Journal of Plant Protection and Pathology

Journal homepage & Available online at: www.jppp.journals.ekb.eg

Effect of Adding Chamomile and Cinnamon Oils to Larval Diet of Silkworm *Bombyx mori* L. on its Biological Parameters

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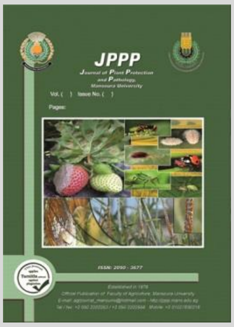

ABSTRACT

The quality and quantity of silk produced by the mulberry silkworm, *Bombox mori* L. are highly influenced by larval feeding, larval ages, care, and types of races. This work investigated effects of adding chamomile and cinnamon oil to larval food on some biological parameters of *B. mori*. For this purpose, cinnamon and chamomile oils were used in different concentrations (for cinnamon: 0.5 ,0.75, 1%; for chamomile: 1%, 2% and 3%). The results showed that the cinnamon 1% highly increased, in a significant way, the weights of larvae, cocoon and pupae compared with the other concentrations. Further, cinnamon 1% also gave the heaviest weights of larvae and fresh cocoon with an increase of 4.368 and 3.229 g, respectively. In addition, the counts of haemocytes, in general, and especially oenocytoides were higher in those larvae fed on diet containing 1% of cinnamon oil. Furthermore, this concentration increased the immunity of larvae. Furthermore, the best concentration for accelerating the development of B. mori larvae as well as their cocoon weight was found to be 1% of cinnamon oil. From the economical point of view, these results are promising and these oil additives to larval diet could be recommended to improve the sericulture parameters.

Keywords: Nutrition, essential oils, silkworm cocoon, haemocytes.

INTRODUCTION

The mulberry silkworm, *Bombyx mori* L. (Bombycidae, Lepidoptera) is one of the most economically important insects not only on the national level but also internationally. The production of high quality and quantity of natural silk depends mainly on larval feeding (Parra, 1991). The mulberry silkworm, *B. mori*. is reared successfully as the main source of natural silk. Recently, considerable attention has been given to improve rearing techniques of silkworms to increase the production of raw silk in Egypt to meet with the higher demands for industrial purposes. Therefore, it has been reported that better production of cocoon crops and eggs is possible when mulberry leaves are supplemented with certain nutritional materials (Zannoon, 1994; Ashour, 1997; El-Sayed *et al*., 1998).

Different parts of plant and plant extracts are used by many researchers for immunization of mulberry leaves, as a food of mulberry silkworm *B. mori*. Jeyapaul *et al.* (2003) estimated food consumption and utilization of mulberry leaves by silkworms after treated with extracts of *Alternanthera sessilis*, *Coffea arabica*, and *Eichharnia Crassipes*, whereas, Eswaran and Sovarkodiyone (2004) used extracts derived from green leaves of *Amaranthus viridis* and tapioca flour for fortification of mulberry leaves; Bohidar and Choubey (2005) used 18 different plant extracts for the same purpose; and Weerakkody *et al.* (2010) indicated that the cinnamon, clove, rosemary, oregano and dill had strong antimicrobial activity. *Cinnamomum osmophloeum* had excellent antifungal, antibacterial, antipathogenic, antitermite, and anti-inflammatory activities.

The haemocytes of lepidopteran insects such as the silkworm are morphologically and functionally divided into five main subsections: granulocytes (GR), plasmatocytes (PL), prohemocytes (PR), spherulocytes (SP), and oenocytoids (OE) (Ribeiro and Brehelin, 2006; Strand, 2008). In general, prohemocytes are considered as multipotent progenitor cells or stem cells giving rise to the other subsets (Yamashita and Iwabuchi, 2006). Granulocytes and plasmatocytes are the only haemocyte subsections able of adhering to foreign surfaces, and both usually comprise more than 50% of the haemocytes in blood circulation of the larval stage. Furthermore, plasmatocytes and granulocytes are included in most cellular defense responses (Lavine and Strand 2002 and Strand 2008). Oenocytoids are rich in prophenoloxidase and mainly participate in melanisation, whereas the function of spherulocytes are unknown (Nakahara *et al.*, 2009 and Nakahara *et al.*, 2010). There are few studies on the effects of adding cinnamon and chamomile to nutritional diet of *B. mori* larvae on their fitness.

