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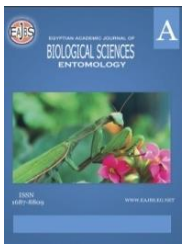

Laboratory Evaluation of Certain Carbohydrates Sources as Nutritive Diets for *Trichogramma evanescens* (Westwood)

Amany Nady Siam[1] and EL-Kholy, M.Y.[2]

1. Department of Cutworms and Mole Crickets, Plant Protection Research Institute, Agricultural Research Center, Egypt.
2. Department of Pests and Plant Protection, National Research Center, Dokki, Cairo, Egypt.

E-mail* : melkholy2000@yahoo.com

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ABSTRACT

Laboratory feeding is an effective factor for *T. evanescens* efficiency. Mainly, mass-rearing programs rely on sugar requirements in parasitoid nutrition. In this study, different carbohydrates sources were evaluated on the biological parameters of *T. evanescens* (Westwood) under laboratory conditions. Females fed on sugar cane honey; *Pritchardia* dates honey and licorice roots extract had the highest fecundity, produced the highest rates of emerged individuals, lived the longest days, and produced the highest rates of females in progeny than those fed on 10% sucrose solution. While starved females had the lowest fecundity and the produced adults emergence rates, lived the shortest days and produced the lowest rates of females. Generally, the highest general productivity was produced when *T. evanescens* females fed on sugar cane honey, *Pritchardia* dates honey and licorice plant extract. This finding implies that alternative carbohydrates sources could be introduced as diets in mass-rearing programs of *T. evanescens* for improving their performance in fields.

INTRODUCTION

Trichogramma parasitoid is a very small wasp; it is an important bioagent used in biological control programs to destroy the eggs of many lepidopteron pests. The successful mass rearing of *Trichogramma* parasitoid under laboratory conditions depends on many parameters; one of them is the feeding of its adults, it is considered the most important parameter as it ensures sustainable and continuous production. Mass rearing programs often take the requirement of sugar into consideration, as insects seem to use carbohydrates as an energy resource (Rivero and Casas, 1999, Mondy *et al*., 2006) also, it helps to enhance the biological control efficacy of *Trichogramma* parasitoid (Tian *et al*., 2021). Nevertheless, introducing new nutritive sources based on many trials for the shortage of the nutritive sources information of natural enemies in fields (Wackers, 2005). The former laboratory evaluations indicated that carbohydrates sources for adult parasitoids significantly increase parasitoid longevity as those of Leatemia *et al*., 1995; Mc Dougal and Mills, 1997, as well, it increases females' potential fecundity (Waage and Ng, 1984). So, adult parasitoid nutrition is very critical for its efficiency. In addition, the cheapest diets for *Trichogramma* parasitoid should be also taken into consideration

through its mass-rearing process with the highest efficiency of the resulting parasitoids (Ali and Wright, 2020). Many authors evaluated the impacts of different nutritive sources as the plant nectars and pollen, honeydew, bee honey, sucrose solution, sugar cane honey as diets for adults on the parasitoids fitness components, as Wackers., 2005, who investigated certain artificial diets comprising carbohydrates to select food supplement in mass rearing to enhance fecundity and longevity of the resulting parasitoids under laboratory conditions. Honey and sucrose solution-based diets were reported to increase parasitization rates of *T. euproctidis* Girault (Hymenoptera: Trichogrammatidae) (Leatemia *et al*., 1995), (Abd EL-Hafez *et al*., 1999), (Karimi & Hatami, 2010) and (Tuncbilek *et al*., 2012). Our nature is rich in various and cheap nutritive sources utilized by people either for feeding or medicinal purposes like the *Pritchardia* palm (Family: Arecaceae) which is a species of palm trees in the genus *Pritchardia*. Its fruits are borne in a terminal cluster with simple or compound branches of an actuate inflorescence. Those fruits called hawane were eaten by early Hawaiians, its flavor is sweet. It has a relevant concentration of nutrients and bioactive compounds with importance for human health (Lescano *et al*., 2018). A sweet extract was prepared from *Pritchardia* dates for *T. evanescens* adults feeding for the improvement of its fitness components, The 2nd nutritive source is sugar cane honey, it is the final byproduct of the boiling cycle in the sugar-making process, and it was evaluated as a cheap source of carbohydrates on the efficiency of resulting *T. evanescens* as it contains high content of vitamins and minerals (Siam, 2018). Finally, the licorice plant (*Glycyrrhiza glabra*), a perennial herb legume (Family: Fabaceae), the extracts of its roots contain certain active compounds acting as an activator for plant growth (Al Jebouri *et al*., 2010) and an extract was excluded from its roots (Siam and El-Genaidy, 2021). The manipulation of introducing unfamiliar or an extraordinary nutrition source is required as the availability and quality of food plays an important role in *Trichogramma* parasitoids' performance acting as biocontrol agents. All those nutritive sources are useful, healthy and safe products. Accordingly, this work was done to evaluate the impacts of those nutrients on the fitness components of the parasitoid aiming at the improvement of the produced trichogrammatids and raising their efficiency in fields. In this study, the evaluation of five nutritive sources; Sugar cane honey, *Pritchardia* dates honey, licorice roots extract, sucrose 10% solution and water on the fecundity, adults' emergence percentage, longevity, produced female's ratio in progeny and the general productivity of *T. evanescens* was carried out aiming at their improvement through the mass rearing and production processes.

