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Effects of diets added with mulberry cake on development and oviposition of white-spotted flower chafer, *Protaetia brevitarsis* (Coleoptera: Cetoniidae)

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Abstract

This study was carried out to investigate the development and oviposition characteristics of *Protaetia brevitarsis* fed mulberry fermented sawdust added with mulberry cake. The results of rearing of *P. brevitarsis* larvae on oak fermented sawdust and mulberry fermented sawdust added with different ratio of mulberry cake, the development periods of larvae feed sawdust with mulberry cake were about 41.6~48.5 days and shortened by 117 or more than that without treatment. The development periods were shorter as the ratio of mulberry cake added increased and there was no difference according to the kind of sawdust. But the weight of the larvae raised in mulberry fermented sawdust added with mulberry cake was heavier than that of the larvae raised in oak fermented sawdust added with mulberry cake. When mass rearing *P. brevitarsis* larvae in oak fermented sawdust, the addition of 5% mulberry cake was appropriate. Also, the average number of laying eggs of female who fed diet with mulberry cake was 12.3% higher than that of untreated.

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Introduction

In recent years, insects have attracted attention due to their high nutritional value, high growth ability, and mass rearing possibility at small areas, and they are emerging as future food resources (FAO, 2013). In Korea, interest in edible insects has increased, worm, crickets, beetles, and white spotted flower chafer have been registered as food (Song *et al*., 2017b), and farms that rearing insects growing year by year.

P. brevitarsis is part of the family of Cetoniidae belong to the order Coleoptera, which is traditionally known to be effective in the treatment of liver diseases such as liver cancer, liver cirrhosis and hepatitis, and adult diseases such as inflammatory diseases, tetanus and paralysis (Kang *et al*., 2001). As of 2017, the number of farm households that keep white spotted flower chafer in Korea is 1,195, which accounts for 55.9% of then number of domestic industrial insect rearing farm. The sales amount of white spotted flower chafer is 16.6 billion won, accounting for 48% of total insect sales, which is the largest in the domestic industrial insect market (MAFRA, 2018b). Among the total production costs for white spotted flower chafer rearing, the feed cost accounts for 32% (Lee *et al*., 2018). Therefore, in order to reduce the production cost, it is necessary to develop a food source to edible insects containing safe and high nutrients (Song *et al*., 2017b). It has been reported that larval development is promoted when a

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certain ratio of livestock feed, aloe vera, soybean curd cake, sweet pumkin and sweet persimmon added to oak fermented sawdust and rearing with spent mushroom substrate ((Kang *et al.*, 2012; Yoon, 2017; Song *et al*., 2017b; Lee *et al.*, 2018; Song *et al.*, 2018). Therefore, there is a high possibility of white spotted flower chafer larvae food additives using agricultural by-products for the economical production of white spotted flower chafe larva.

As mulberry is the fruit of *Morus alba*, mulberry was grown in 1,309 ha and produced 5,637 tons in 2017 (MAFRA, 2018a), Mulberry is a high nutrient fruit with a high content of glucose, sucrose, sugar, fiber and vitamin C, etc. (Park *et al.*, 2008). Because mulberry has a short storage period, it is being processed into a product using a juice extract or an extract. A large amount of mulberry cake was produced as a by-product during processing, and most were being discarded (Jeon *et al*., 2012). But it was predicted that mulberry cake will be used as a functional material because it was abundant in nutriental and dietary fiber such as crude protein 18.3%, crude fat 16.3%, crude fiber 14.7%, crude ash 4.9%, etc. (Jeon *et al.*, 2012).

Therefore, this study was conducted to fine out the effects of mulberry cake addition on the development and oviposition of white spotted flower chafer to improve productivity.

Materials and Methods

Experimental insects and Diets

P. brevitarsis used in experiments were purchased from a private seller (Jangsu-gun, Jeollabuk-do, Republic of Korea). The purchased adults were reared in insect rearing facilities at Sericulture and Entomology Experiment Station of Jeonbuk Agricultural and Extension Services at 25±1°C, 50~60 relative humidity and a light : dark cycle of 16h : 8h. Adults of *P. brevitarsis* were oviposited in the plastic box (543 mm long × 363 mm wide × 188 mm high) with enough fermented oak sawdust and mulberry sawdust, respectively, and fed banana to adults. The oviposited eggs were collected at weekly intervals for this experiment. The oak fermented sawdust and mulberry fermented sawdust were purchased at insect rearing farm in Jangsu-gun and Gochang-gun, respectively. Mulberry cake were collected at Processing center of Buan-gun Agricultural Technology Center, and dried for 3 days in a hot air dryer (55~60°C) after removing the extra juice. The dried mulberry cake was pulverized and stored at 4°C for use as a food additive.