MATERIALS AND METHODS

This study was conducted during spring season of 2021 in silkworm laboratory that belonging to Economic Entomology Department, Faculty of Agriculture, Mansoura University. It was started in the fifth instar larvae. It was using bulgarian races (Q_2*V_2*H_1*UV) of the mulberry silkworm *Bombyx mori* L. It was fed with the native mulberry leaves, *Morus alba* variety Balady. Certain oils of herbs were used as supplementary nutrients to enhancement feeding of the

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DOI: 10.21608/jppp.2022.118966.1058

silkworm *B. mori*. The oils extracted from Chamomile (*Matricaria chamomilla*) and Cinnamon (*Cinnamomum Zeylanicum*) were evaluated. These oils were obtained from Elhawag for Natural Oil. It was taken 10 days.

There were three concentrations of each oil were prepared. Concentration of cinnamon oil were 0.5%, 0.75 and 1%. They were prepared as follows: 0.5 ml of oil and 0.5 ml of acetone were adding with 99 ml of distilled water until reached 100ml; 0.75 ml of oil and 0.5 ml of acetone were adding with distilled water until reached 100 ml and 1 ml of oil and .5 ml of acetone were mixed with distilled until reached 100 ml. Chamomile oil concentrations were 1%, 2% and 3%. They were prepared by adding 1 ml of oil and 1 ml acetone to distilled water until reached to 100 ml, 2 ml of oil and 1 ml acetone to distilled water until reached to 100 ml, and 3 ml of oil and 1ml of acetone to distilled water until reached to 100ml.

Fifth instar larvae were divided into three experimental groups. The first group treated with cinnamon concentrations (0.5%, 0.75% and 1%), the second with chamomile concentrations (1%, 2%, and 3%), and the third one was considered as control (not treated). Thus, seven treatments were performed. Each concentration replicated three times and each replicate consisted of 50 larvae. Fresh mulberry leaves were soaked in each concentration for 15 minutes (leaf treated method) and then were dried in air for 20 minutes before being used in feeding trials. The fifth instar larvae were fed with treated leaves four times every day.

After feeding and pupation, the larval and cocoon parameters were estimated in each treatment. The weight of larvae was estimated by weighing 30 fifth instar larvae in different days and the percentage of daily increase in weight (DIW%) was calculated for each group. Pupal weight, Cocoon weight, cocoon shell weight, and cocoon shell ratio were estimated by using standard protocol in sericulture that was described as follows:

The relative growth rate was [G.R = G/W.T], where G, the fresh weight gain was computed as the difference between the initial and final weight of larva in each replication; W, the mean weight of the last instar during the feeding period, T (Etebari *et al*., 2004).

The ratio between the shell weights of cocoon and the whole weight of the cocoon expressed as cocoon shell ratio (Rajitha and Savithri, 2015). It is calculated by using the following equation.

$$\text{Cocoon shell ratio} = \frac{\text{Cocoon shell weight}}{\text{Cocoon weight}} \times 100$$

The haemolymph samples from the fifth instar larvae were taken after 72 hrs of feeding on treated leaves for all groups. To estimate the differential haemocytes count (DHC), 100 cells were identified to their typical haemocytes type after staining a smear of haemolymph with Wright's stain (Gad, 1996).

Data of different treatments were subjected to one-way ANOVA and means separated by Duncan's Multiple Range Test. analyses were performed using the computer program of CoHort Software (2004).

RESULTS AND DISCUSSION

Some biological and economical parameters of silkworm larvae are presented in the Tables (1 and 2). The larvae weight in the fifth instar significantly differed among the different concentrations of cinnamon and chamomile oils and control (Table l). The highest weight (4.368 gm) was recorded for the fifth instar larvae, in the last day of development, that feed with leaves treated with 1% of cinnamon oil, whereas the lowest weight (3.301 gm) was recorded for larvae in control treatment. Results showed that the weights of larvae treated with different concentrations of cinnamon were significantly higher than those either treated with chamomile oil or did not treat (i.e. control). The cinnamon 1% concentration was the efficient concentration among all concentrations tested. Growth rate was highly in the cinnamon oil rather than chamomile oil and control. It gave the highest growth rate (8.63 %). The weight of larvae in control treatment was less than those treated with different concentrations of chamomile and cinnamon oils.

As shown in Table (2), the highest weights of cocoon, pupa, and cocoon shell were significantly higher for *B. mori* larvae that fed with 1% cinnamon oil compared to all other different treatments. Similarity, the highest silk content ratio (24.189%) was recorded for the cocoon spun by *B. mori* larvae fed in 5th instar on mulberry leaves treated with cinnamon oil 1%. Generally, the treatments of cinnamon and chamomile oil proved to be the superior compared to control treatments. They achieved silk content ratios of 24.189 and 23.486 %, respectively, whereas control treatment achieved 22.153 %. Generally, all different concentrations of oil used improved biological criteria over the control.

Table 1. Effect of cinnamon and chamomile oils on duration, weight of larval stage and G.R of *Bombyx mori* .