MATERIALS AND METHODS

All the experiments concerning the quality of *T. evanescens* were conducted at Fayoum laboratory, Plant Protection Research Institute, Agricultural Research Centre, Egypt, under the optimum laboratory conditions of insects rearing; (25±2^{0}C), (72±5% R.H.) and (14:10 L:D). *T. evanescens* were reared on eggs of *Sitotroga cerealella***.**

1. *Sitotroga cerealella* Rearing:

Rearing *S. cerealella* on soft wheat as the rearing medium was done as a modified method reported by Hassan (1995).

2. *Trichogramma evanescens* rearing:

T. evanescens was reared on the host eggs of *S. cerealella* which were <24 hrs. old. The fresh host eggs were glued to cards of paper (21×15cm) then exposed to *Trichogramma* parasitoid in transparency jars of 2 litters capacity provided with drops of the nutritive source as a diet and covered with cloth-wrapped cotton. The egg cards were replaced daily to prevent super-parasitism, and then the parasitized egg cards were kept in

clean jars.

3. Preparation of *Pritchardia* Dates Honey:

It was prepared as described by (Siam, 2018) where, ripe fruits were collected, washed with water, and then the outer cover of the fruit is pulled off the stone seed, and then weighed. About one kilogram of the dates is covered with one litter of water in a wide pot, boiled on low heat with stirring with a wooden spoon until the mixture thickened. The thick, boiled mixture poured through two mesh cheesecloths. The cloth squeezed well to retrieve more juice as possible from the cooked date mixture. Then it was returned to heat till be thicker, cooled and kept in a refrigerator.

4. Preparation of Licorice Plant Extract:

The aqueous licorice plant extract was prepared according to Siam and EL-Genaidy (2021) as follows: Soaking about 100 gm of licorice roots with 175 ml of 6% acetic acid solution for at least 6 hrs., then, precipitated it through filter paper. About 500 ml of distilled water was added and left for 12 hrs. Finally, another 500 ml of distilled water was added and left for another 12 hrs., till the extract turns colourless.

5. Experimental Assay:

Experiments were conducted to evaluate the impacts of the optimum and available alternative nutritive source as a laboratory diet for *T. evanescens* adults aiming at the production of high-quality parasitoids with high performance in fields. The tested nutritive sources were; sugar cane honey, *Pritchardia* dates honey, licorice plant extract, sucrose 10% and water. The control group was females with no nutritive source. About 25 females for each treatment were released individually on 70 fresh *Sitotroga* eggs in a wide test tube of 4×8.5cm and provided with a droplet of the tested nutritive source on the eggs card. Starved females were left as controls. All replicated treatments were observed daily to count the mortality and longevity of females. Fecundity was calculated for each female at each treatment by counting the parasitized host eggs, and then the emergence percentage of adults and the ratio of produced females in progeny were determined. Finally, the general productivity was calculated for each treatment according to Tshernyshev and Afonina (1995) as the following:

General productivity = Rate of adult emergence × Rate of parasitoid female × fecundity

6. Statistical Analysis:

The biological parameters of *Trichogramma* females fed on the tested nutritive sources were compared using one way ANOVA test. All statistical analyses were carried out by IBM- SPSS for windows (v. 25). Variance Analysis (ANOVA) was used to process the obtained data, separation of means was done by Duncan's Multiple Range Test (Duncan, 1955).