Development of *P. brevitarsis* at Different Ratios of Mulberry Cake

A larva of first day of hatching were raised in petridish (φ100 × 40mm) filled with oak fermented sawdust and mulberry fermented sawdust mixed with 10%, 5%, 3% mulberry cake, and larva developmental periods, larva weight, pupa periods and adults weight were measured. The instar of larva was classified by measuring widths of the head capsule and larval weight was weighted at every two days using a laboratory precision scales. The pupal periods were from the day when the cocoon was formed until the time when the adult passed through the cocoon. Experiments were conducted in incubators (VS-1203PFCL) with 3 replicated of 20 newly hatched larvae per repetition at 25°C and 16(L):8(D). The feeding of larvae was replaced if necessary according to the amount of feces, and water was supplied with a small sprayer to prevent the fermented sawdust from drying as needed during the survey. Also, because post-fermentation was carried out after mulberry cake added to fermented sawdust, it was supplied to larva about 30 days after the addition of mulberry cake.

Establishing of time for sale of larvae according to larval mass rearing

In order to establish of time for sale of white spotted flower chafer larvae according to the ratio of mulberry cake additions, 100 larvae on the first day of hatching were reared in the plastic box (543mm × 363mm × 188mm) filled with oak fermented sawdust added with 10%, 5%, 3% mulberry cake. And the number of larvae 2.5 g or more was examined at regular intervals from 35 days to 95 days.

Oviposition characteristics

In order to investigate the oviposition characteristics of *P. brevitarsis* rearing with fermented sawdust added mulberry cake, one pair of adults emerged newly were reared in petridish (φ120 × 80mm) filled with fermented sawdust and the number of eggs deposited was measured every 7 days. Bananas were fed with

adult food. Also, in order to investigate the effect of the addition of mulberry cake on oviposition of *P. brevitarsis*, newly emerged adults from the cocoon purchased from a farmer (Jangsu-gun, Jeollabuk-do, Republic of Korea) were collected daily and treated in the same manner as above. Mulberry cake was served 0.3 g with banana in a disposable weighing dish (40 × 40 mm), and it was exchanged every day. All experiments were conducted in incubators (VS-1203PFCL) with 25±1°C, RH 50±10%, and L:D=16:8.

Statistical analysis

The data were expressed as mean±SD. Statistical difference at $P<0.05$ between the groups were analyzed by ANOVA analysis and Duncan test using the SAS 9.1 program.

Results and Discussion

Development of *P. brevitarsis* at Different Ratios of Mulberry Cake

The results of rearing of *P. brevitarsis* larvae on oak fermented sawdust and mulberry fermented sawdust added with different ratio of mulberry cake (Tables 1 and 2), the development periods of larvae feed sawdust with mulberry cake were about 41~48 days and shortened by 117 or more than that without treatment. In particular, the development periods of third instar was 21~25 days, which is very short compared to the control. In case of adding 10% soybean cake to oak fermented sawdust, the development periods of third instar of *P. brevitarsis* was reduced by 40% compared to control (Song *et al*., 2017a). When adding 3~15% of aloe vera to oak fermented sawdust, the development periods of *P. brevitarsis* larvae was reduced from about 10 weeks to 5~6 weeks (Kang *et al.*, 2012). Also, when adding 2.5~5% of livestock diet to oak fermented sawdust, the development periods of *P. brevitarsis* larvae was reduced more than 35 days (Song *et al*., 2018). Therefore, it was thought that the addition of mulberry cake further promoted the development of *P. brevitarsis* larvae. As the rate of mulberry cake addition increased, the larval development period tended to decrease.

