult diet protein (essential and non-essential amino acids, vitamins and minerals). Medfly females can conserve sperm from different mates to fertilize their eggs. From an evolutionary point of view, the storage of sperm in a stratified pattern by medfly females may initially favour the fresher ejaculate from the last male. However, as the second male's sperm gradually depleted, the sperm from the first male becomes increasingly available for fertilization. Samuel *et al*., (2013) studied the role of some biogenic amines on the morphological features of males produced sperms, sperm transfer and females oviduct of the stable fly, *Stomoxys calcitrans* (L.). They found that the presence of some biogenic amines affect the nervous system of male testes and suggested these biogenic amine may also be involved in stable fly mating behavior by influencing sperm transfer, eggs fertilization and finally female oviposition. As all treatments were kept under the same circumstances, we can deduce that protein supplements contained vitamins and minerals (FA, FB and FC) affected the males' sperms features, sperm transfer and provided an advanced transcriptome via repeated mates and affected the embryos and increased hatchability.

Table 2. Effect of protein supplements on flies' maturity parameters in days ± SE

Maturity parameters	Protein supplements				
	PS	YE	FA	FB	FC
			Parents' generation		
First couple after flies' emergence	2.25±0.25	2.51±0.29	1.53±0.29	1.75±0.26	1.26±0.27
			F=15.75, P<0.0001, MS=0.046		
First egg laid after coupling	2.53±0.28	2.01±0.09	1.54±0.89	1.25±0.31	1.23±0.35
			F=14.70, P<0.0001, MS=0.135		
Egg incubation	3.01±0.12	2.93±0.13	2.80±0.11	2.92±0.23	3.02±0.02
			F=1.034, P=0.4202, MS=0.030		
Larval duration	8.01±0.41	8.25±0.33	8.30±0.30	8.75±0.43	8.32±0.34
			F=1.46 (ns), P=0.2621, MS=0.440		
Pupal duration	9.21±0.31	9.75±0.61	8.82±0.30	9.31±0.34	9.01±0.03
			F=7.11, P=0.0017, MS=0.090		
			First generation		
First couple after flies' emergence	2.41±0.21	2.21±0.20	1.43±0.13	1.23±0.16	1.01±0.04
			F=39.16, P<0.0001, MS=0.046		
First egg laid after coupling	2.28±0.17	2.29±0.23	1.28±0.31	1.19±0.10	1.09±0.11
			F=23.35, P<0.0001, MS=0.135		
Egg incubation	3.01±0.09	2.94±0.04	2.82±0.12	3.02±0.12	3.03±0.02
			F=1.19, P=0.3538, MS=0.015		
Larval duration	7.44±0.43	7.69±0.27	7.48±0.25	7.23±0.12	7.13±0.16
			F=2.26 (ns), P=0.2621, MS=0.084		
Pupal duration	9.16±0.13	9.22±0.20	9.20±0.11	9.04±0.04	9.02±0.02
			F=0.645 (ns), P=0.6356, MS=o.058		
			Second generation		
First couple after flies' emergence	2.21±0.23	2.23±0.27	1.27±0.15	1.21±0.14	1.03±0.10
			F=29.93, P<0.0001, MS=0.064		
First egg laid after coupling	2.14±0.14	2.24±0.21	1.41±0.23	1.04±0.07	1.01±0.10
			F=19.24, P<0.0001, MS=0.097		
Egg incubation	3.02±0.04	3.01±0.03	3.01±0.01	3.08±0.02	3.01±0.04
			F=0.271, P=0.8924, MS=0.002		
Larval duration	7.30±0.21	7.33±0.18	7.40±0.23	7.10±0.12	7.12±0.19
			F=0.449 (ns), P=0.7714, MS=0.045		
Pupal duration	9.18±0.14	9.20±0.21	9.19±0.13	9.12±0.11	9.02±0.02
			F=0.754 (ns), P=0.5699, MS=0.068		

MS=Mean Squares

Table 3. Mean different biological parameters of *C.capitata* in parents and two consecutive generations ±SE under five flies' food supplements