RESULTS

1. Effect of Tested Nutritive Sources on Fecundity:

The fecundity of *T. evanescens* females fed on the tested nutritive sources differed significantly ($P<0.05$). Unfed females parasitized 20.2 *Sitotroga* eggs which were the lowest number among the tested groups, while the highest number 65.3 parasitized *Sitotroga* eggs was recorded with females fed on sugar cane honey and on *Pritchardia* dates honey 64.9 eggs, followed by 59.05 parasitized *Sitotroga* eggs by females fed on licorice plant extract. Only water-fed females parasitized 27.3 *Sitotroga* eggs, while the sugar solution diet caused females to parasitize 42.95 *Sitotroga* eggs (Table 1. Fig.1 a).

Table 1: The effect of tested nutritive sources on the fitness components of resultant *T. evanescens* (Mean ± SD).

Tested nutritive sources	Fecundity	Adult emergence percentage	Life days	Ratio of produced females in progeny
Sugar cane honey	65.3±1.720	97.15% ±1.137	5.7 ±0.865	75.85% ±1.348
***Pritchardia* dates honey**	64.9±2.049	95.9% ±0.788	5.5 ±0.513	75.35% ±1.156
Licorice plant extract	59.05±1.701	95.75% ±0.851	5.35 ±0.671	74.0% ±2.0
Sucrose 10%	42.95±3.170	90.85% ±1.387	3.9 ±0.852	64.65% ±1.843
Water	27.3±2.886	74.45% ±2.724	3.65 ±0.671	61.05% ±2.585
Control	20.2±2.308	63.15% ±2.661	1.45 ±0.605	55.8% ±3.019
	F= 1366.900	F= 1254.339	F= 105.484	F= 314.366

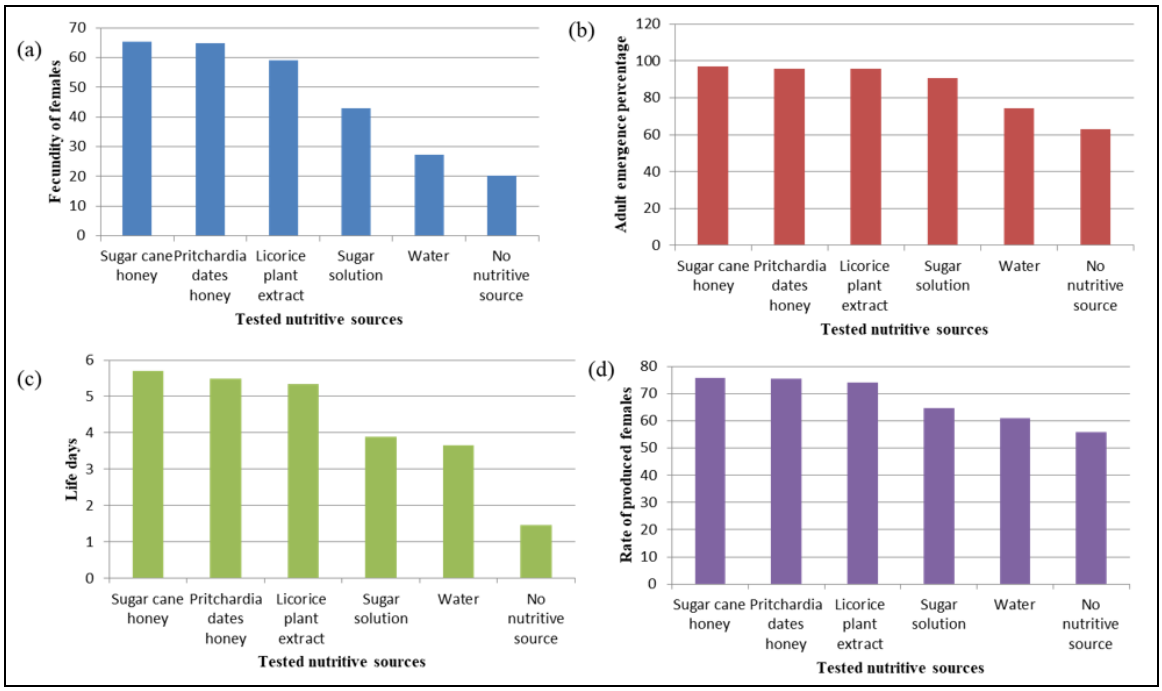

Fig. 1: The effect of tested nutritive sources on the fitness components of resultant *T. evanescens*.

