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# The Effect of Antiseptic and Sugar Solution on Colony Development of the Bumblebees, *Bombus ignitus* and *B. terrestris*

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**We investigated possible effect of different concentration of sugar solution and addition of antiseptic in the solution on oviposition and colony development of *Bombus ignitus* and *B. terrestris*. The rates of oviposition, colony foundation and progeny-queen production of *B. ignitus* were 1.2–3.0 fold higher in the 40% sugar solution than those of the 50% sugar solution. The rates of oviposition, colony foundation and progeny-queen production were 1.1–2.6 fold higher in the 40% sugar solution added in 0.3% sorbic acid as antiseptic than those of the 40% sugar solution. Further, the death rate within one month was 1.7 fold lower in the 40% sugar solution added in 0.3% sorbic acid than that of 40% sugar solution alone. In the comparison of the colony development tested using imported sugar solution, the Beehappy®, the 40% sugar solution added to antiseptic and the 40% sugar solution without antiseptic, the 40% sugar solution added to antiseptic was about equal to the Beehappy® in colony development of *B. terrestris*. Further, the number of adults produced was 1.2–3.0 fold higher in the 40% sugar solution added to antiseptic than that of the Beehappy®. Therefore the 40% sugar solution was more effective than the 50% sugar solution, and the 40% sugar solution added to antiseptic was the most effective in colony development and mass rearing of bumblebee.**

**Key words**: Antiseptic, Bumblebee, *Bombus ignitus*, *Bombus terrestris*, Colony development, Oviposition, Sugar solution

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## Introduction

Bumblebees are an important pollinator of various greenhouse crops, particularly effective in pollinating for the night shade family, which includes tomato and eggplant. (Buchmann and Hurley, 1978; Free, 1993). Bumblebees provide farmers the opportunity to decrease the labor costs of pollination and promise a good yield both in quantity and in quality (Iwasaki, 1995). We chose *Bombus ignitus* out of seven Korean native bumblebees, because the species showed the best result both in artificial multiplication and in pollinating ability (Yoon *et al.*, 1999). Now, we are studying an artificial year-round mass rearing of *B. ignitus*, because the species is the most reliable native bumblebee in crop pollination (Yoon and Kim, 2003; Yoon *et al.*, 2004).

In artificial mass-rearing of bumblebee, every author used pollen and honey obtained from honeybee hives (Griffin *et al.*, 1991; Ono *et al.*, 1994; Tasei and Aupinel, 1994; Hannan *et al.*, 1998), though the supplying method varied a little among various experiments. To enhance colony development, the study of feeding is of significance. To deposit food on larva in *B. ignitus*, workers put their mouth into the larval cell through an orifice (Katayama, 1973, 1975). In the majority of cases the number of orifices in the wax envelope made by the queen and/or workers when feeding the larvae corresponded to the number of larvae present in the egg cell. Queen–worker differentiation in social insects depends primarily on nutritional and social factors (De Wilde and Beetsma, 1982; Wheeler, 1986). The mechanisms regulating the rearing of new queens in bumblebees, as well as the process of developmental differentiation into queens and workers are not yet fully understood. The production of new bumblebee queens has been related to factors such as the availability of food, the relative number of workers involved in foraging and nursing and the presence of the queen (Pomeroy and Plowright, 1982; Duchateau and Velthuis, 1988).

As mentioned in a previous paper (Park *et al.*, 2004), sugar solution is more useful than honey solution on mass rearing of bumblebees in cost. In this study, we conducted to investigated whether or not different concentration of sugar solution and addition of antiseptic in the sugar solution have any effects on oviposition and colony development of *B. ignitus* and *B. terrestris*

## Materials and Methods

### Origin of experimental insects

Experimental insects were artificially hibernated 2nd and 5th generation queens obtained from *Bombus ignitus* and *B. terrestris* colonies year-round reared in a controlled climates room (28°C, 65% R. H. and continuous darkness). For artificial hibernation, queens were hibernated for 4 months at 2.5°C to preserve them in a bottle filled with perlite and keep it around 80% R. H.

### Indoor rearing

The basic colony-rearing technique was followed as described in Yoon *et al.* (2002). The queens were reared in three types of cardboard (1.5 mm thick) boxes each for nest initiation (10.5×14.5×6.5 cm: small box), colony foundation (21.0×21.0×15.0 cm: medium box), and colony maturation (24.0×27.0×18.0 cm: large box). Each box had a wire net window on its lid for ventilation. The sizes of these windows were 5.5×6.5 cm, 7.0×14.0 cm and 10.0×20.0 cm, respectively. Queens were first confined individually in small boxes for colony initiation and remained there until oviposition. To stimulate the egg laying, two narcotized old *B. ignitus* and *B. terrestris* worker 10 – 20 days aged after emergence was added to each queen (Yoon and Kim, 2002). When the adults emerged from the first brood, the nest was transferred to a medium box for colony foundation, and left there until the number of workers reached 50. The nest was thereafter moved to the big box for further colony development.