Table. 1. Development of *P. brevitarsis* fed oak fermented sawdust added with mulberry cake at different ratio at 25°C

Addition ratio (%)	Larva(days)				Pupa (days)
	1st instar	2nd instar	3rd instar	total	
10	8.5±0.84a	12.2±3.41ab	21.0±2.34a	41.7a	32.6±2.65b
5	8.7±1.21a	10.9±3.63a	22.0±2.67a	41.6a	30.5±2.32a
3	9.0±1.27a	13.7±3.69b	25.2±3.13b	47.9b	31.5±2.92ab
0	11.7±1.81b	21.6±6.29c	135.6±21.40c	168.9c	38.0±5.89c

* Mean±SD, DMRT 5%.

Table. 2. Development of *P. brevitarsis* fed mulberry fermented sawdust added with mulberry cake at different ratio at 25°C

Addition ratio (%)	Larva(days)				Pupa (days)
	1st instar	2nd instar	3rd instar	total	
10	8.8±0.76a	12.0±2.95a	21.3±2.45a	42.1a	34.4±5.52a
5	9.8±0.85b	13.1±3.76a	22.4±2.75a	45.3a	32.0±4.44a
3	9.7±0.71b	13.8±4.50a	25.0±9.18a	48.5a	38.5±9.28b
0	9.9±0.89b	20.7±4.70b	135.1±20.24b	165.7b	42.1±4.36c

* Mean±SD, DMRT 5%.

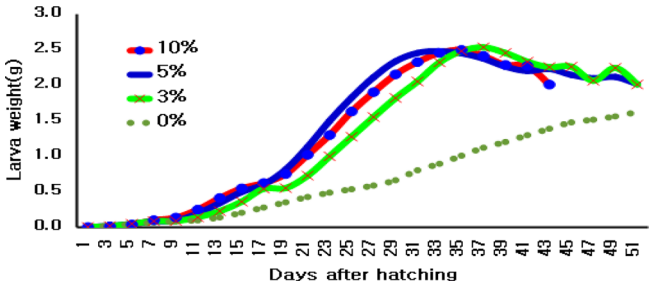

Fig. 1. Body weight of *P. brevitarsis* larvae fed fermented oak sawdust added with mulberry cake at different rate at 25°C.

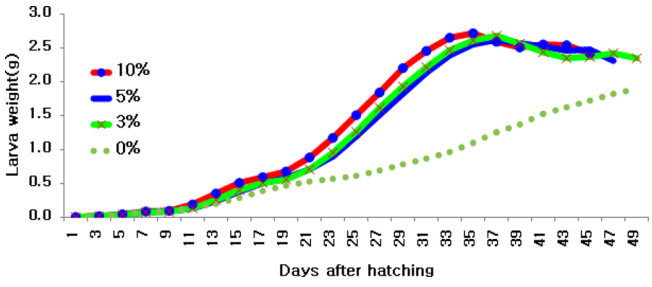

Fig. 2. Body weight of *P. brevitarsis* larvae fed fermented mulberry sawdust added with mulberry cake at different rate at 25°C.

There was no difference according to the addition ratio of the mulberry cake in the mulberry fermented sawdust. But in oak fermented sawdust, there was a significant difference in 3% addition, so it was considered that the addition of 5% or more was effective. There was no difference in development period of *P. brevitarsis* larvae depending on the kind of sawdust. Protein and other nutrients contribute to shortening the larval development of *P. brevitarsis* (Song *et al*., 2018), mulberry cake was also rich in nutrients such as protein and was considered to be an effective additive to shorten the development period of *P. brevitarsis* larvae. The weight of the *P. brevitarsis* larvae due to addition of the mulberry cake increased rapidly in all treatment from the beginning of the third instar compared to the no addition, and the weight of the larvae decreased slightly after the peak weight point (Fig. 1 and Fig. 2). An increase in the weight of larvae in the mulberry cake 5% addition to oak fermented sawdust was faster than 3% and 10% additive (Fig. 1). In mulberry fermented sawdust, 10% of the added mulberry cake tended to increase the weight of larvae faster (Fig. 2). The maximum weight of the larvae of oak fermented sawdust and mulberry fermented sawdust was 2.56~2.89g, which was not significantly different from that of the control which had a long development period. However, the time required to reach the maximum weight has been greatly reduced (Table 3). The addition of mulberry cake to mulberry fermented sawdust tends to be higher than the weight of oak fermented sawdust, which was thought to be due to differences in nutritional content of basic fermented sawdust. Yoon (2017) showed that the addition of apple powder, squash powder and aloe powder had a high increase in body length and body width of the white spotted flower chafer larvae, and also has a significant effect on the weight gain rate. Therefore, it was advantageous to supply them as additives. Song *et al*. (2017b) showed that when fed oak fermented sawdust with 10% soybean curd cake to the larvae of *P. brevitarsis*, the periods until the weight of the larvae increased to 2.5 g available for sale was about 5 weeks of feeding, which was faster than untreated. And, it was believed that feeding soybean curd cake will enable to produce insects efficiently. Song *et al.* (2017b) was suggested that the development of food sources containing high nutrients was necessary because nutrients in the food sources directly affect the growth of insects in the rearing edible insects. As the addition of mulberry cake to fermented sawdust was effective in increasing to the available weight of the *P. brevitarsis* larvae in a short period of time, it was thought to be useful as a feed additive for the *P. brevitarsis* larvae. The weight of the emergenced adults in larvae raised with fermented sawdust with mulberry cake tended to be lighter than that of untreated larvae with long growth period (Table 3, Table 4).