Biological Parameter	PS	YE	FA	FB	FC
		Parents' generation			
Laid eggs/female	3.98±0.11	3.66±0.19	3.68±0.41	4.13±0.15	4.08±0.12
		F=25.99, P<0.0001			
Egg-larval recovery %	91.45 ±3.04	83.70 ±5.01	95.86 ±1.77	96.30 ±0.52	97.53 ±0.44
		F=4.32, P=0.0025			
Pupal recovery %	90.71±2.71	90.61±2.69	98.43±1.74	97.85±3.38	99.17±2.77
		F=13.18, P<0.0001			
Flies emergence %	90.92±2.32	87.23±4.31	99.32±1.80	98.71±0.62	99.81±0.55
		F=21.87, P<0.0001			
		First generation			
Laid eggs/female	2.79±0.024	2.65±0.015	2.32±0.043	2.81±0.018	2.72±0.025
		F=52.94, P<0.0001			
Egg-larval recovery %	98.63 ±0.34	98.40 ±5.01	96.83 ±1.77	98.83 ±0.52	98.03 ±0.44
		F=1.71 (ns) , P=0.1502			
Pupal recovery %	99.29±0.37	99.12±0.35	98.31±1.27	98.64±0.35	98.39±0.37
		F=2.70, P= 0.0328			
Flies emergence %	98.34±0.59	99.35±0.37	95.24±3.18	99.21±0.35	99.18±0.37
		F=3.46, P=0.0109			
		Second generation			
Laid eggs/female	4.29±4.04	4.24±7.09	3.88±2.29	4.26±5.13	4.25±5.12
		F=49.87, P<0.0001			
Egg-larval recovery %	98.37 ±0.30	97.46 ±0.41	94.64 ±1.04	99.86 ±0.52	97.17 ±0.38
		F=1.48 (ns) , P=0.2102			
Pupal recovery %	99.49±0.27	99.14±0.39	98.25±1.02	99.27±0.32	98.26±0.30
		F=3.88 , P= 0.0050			
Flies emergence %	99.18±0.37	99.10±0.58	97.97±1.19	99.56±0.35	99.20±3.05
		F=2.22, P=0.0695			

Numbers of eggs were transformed by log $_{x+1}$

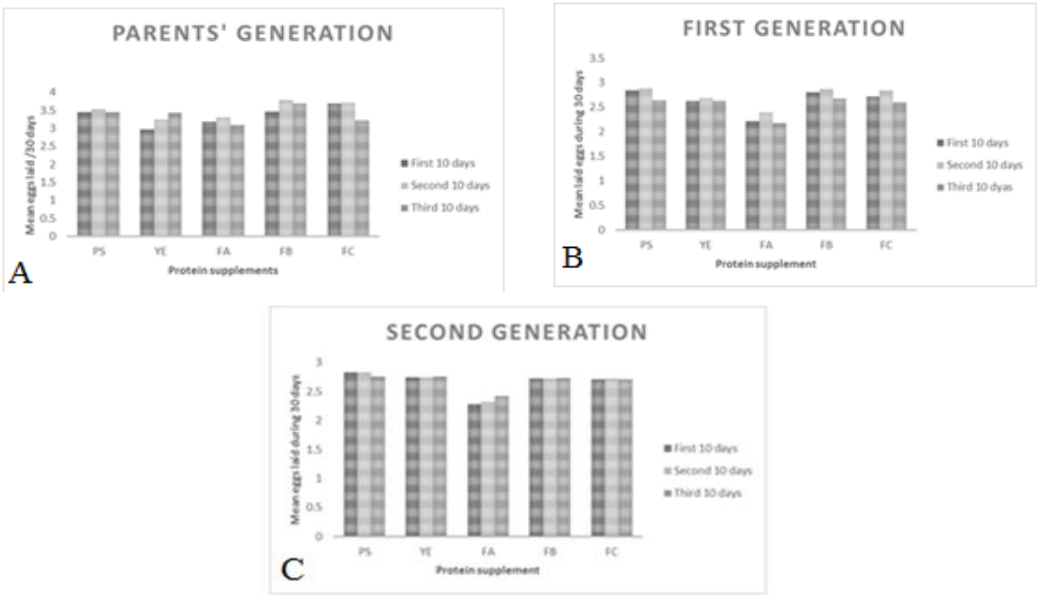


Fig.1. Effect of protein supplements on eggs laid by females during 30 days test.
Laid eggs number were transformed by log $_{x+1}$.