Sugar solution and pollen dough were provided *ad libitum*. The pollen dough was made from sugar solution and fresh pollen collected from an apiary (v : v = 1 : 1).

### Effect of antiseptic, concentration of sugar solution and kinds of nectar on colony development of *B. ignitus* and *B. terrestris*

To examine effect of antiseptic and sugar solution on oviposition and colony development of *B. ignitus* and *B. terrestris*, the following environmental conditions were provided. Concentration of sugar solution was defined as 40% ad 50%, as results of a previous paper (Park *et al.*, 2004). Antiseptic was sorbic acid (Junsei Chemical Co.) used in artificial diet of insects and its concentration was 0.3%. The numbers of *B. ignitus* queens allotted to this experiment were 30 and were 3 replicates. In addition, the colony development of Beehappy®, sugar solution impor-ted from Korppert company, 40% sugar solution added in antiseptic, and 40% sugar solution without antiseptic was compared. The numbers of *B. terrestris* queens allotted to this experiment were 50.

The developmental ability of each colony was estimated by rate of oviposition, colony foundation and progeny-queen foundation, production of progeny, and period up to first adult emergence. Colony foundation here indicates that more than 50 workers emerged in a colony. Period up to first adult emergence designates the duration from the first oviposition to the first adult-emergence. The queens that did not oviposit in 60 days were excluded from the number of oviposited colonies.

Statistical analysis was done with Chi-square test and Tukey's pairwise comparison test (MINITAB Release 13 for Windows, 2000). The Chi-square test was used to compare colony development of *B. ignitus* and *B. terrestris* by effect of antiseptic and sugar solution. Tukey's pairwise comparison test was used to examine the durations until colony foundation and first adult emergence, as well as the number of adults produced.

## Results and Discussion

### Effect of antiseptic and sugar solution on oviposition and colony development of *B. ignitus*

We investigated effect of antiseptic and sugar solution

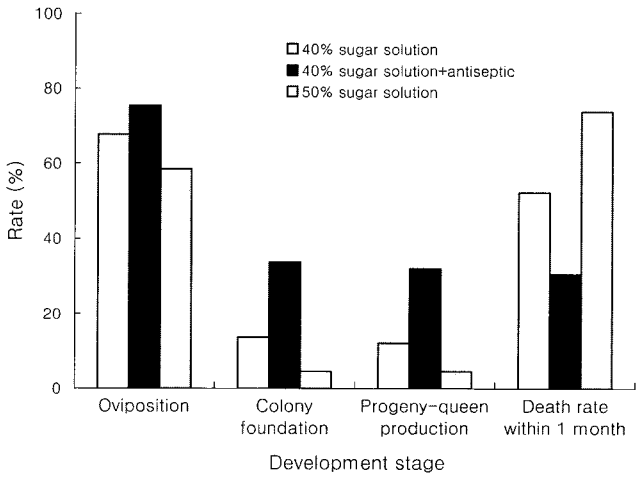


**Fig. 1.** Comparison of colony development of queen of *B. ignitus* at antiseptic and concentration of sugar solution. For the statistical analysis, a Chi-square test was used for each developmental stage: $x^2 = 4.233$, $p > 0.05$ (N.S.) for oviposition; $x^2 = 20.163$, $p > 0.001$ for colony foundation; $x^2 = 19.365$, $p > 0.001$ for progeny-queen production; and $x^2 = 25.79$, $p > 0.001$ for death rate of one month. The numbers of *B. ignitus* queens allotted to this experiment were 30 and were 3 replicates.