2. Effect of Nutritive Sources on Adult Emergence Percentage:

Sugar cane honey caused the highest individuals emergence percent with an average of 97.15%. It was significantly different (P<0.05, F=1254.339), followed by those from *Pritchardia* dates honey and licorice plant extract. The rates of their emergence averaged 95.9% and 95.75% respectively, compared with 63.15% of adults who emerged from starved females. Sugar solution and water resulted in 90.85% and 74.45% of individuals, respectively (Table 1. Fig.1 b).

3. Effect of Tested Nutritive Sources on Longevity:

Females fed on sugar cane honey lived 5.7 days, followed by those fed on *Pritchardia* dates honey and licorice plant extract which lived nearly the same life period (5.5 and 5.35 days) respectively, compared with unfed females which lived the shortest life span 1.45 days. Water-caused *Trichogramma* females lived for 3.65 days while adding sugar solution prolonged the life span of females to 3.9 days (Table 1, Fig.1 c).

4. Effect of Tested Nutritive Sources on Produced Females' Ratio in Progeny:

The rates of produced *T. evanescens* females differed significantly with the tested nutritive sources (P<0.05, F=314.366). Unfed females resulted in the lowest rates with an average of 55.8%. *Trichogramma* females fed on water recorded 61.05% of the produced females in progeny. The highest rates were recorded with females fed on sugar cane honey, *Pritchardia* dates honey with the values of 75.85% and 75.35% respectively, followed by those fed on licorice plant extract with the value 74.o% females and 64.65% females from females fed on a sugar solution (Table 1. Fig.1 d).

5. The General Productivity of Resultant *T. evanescens* Females:

The tested nutritive sources; sugar cane honey and *Pritchardia* dates honey recorded the highest general productivities with the values of 48.118 and 46.897 females/female respectively, followed by 41.839 females/female from licorice plant extract. The lowest general productivity was 7.118 females / female recorded from unfed females. Sugar solution and water raised the general productivity of *T. evanescens* females to 25.226 and 12.408 females/female respectively (Fig. 2).

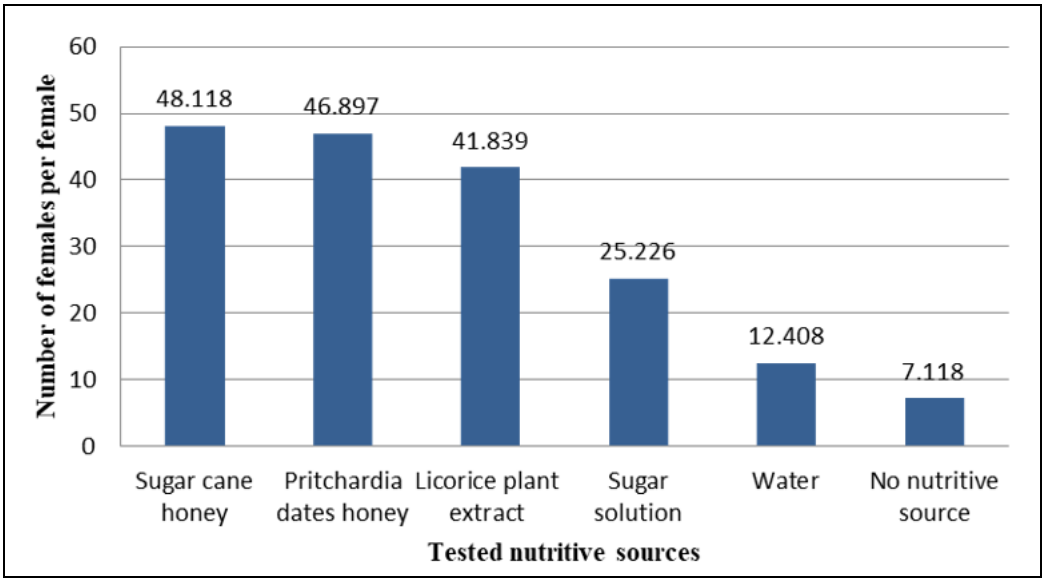

Fig.2: The General Productivity of Resultant *T. evanescens* Females after feeding with tested nutritive sources