Establishing of time for sale of larvae according to larval mass rearing

The cumulative ratio of larvae over 2.5 g was investigated by using oak fermented sawdust adding with mulberry cake (Fig. 3). The cumulative ratio of larvae over 2.5 g were 53~60% on the 40th day after hatching and 86~96% on the 45th day. On the other hand, 3% mulberry cake added group showed 67% on 45th day and 86% on 55th day, and the rate of larvae with slow growth tended to be slightly higher than 5~10% added group.

During the rearing period, the survival rate of *P. brevitarsis* larvae was 88~91% higher than untreated group. As a result of rearing larvae by adding livestock feed to oak fermented sawdust, the survival rate of larvae was more than 80% (Song

Table 3. Maximum larval weight and mean periods of larvae with maximum weight by supplying fermented sawdust added with mulberry cake at different rate at 25°C

Addition ratio of mulberry cake (%)	Maximum larval weight(g)		Duration to reach max. weight(days)	
	Oak	Mulberry	Oak	Mulberry
10	2.56±0.242b	2.89±0.213a	34.4±3.48a	35.4±2.85a
5	2.65±0.249ab	2.78±0.314b	34.2±3.83a	37.7±3.22b
3	2.65±0.252ab	2.76±0.268b	38.4±4.10b	37.7±2.76b
0	2.76±0.285a	2.89±0.340a	162.1±9.80c	155.4±11.25c

* Mean±SD, DMRT 5%.

Table 4. The average weight of *P. brevitarsis* adults reared with fermented sawdust added with mulberry cake at different rate at 25°C

Diet	Sex	Adults weight(g) by addition with mulberry cake			
		10%	5%	3%	0%
Oak sawdust	Female	0.654±0.062	0.637±0.070	0.593±0.077	0.776±0.110
	Male	0.658±0.032	0.617±0.066	0.596±0.048	0.942±0.126
Mulberry sawdust	Female	0.762±0.136	0.740±0.123	0.760±0.111	0.941±0.126
	Male	0.774±0.107	0.784±0.109	0.806±0.168	0.956±0.138

* Mean±SD.

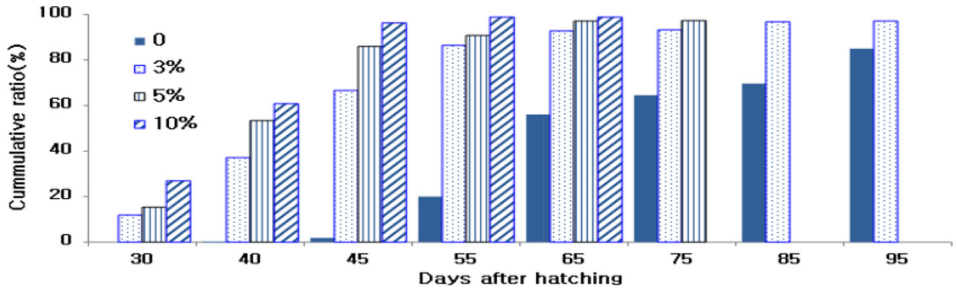

Fig. 3. Cumulative ratio of *P. brevitarsis* larvae over 2.5g among the larvae fed fermented oak sawdust added with mulberry cake at different rate at 25°C.

et al., 2018). Therefore, it was judged that mulberry cake could be safely used for the rearing of *P. brevitarsis* larvae, and considering the cumulative ratio over 2.5 g and survival rate, 5% addition of mulberry cake was considered to be the most economical for rearing *P. brevitarsis* larvae. As a result, when the larva weight was 2.5 g or more in the white spotted flower farm was determined to be shipped (Song *et al*., 2018), it was judged that the larva shipment would be possible after 40 days when the mulberry cake was added 5%.