Pupal recovery flies emergence and sex ratio:

Data listed in Table (3) revealed that, the chemically composed protein treatments used to feed flies resulted in high pupal recovery more than the standard feeding protein. The highest pupal recovery percentage (99.17%) was recorded on using the protein alternative source FC while the least was resulted on using YE (90.61%). Pupal recovery percentages showed significant difference (P<0.05) when compared the treatments (F=13.18, P<0.0001). PS and YE reflected low significance (P>0.05) in pupal recovery (F=0.36, P=0.0081). Pupal recovery increased significantly (P<0.05) on using the protein source alternative FA more than that of PS (F=8.72, P=0.0198). Also, pupal recovery reflected significant difference ((P<0.05) on using the protein alternative FB more than PS (F=20.72, P=0.0001) and increased significantly (P<0.01) in case of using the protein alternative FC (F=31.22, P<0.0001). We suppose that flies nutrition may play a key role in eggs quality and may be embryo genetics. Flies emergence percentages produced by parents' generation showed a similar pattern and seem to be influenced by using different protein feeding sources (Fig.2). The highest flies' emergence percentage accompanied with the use of the protein FC (99.75%) while the least was observed on the use of the alternative YE

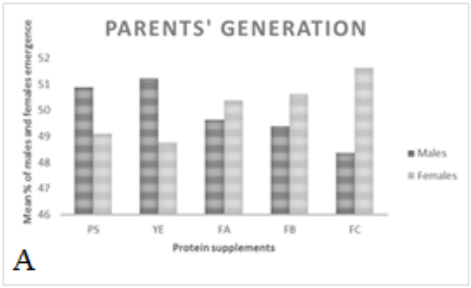
(87.23%). Mean percentages of flies emergence showed significant differences (P>0.05) when all treatments compared (F=21.87, P<0.0001). Treatments of PS and YE showed low significance (P<0.05) (F=0.49, P=0.0073) while PS and FA reflected significant difference (14.80, P<0.0001). Significant difference (P<0.05) floated on comparing flies emergence resulted from using PS and FB (F=17.72, P<0.0001) and PS with FC (F=29.72, P<0.0001). Comparison among flies emergence resulted of using protein FA, FB and FC reflected non-significant difference (P>0.05) (F=1.54, P=0.2193). Male flies emergence percentages obtained cleared significance (P>0.05) among treatments (F=13.24, P<0.0001). The highest male flies' emergence percentage was recorded on using YE (51.23%) while the least accompanied FC (48.36%). PS and YE protein sources resulted in low significance (P<0.05) in male flies emergence (F=2.10, P=0.0195) and PS compared to FA (F=1.71, P=0.1533). Male flies emergence percentages resulted in using FB decreased significantly (P<0.05) when compared to PS (F=5.45, P<0.0001) and with FC (12.08, P<0.0001). Male flies emergence percentages were not significantly different (P>0.05) on using the protein alternatives FA, FB and FC (F=0.08, P=0.9264). Female flies emergence percentages showed different pattern and resulted in significance (P>0.05) among all protein treatments (F=28.004, P<0.0001). The highest and the least female flies' emergence percentages was recorded on the use of the protein alternatives FC (51.64%) and YE (48.77%), respectively. On comparing the females flies emergence percentages resulted from the use of the standard protein PS and the alternative YE, it increased non-significantly (P>0.05) (F=1.67, P=0.0035). The protein alternative FA and FB increased the female flies emergence significantly (P<0.001) than the standard protein PS (F=8.07, P<0.0001) and (F=8.09, P<0.0001), respectively. In addition, the protein alternative FC increased the female flies emergence percentage significantly (P<0.001) as compared to the standard protein PS (F=10.07, P<0.0001). There was no significance (P>0.05) among female flies emergence percentages resulted in the use of the protein alternatives FA, FB and FC (F=4.02, P=0.0214). The accumulation of sperm from different males will increase the overall genetic variability of the offspring and will ultimately affect the effective population size. From an applicative point of view, the dynamics of sperm storage and their temporal use by a female may have an impact on the sex ratio (Scolari et al., 2014).

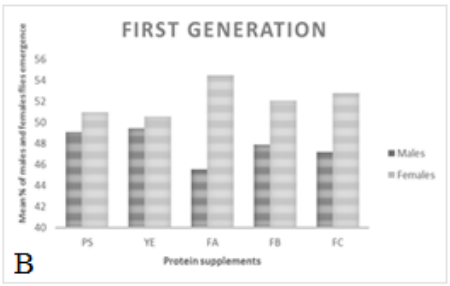
Flies Mortality:

Mortality recorded of flies fed on different protein sources showed non-significant differences. The highest flies' mortality percentages recorded along the 30 days test was due to feeding on PS followed by FC, FB, FA and YE, respectively (Table 4).