Concentration	Larvae weight		Larval	G.R
	1st day of 5th	Last day of 5th	duration	(growth rate)
Cinna. 5 %	0.9756a	4.169c	9.33e	8.21
Cinna. 0.75%	0.9726a	4.255b	9.25f	8.34
Cinna. 1%	0.9727a	4.368a	9.00g	8.63
Cham. 1%	0.9760a	3.608e	9.42d	7.75
Cham. 2%	0.9724a	3.710d	9.50c	7.77
Cham. 3%	0.9742a	3.505f	9.58b	7.54
Control	0.9732a	3.301g	10.00a	7.00

Table 2. Effect of different concentration of cinnamon and chamomile oils on Cocoon criteria of *Bombyx mori*.

Concentration	Cocoon criteria			
	Cocoon weight (g)	Pupal weight (g)	Cocoon shell weight (g)	Cocoon shell ratio (%)
Cinna. 5%	3.025c	2.282c	0.734b	23.926ab
Cinna. 0.75%	3.126b	2.351b	0.749ab	24.052ab
Cinna. 1%	3.229a	2.458a	0.766a	24.189a
Cham. 1%	2.859d	2.180d	0.672c	23.674ab
Cham. 2%	2.740e	2.124e	0.654cd	23.522b
Cham. 3%	2.726e	2.104e	0.646d	23.486b
Control	2.325f	1.784f	0.514e	22.153c

The light microscopic observations of the stained larval hemolymph demonstrated presence of four morphologically distinct types of heamocytes: prohaemocytes, granulocytes, plasmatocytes and Oenocytoides. All treatments had effects on the count of differential haemocytes in fifth instar larvae of *B. mori*. The counts of prohaemocytes were observed less in different concentrations of cinnamon and chamomile oils. Furthermore, both cinnamon 1 and 3% concentrations caused a significant decrease in the number of prohaemocytes to be 6.66 cells compared with 11.7 cells in control (Fig. 1). Moreover, the number of plasmatocytes did not significant differ among all treatments. The less number of plasmatocytes were in treatment of cinnamon 1%. The same count was estimated in the number of granulocytes. The lesser count number of granulocytes was 27.33 in cinnamon 3% than different concentrations of oils. In contrast, increasing the concentration of cinnamon led to an increase in number of oenocytoides to be 8.33, 10 and 12 cells in 1, 2, and 3%, respectively. Whereas number of oenocytoides was 6 cells in control. In general, cinnamon 1% had the highest effect of on haemocytes and raised up the immunity of silk worm larvae. In the meantime, chamomile oil treatments (Fig. 1) significantly decreased the number of prohaemocytes , granulocytes and plasmatocytes, but increased the number of oenocytoides.

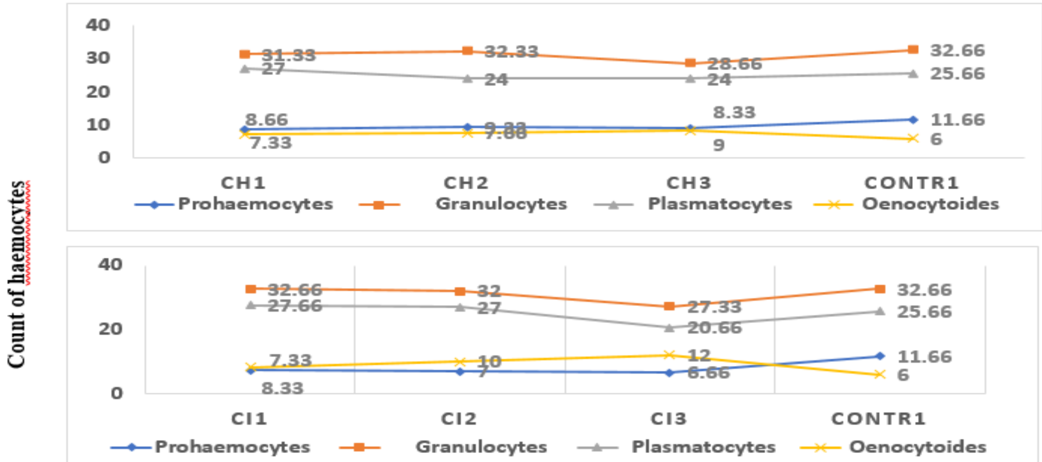

Fig. 1. Haemocyte counts in 5th instar larvae of *Bombyx mori* that treated with different concentrations of cinnamon, chamomile oils and not treated.