on developmental characteristics, rates of oviposition, colony foundation and progeny-queen production, of artificially hibernated *B. ignitus* queen (Fig. 1). The rate of oviposition of 40% sugar solution was 67.7%, which was 1.2 fold higher than that of the 50% sugar solution. The rate of oviposition of the 40% sugar solution added in 0.3% sorbic acid as antiseptic was 75.4%, which was 1.1 – 1.2 fold higher than that of the 40% and the 50% sugar solution. There was no significant differences in the oviposition rate of *B. ignitus* queen in effect of antiseptic and sugar solution at $p < 0.05$ by Chi-square test ($x^2 = 4.233$, df = 2, $p = 0.120$) (Fig. 1). In case of the colony foundation rate, the 40% sugar solution added in antiseptic showed the best performance as 33.9%, which was 2.5 – 7.4 fold higher than that of the 40% and the 50% sugar solution and the rate of colony foundation of the 40% sugar solution without antiseptic was 3.0 fold higher than that of the 50% sugar solution. The colony foundation rate of *B. ignitus* was very affected by the concentration of sugar solution and antiseptic addition in the sugar solution ($x^2 = 20.163$, df = 2, $p = 0.001$) (Fig. 1). The rate of progeny-queen production was also compared depending on antiseptic and different sugar solution. As shown in Fig. 1, the rate of progeny-queen production of the 40% sugar solution added in antiseptic was 32.3% and this value was also 2.6 – 7.0 fold higher than that of the 40% and the 50% sugar solution and there was statistically significant differences in the progeny-queen production rate of *B. ignitus* queen in effect of antiseptic and sugar solution at $p < 0.001$ by Chi-square test ($x^2 = 19.365$, df = 2, $p = 0.001$). In case of the death rate within one month, the 40% sugar solution added in antiseptic was 30.8%, which was 1.7 – 2.4 fold lower than that of the 40% and the 50% sugar solution, and the 40% sugar solution was 1.4 fold lower than that of the 50% sugar solution. The death rate within one month of *B. ignitus* was significantly affected by concentration of sugar solution and antiseptic addition ($x^2 = 25.793$, df = 2, $p = 0.001$) (Fig. 1).

Table 1 shows the duration up to preoviposition, colony foundation, and adult emergence from *B. ignitus* queen in antiseptic addition and concentration of sugar solution. The duration up to preoviposition at the 40% sugar solution without antiseptic, the 40% sugar solution added antiseptic and 50% sugar solution was 11.1 – 11.7 days and no significant difference in antiseptic addition and concentration of sugar solution at $p < 0.05$ by Tukey's pairwise comparison test (F = 0.13, df = 2.125, $p = 0.874$). The duration up to colony foundation of the 40% sugar solution was 56.6 days. It was 2.8 – 3.6 days longer than that of the 40% sugar solution added in antiseptic and the 50% sugar solution. But the colony foundation period was not affected by antiseptic and concentration of sugar solution (F = 0.13, df = 2, 125, $p = 0.874$). The period up to first worker emergence was 28.5 days, which was 3.6 – 4.2 days longer in the 50% sugar solution than that of the 40% sugar solution without antiseptic and the 40% sugar

**Table 1.** Duration up to preoviposition, colony foundation, and adult emergence from queens of *B. ignitus* at antiseptic and concentration of sugar solution

| Concentration of sugar solution (%) | n[a] | Preoviposition (days)[b] | n[a] | Colony foundation (days)[b] | First adult emergence (days)[b] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | n[a] | Worker | n[a] | Male | n[a] | Queen |
| 40 | 41 | 11.7±6.8 | 9 | 56.6±5.8 | 15 | 24.9±3.2 a | 12 | 67.8±21.0 | 9 | 79.8±14.0 |
| 40+antiseptic | 49 | 11.4±6.0 | 22 | 53.8±7.6 | 31 | 24.3±4.7 ab | 21 | 58.8±16.1 | 18 | 74.4±15.0 |
| 50 | 38 | 11.1±4.8 | 2 | 53.0±5.7 | 10 | 28.5±4.0 ab | 3 | 51.0±24.8 | 3 | 74.7± 4.2 |

[a]n means the number of colony surveyed.
[b]The figures stand for means± SD. Means followed by different letters in the same column are significantly different at $p<0.05$ by Tukey's pairwise comparison test.

**Table 2.** Number of adults produced from queens of *B. ignitus* at antiseptic and concentration of sugar solution

| Concentration of sugar solution (%) | Number of adults produced | | | | | | n[a] | Longevity of foundation queen (days)[b] |
|---|---|---|---|---|---|---|---|---|
| | n[a] | Worker[b] | n[a] | Male[b] | n[a] | Queen[b] | | |
| 40 | 10 | 104.2±35.5 | 10 | 176.7±68.7 | 8 | 10.3±09.8 | 15 | 95.9±27.9 |
| 40+antiseptic | 15 | 105.0±43.0 | 15 | 196.7±71.2 | 15 | 25.5±29.9 | 29 | 88.1±28.5 |
| 50 | 3 | 101.7±61.7 | 3 | 210.7±70.8 | 3 | 29.0±22.1 | 7 | 67.7±20.5 |