Table 4. Flies mortality after feeding on protein food supplements

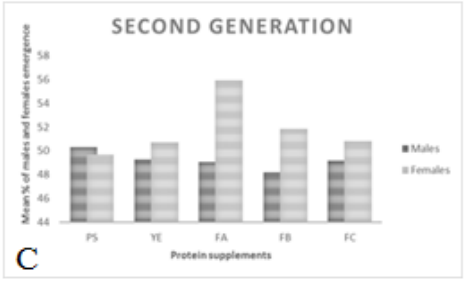
Mean mortality % of flies ± SE									
PS		**YE**		**FA**		**FB**		**FC**	
Parents' generation									
♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
24.54±0.11	24.12±0.10	12.64±0.07	8.09±0.06	11.03±0.07	15.87±0.80	23.76±0.55	31.83±0.13	19.91±0.09	22.86±0.12
First generation									
10.40±0.34	5.62±0.10	3.20±0.13	6.09±0.17	4.03±0.09	4.87±0.14	3.21±0.06	8.03±0.15	4.01±0.08	5.62±0.12
Second generation									
3.95±0.05	5.31±0.11	3.37±0.13	2.11±0.17	2.93±0.09	2.07±0.14	3.35±0.06	2.79±0.15	3.81±0.08	4.71±0.12





Protein supplement	PS	YE	FA	FB	FC
Sex ratio	0.49	0. 49	0.50	0.51	0.52

Protein supplement	PS	YE	FA	FB	FC
Sex ratio	0.51	0. 51	0.58	0.52	0.53



Protein supplement	PS	YE	FA	FB	FC
Sex ratio	0.49	0.51	0.53	0.52	0.52

Fig.2. Effect of protein supplements on percentages of males and females emergence and sex ratio.

There was no significance between mortality recorded for males and females. Male and female flies fed on PS reflected non-significant difference (P<0.05) in mean percentage of mortalities (F=1.00, P=1.00). The same was observed for males and females fed on YE (F=1.12, P=0.6027), FA (F=1.36, P=0.4174), FB (F=0.64, P=0.2267) and FC (F=0.63, P=0.2322). There was no significance (P>0.05) among mean percentages of male flies mortality in all treatments (F=0.32, P=0.8668) and female flies mortality (F=0.59, P=0.6724). There were no mortalities recorded for male and female flies fed on PS, YE, FA and FB on the first ten days but flies fed on FC recorded one male and one female mortality on the ninth day. There were no male or female mortalities recorded on the next ten days when fed on YE , FB and FC protein source alternatives while flies fed on PS recorded one male and two females' mortalities on the 12th and 20th days, respectively. In addition, flies fed on FA recorded two males mortality on the 14th day while one female mortality was recorded on the 12th, 14th and 17th days, respectively. All treatments recorded both sexes flies mortality on the last ten days. Flies fed on PS the standard protein showed two males and one female mortalities on the 25th and other two males and one female on the 26thday while one males and two females died on the 30th day. Flies fed on YE showed the least mortalities among all protein feeding sources. Flies started to record two males and two females' mortality on the 26th day and one male on the 29th day. Flies fed on FA recorded one male and one female on the 23th day. FB treatment showed one male mortality on the 22th and 24th days and two males on the 26th day followed by one male on the 27th day.

Flies fed on FC recorded two males and two females' mortality on the 22nd day. *C.capitata* flies had access to protein source feeding immediately after eclosion, increased life expectancies (Harwood *et al*., 2013). Males and females fed on the FC, FB and FA showed longer life span more than those fed on PS. Protein intake is not the only reason for flies' longevity. Riboflavin was found to increase life span of *D. melanogaster* via anti-oxidative stress pathways involving enhancing the activity of SOD1 (superoxide dismutase) and CAT (catalase) and inhibiting LF (Lipofuscin) accumulation (Zou *et al*., 2017).Micronutrients as vitamins and minerals play a significant role in prolonging flies life span. Fanson and Taylor, (2012) proved the *B. tryoni* flies that fed on protein enriched with vitamins and minerals lived longer than those fed on only protein. These findings may explain the longer life span of *C.capitata* flies that fed on the chemically composed protein supplements more than the standard protein. The hatched larvae of all treatments were reared on the standard larval medium described by Tanaka *et al*., (1969). Pupal recovery represents an important value in Mediterranean fruit fly, *C.capitata* mass-rearing programs.