Oviposition characteristics

The number of laying eggs of adults emerged from pupa raised with oak fermented sawdust with mulberry cake 5% were investigated for 10 weeks. As a result, only one of 12 females oviposited at 10 weeks after emergence and the rest did not spawn (Table 5). Adults with long larval development period started to lay eggs within 7 days after emergence and continued to lay eggs for about 9 weeks (Moon *et al*., 2018), and further studies on the relationship between the larval development periods and oviposition are needed. In the white spotted flower chafer-rearing farm, the larval development period varies from 35 days to 70 days (Song *et al*., 2017a) and 3th instar larvae were stored at 10°C for a certain period of time for successive rearing, so it is necessary to establish a larval management system for larval selling and continuous

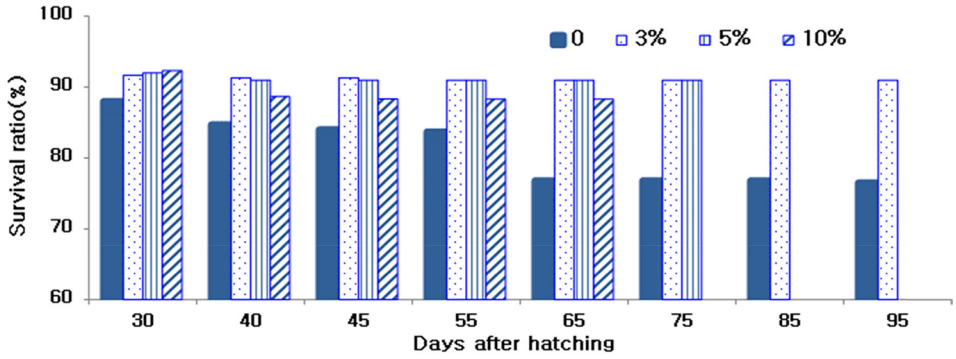

Fig. 4. Survival ratio(%) of *brevitarsis* larva fed oak fermented sawdust added with mulberry cake at different rate at 25°C.

Table 5. Number of eggs of *P. brevitarsis* adults emerged in the larva raised with oak fermented sawdust mixed with mulberry cake

No. of tested(pair)	Spawning adults(pair)	No, of eggs
12	1	5

* Survey periods : 10 weeks.

growth. As a result of investigating fecundity of *P. brevitarsis* by adding mulberry cake to banana, which is an adult food, the average fecundity was 85.5 eggs (Table 6), which was 13.2% higher than that of the untreated. But, the number of laying eggs among female was significantly different. There was a significant different in fecundity among the temperature and photoperiod, with the highest number of eggs (110.8 eggs) at 30°C (L:D=16:8) (Kim et al., 2018). The highest number of eggs were 133 eggs at 25°C (L:D=16:8) in this experiment. Therefore, it was thought that more eggs would be oviposited if they are grown at 30°C (L:D=16:8) by adding mulberry cake to adult food. Also, it was thought that there was a problem in the method of supplying mulberry cake because adults did not consume mulberry cake supplied with bananas. Therefore, further research on the method of supplying mulberry cake such as jelly were needed.

As a result, the addition of mulberry cake to fermented sawdust could shorten the development period of *P. brevitarsis* larvae, which would contribute to the increase of income of edible insect rearing farm through reduction of production cost. Future research on functional nutrients and harmful ingredients of larvae according to the addition of mulberry cake was needed.

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Table 6. Number of eggs and oviposition periods of *P. brevitarsis* adults fed diet added with mulberry cake at 25°C

Diet	No. of tested (pair)	No. of eggs/female		Oviposion periods (weeks)
		Mean	Range	
Banana+mulberry cake	23	85.5±28.39a	40 ~ 133	8.6±2.21
Banana	32	75.1±23.83b	44 ~ 145	9.0±2.03

* Mean±SD, DMRT 5%.

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