DISCUSSION

This work was established as a part of a series that the authors attend to work on. That series aims at the improvement of the mass rearing of *T. evanescens* under laboratory conditions. As the individual's feeding is good, available and sufficient, the resultant parasitoid is more efficient. Obtained results revealed the possibility of introducing unfamiliar nutritive sources for *T. evanescens* adults as a diet to establish sustainable production of the parasitoid and to reduce expenses of its mass rearing. The present results proved that sugar cane honey, *Pritchardia* dates honey and licorice plant extract was served as available and cheap sources of alternative carbohydrates sources for *Trichogramma* parasitoid, those sources improved their fitness components. Sugar cane honey is a very dark viscous liquid with a high concentration of vitamins and minerals, *Pritchardia* fruits honey is a delicious, sweet liquid and it is easy to be prepared as described by Siam (2018). While the licorice plant (*Glycyrrhiza glabra*) is a perennial herb legume, its root extract contains certain active compounds mainly utilized as a

fertilizer for plant growth (Al-Jebouri *et al*., 2010). Present results revealed that the females of *T. evanescens* fed on either sugar cane honey or *Pritchardia* dates honey followed by those fed on licorice plant extract had more fecundity than those fed on the remaining diets. The obtained results are consistence with those of Siam *et al.,* (2014) who assessed sugar cane honey as a nutritive diet for efficiency *T. evanescens* females, and they reported that *T. evanescens* parasitized the highest number of *Sitotroga* host eggs, survived long days and recorded the highest general productivity. Also, Siam (2018) supported this finding when she prepared *Pritchardia* dates honey for *T. evanescens* feeding for the first time and she advised with a provision that sweet honey as a nutritive source for trichogrammatids at laboratory mass rearing. In this study, the licorice plant extract was tested as a nutritive diet for trichogrammatids and the results revealed that the females fed on that extract had high fecundity and it had no harmful effects on the fitness components of resultant parasitoids. The licorice plant extract was previously evaluated under laboratory conditions on *T. evanescens* in a work carried out by Siam and EL-Genaidy (2021), their results proved its safety on the resultant trichogrammatids. Many experiments were conducted on the feeding of adult *Trichogramma* species based on sugar requirements in different sources as those of Tuncbilk *et al*., (2012) who declared that the females of *T. euproctidis* were affected by the type of the offered nutritive source which comprised the molasses of either beet or grape, bee honey, syrups of sucrose. In another work, Abd El-Hafez *et al*., (1999) advised introducing either pure bee honey, honey mixed with yeast, or sucrose solution as diets for trichogrammatids under laboratory conditions. Lundgren and Heimpel (2002) concluded that feeding *Trichogramma* females with honey caused the highest percentage of adult emergence. Salijoqi and Khajjak (2007) advised the provision of honey as a nutritive diet for *T. chilonis* females aiming at the highest female fecundity over those fed on sucrose solution. In addition, many authors advised feeding *Trichogramma* species with honey as it increased the fitness components of the resultant females (Karimi and Hatami, 2010; Paraiso, *et al*., 2011; Leatemia *et al*.,1995; Gurr & Nicol, 2000 and Wacker, 2005. Ozder and Demirtas (2017) reported that feeding *T. brassicae* with honey mixed with *acacia* nectar or with apple syrup raised the fecundity of *Trichogramma* females.

5. Conclusion:

The types of available food take an important part in *Trichogramma* parasitoid efficiency as an important and promising bioagent in biological control programs. From the above-mentioned results, it might be concluded that sugar cane honey, *Pritchardia* dates honey and licorice plant extract are cheap sources of carbohydrates and could be used as innovative and favorable diets for *T. evanescens* adults in the mass rearing processes for the establishment of sustainable production of parasitoids with high quality and high performance in fields.

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