Larval and pupal measurements:

The data in Table (5) described the measurements of larvae and pupae produced from flies fed on all the tested proteins. The longest full-grown larvae accompanied the feeding of flies on the protein alternative FC and the shortest was related to FA reflecting significance (F=19.00, P=0.0145). All protein treatments cleared that, significance (P<0.05) in larval length (F=3.69, P=0.0208). The width of larvae at all treatments showed significance (P<0.05) (F=3.93, P=0.0164). Larvae produced from flies fed on the standard protein PS showed the highest width where the lowest was related to the protein alternative FC and reflected significance (P<0.05) (F=1.50, P=0.7040). Mean pupal weight of all treatments showed significance (P<0.05) (F=27.24, P<0.0001). Mean pupal weight of pupae produced from flies fed on the protein alternative FB increased non-significantly (P>0.05) than the other treatments (F=1.00, P=1.00).

Table 5. Measurements of larvae and weights of pupae produced from flies fed on food supplements at parents and successive generations

PS	YE	FA	FB	FC
		Mean length/larva in cm ± SE		
0.404±0.005	0.412±0.002	0.388±0.011	0.406±0.002	0.416±0.002
		F=3.96, P=0.0208		
		Mean width/larva in cm ± SE		
0.114±0.003	0.106±0.002	0.104±0.003	0.104±0.003	0.102±0.001
		F=3.93, P=0.0164		
		Mean weight/pupa in gram ± SE		
0.0130±0.001	0.0130±0.001	0.0130±0.002	0.0150±0.002	0.0129±0.001
		F=27.24, P<0.0001		

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دراسة عن بعض مكملات البروتين كبدائل للبروتين المُحَلَل الأساسى لحشرة ذبابة فاكهة البحر المتوسط *Ceratitis capitata* كنظام غذائى منتظم

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معهد بحوث وقاية النباتات - 7 ش نادى الصيد-الدقى - جيزة

تحتاج الحشرات لنظام غذائى منتظم يحتوى على مصدر للطاقة و البروتين والفيتامينات وبعض الأملاح المعدنية. وتؤثر المغذيات على جودة وأداء الحشرات مثل النضج الجنسى والقدرة على التزاوج وعدد البيض الذى تضعه الإناث وخصوبته وكذلك عمر الحشرة ووزن العذارى ونسبة خروج الحشرات الكاملة. وفى هذه الدراسة تم اختباركفاءة مستخلص الخميرة (YE) و ثلاث مكملات غذائية (FC) , (FB), (FA) لإمداد الحشرات الكاملة لذبابة فاكهة البحر المتوسط بالبروتين اللازم بالمقارنة مع البروتين المحلل الأساسى (PS). وأثبتت الدراسة أن أقصر فترة للنضج الجنسى للحشرة 1.26 و 1.01 و 1.03 كانت نتيجة لتغذى الحشرات على المكمل البروتينى (FC) في جيل الأباء و الجيل الأول و الجيل الثانى على التوالي. كما أوضحت الدراسة أن أعلى عدد لوضع البيض كان بواسطة الإنثى التي تغذت على المكمل البروتينى (FB) 4.13 و (FC) 4.08 في جيل الأباء و في الجيل الأول (FB) 2.81 و (PS) 2.79 أما الجيل الثانى فقد وضعت الإنثى التي تغذت على البروتين المحلل الأساسي (PS) 4.29 و التي تغذت على المكمل البروتينى (FB) 4.26. وكانت أعلى نسبة مئوية لإنتاج اليرقات من الأفراد التي تم تغذيتها على المكمل البروتينى (FC) 97.53% و الأقل كانت 90.17% مع البروتين الأساسى المحلل (PS). و كذلك كانت أعلى نسبة مئوية لإنتاج العذارى مع المكمل البروتينى (FC) 99.16% و الأقل 90.71% مع البروتين المحلل الأساسى (PS). أسفرت الدراسة أن أعلى نسبة لإنتاج الحشرات الكاملة كانت من جيل الأباء التي تغذت على المكمل البروتينى (FC) 99.81% و الأقل كانت 90.92% مع البروتين المحلل الأساسى (PS). و أظهرت الدراسة أن أقل نسبة مئوية لموت الحشرات تم تسجيلها مع استخدام مستخلص الخميرة (YE) وكانت للذكور 12.06 % و 3.20% و 3.37% في جيل الأباء و الجيل الأول و الجيل الثانى على الترتيب بينما كانت النسبة المئوية لموت الإناث 8.09% و6.09% و 2.11% على الترتيب في ذات الأجيال. وقد بينت الدراسة أنه لا توجد فروقا معنوية في مقاييس اليرقات المنتجة أو وزن العذارى نتيجة تغذى الحشرات الكاملة على المكملات البروتينية بالمقارنة مع البروتين الأساسى المحلل.