Apparently, the present investigation stated that cinnamon and chamomile oils used at different concentrations had significant effect on different parameters of larvae silkworm with different degrees. The application with such oils highly increased the weight of larva, cocoon and pupae compared with control larvae. The 1% cinnamon oil achieved the heavier fresh cocoon, and lead to an increase in haemocytes cells and immunity of larvae. These findings are consistent with those of (Manoharan, 1997); Mahmoud and Yehia (2007); Benţea *et al*. (2011) and Mahmoud et al. (2012) when *Philosamia ricini* Boisd or/and *Bombyx mori* L. larvae fed with the treated mulberry leaves with chamomile. Further, they concluded that the fitness components of *P. ricini* or/and *B. mori* L. larvae were highly influenced by food additives. In the meantime, the current results are in agreement with several authors they reported that the tested nutrients and food additives maximized fecundity of silkworms (El-Sayed, *et al*., 1996; Manoharan, 1997; Yehia, 1998; Mahmoud and Yehia,2007 and Mahmoud *et al*., 2012). As shown in Table (2) the mean weights of larvae and cocoon were significantly varied among treatments with the best performance of pupal weight, cocoon weigh, cocoon shell weight and cocoon shell ration were for larvae fed with mulberry leaves treated with cinnamon 1%. This finding is in the same trend for that obtained by Mahmoud (2014) who found that chamomile at 3%, and fennel 5% and thyme 5% significantly led to heavier weights of larvae, pupae, fresh cocoon than other treatments. The chamomile at a concentration of 3% and thyme at 5% led to an increase in cocoon weight by 18.82 and 21.45%, respectively compared with those untreated larvae. Further, these results are similar with Omar and Fathy (2016) who also found that EEP2 and cinnamon2 lead to heavier weights of cocoon than others treatments. Further, EEP2 lead to faster development of larvae that take eight days in comparison with 9, 9, 10, and 11 days for those treated with EEP1, cinn1, and cinn2 and those untreated, respectively. In this study, the stained haemolymph from larvae treated with different concentrations of cinnamon and chamomile oils after 48 hours of treatment demonstrated four types of haemocytes: granulocytes, prohaemocyte, plasmatocytes and oenocytoides. The same findings are obtained by Cheng *et al*. (2004) and Gad and Dakhaal (2009). The main effect of oil treatments on haemocytes was observed on granular and plasmatocytes (Sharma *et al*., 2008). Generally, the plant crude extracts, containing several biochemical components such as fats, thiamin, riboflavin, vitamin A, and protein. Therefore, determining the optimum concentration of the supplemented additives derived from the plant crude extracts is a fundamental object in supplementation trials for attaining faster growth of *B. mori* with heavier cocoon Though, the best concentration for accelerating the development of *B. mori* larvae as well as their cocoon was found to be 1% of cinnamon. From the economical point of view, these results are promising and these oils could be recommended, as food additives, to improve the sericulture parameters.

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تأثير إضافة زيت البابونج والقرفة إلى غذاء يرقات دودة القز *Bombyx mori L.* و خصائصها البيولوجية

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تتأثر جودة وكمية الحرير الذي تنتجه دودة حرير التوت ، *Bombox mori L.* بشدة بتغذية اليرقات ، وأعمار اليرقات ، ، وأنواع السلالات والعناية بها. اجريت هذه الدراسه لمعرفة تأثير إضافة زيت البابونج والقرفة إلى غذاء يرقات دودة القز *mori B* و خصائصها البيولوجية، لهذا الغرض تم استخدام زيت القرفة والبابونج بتركيزات مختلفة (القرفة: ٠,٥ ، ٠,٧٥ ، ١٪ & البابونج: ١٪ ، ٢٪ ، ٣٪). وأظهرت النتائج أن القرفة بتركيز ١٪ادت الي زيادة كبيرة بصورة معنوية في أوزان اليرقات والشرانق والعذارى مقارنة مع التراكيزات الأخرى. كما اظهرت النتائج ان القرفة ١٪ ادت الي زيادة في وزن اليرقات والشرنقة الطازجة بزيادة قدرها ٤,٣٦٨ و ٣,٢٢٩ جرام على التوالي. بالإضافة إلى ذلك ، كان تعداد خلايا الدم بصفة عامة و الخلايا النبيذية oenocytoides بصفة خاصة أعلى في تلك اليرقات التي تتغذى على غذاء مضاف اليه ١٪ من زيت القرفة. علاوة على ذلك ، فإن هذا التركيز من زيت القرفة يزيد من مناعة اليرقات. علاوة علي ذلك، وجد أن أفضل تركيز لتسريع نمو يرقات *B. mori* ووزن شرانقها هو ١٪ من زيت القرفة. من الناحية الاقتصادية ، فإن هذه النتائج واعدة ويمكن التوصية بهذه الإضافات الزيتية الي غذاء دودة القز لتحسين معايير تربيتها وزيادة جودة وكمية الحرير المنتج منها.