[a]n means the number of colony surveyed.
[b]The figures stand for means± SD. There were no significant in number of adults produced from queens of artificial hibenated *B. ignitus* by concentration of sugar solution factor at $p<0.05$ by Tukey's pairwise comparison test.

solution added in antiseptic. The period of first worker emergence was statistical difference ($F=3.80$, $df=2, 53$, $p= 0.029$). The period up to first male emergence was short at the 50% sugar solution and prolonged in the order of the 50% sugar solution and the 40% sugar solution added in antiseptic. However there was no statistical difference between them ($F=1.41$, $df=2, 33$, $p=0.259$). Besides, the period of first queen emergence was not either affected by antiseptic and concentration of sugar solution although the period of queens emergence at the 40% sugar solution was 5.1 – 5.4 days longer than that of the 50% sugar solution and 40% sugar solution added in antiseptic ($F=0.45$, $df= 2, 27$, $p=0.643$).

The number of adults produced from *B. ignitus* queen at antiseptic and concentration of sugar solution was surveyed with more than 50 workers emerged colonies (Table 2). The numbers of worker produced at antiseptic and concentration of sugar solution was 102 – 105 ($F = 0.01$, $df = 2, 25$, $p = 0.992$). In case of the number of males produced at antiseptic and concentration of sugar solution, the 50% sugar solution was 210.7 ± 70.8 numbers and these values were 14 - 34 numbers more than that of the 40% sugar solution and the 40% sugar solution added in antiseptic. But there was no statistical difference between them at $p < 0.05$ ($F = 0.37$, $df = 2, 25$, $p = 0.692$). In case of the number of queens produced, which is an important point in year-round rearing of bumblebee, the 50% sugar solution queen produced 29.1 ± 22.1 numbers, which corresponded to 1.1 – 2.8 fold of that of the 40% sugar solution and the 40% sugar solution added antiseptic. However there was no statistical difference between them ($F = 1.16$, $df = 2, 23$, $p = 0.332$). The longevity of foundation queens was 95.9±27.9 days, which is 7.8–28.2 days longer in the 40% sugar solution than those of the other regimes though there was no significant difference at antiseptic and concentration of sugar solution ($F = 2.53$, $df=2, 48$, $p=0.090$).

With above results, we supposed that the 40% sugar solution was more effective than 50% sugar solution in colony development and mass rearing of bumblebee and bumblebee queen was significantly affected by antiseptic addition in the sugar solution. In feeding behavior of bumblebee, to deposit food on larva in *B. ignitus*, the worker approaches the egg cell and opens it with her mandibles. Then she puts her head into it, stays motionless for a moment and contracts her abdomen, discharging the food. The droplet of liquid food is deposited on the ventral part of the larva. Because of the cylindrical shape of its body and its curled position, the larva is capable of holding the food and stars to eat it immediately. In the case the larvae are still in a common envelope, the worker closed the orifice in the wax and leaves (Katayama, 1973). Queens grow larger since they obtain more food than worker larvae. This can partly be explained by the longer duration of their development and, directly related to that, the higher frequency of feeding during the last instar (Röseler and Röseler, 1974; Ribeiro *et al.*, 1999). Ono *et al.* (1994) showed that sucrose was inverted into fructose and glucose within a few minutes by adding macerated hypopharyngeal glands from *B. terrestris* workers.

**Effect of kinds of nectar solution on oviposition and colony development of *B. terrestris***

The relationship between kinds of nectar solution and developmental characteristics of *B. terrestris* queen was investigated (Fig. 2). The rate of oviposition of the 40% sugar solution added in 0.3% sorbic acid as antiseptic was 80.0%, which was 1.2 – 1.3 fold higher than that of the Beehappy® and the 40% sugar solution without antiseptic. There was no significant differences in oviposition rate of *B. terrestris* queen at kinds of nectar at $p < 0.05$ by Chi-square test ($x^2 = 3.345$, $df = 2$, $p = 0.188$) (Fig. 2). The rates of colony foundation rate the 40% sugar solution added in antiseptic was equal to that of the Beehappy® as 46.0%, which was 2.3 fold higher than that of the 40% sugar solution. The colony foundation rate of *B. terrestris* was affected by kinds of nectar ($x^2 = 9.631$, $df = 2$, $p = 0.008$) (Fig. 2). The rate of progeny-queen production was also compared depending on kinds of nectar. As shown in Fig. 2, the rate of progeny-queen production of the Bee-

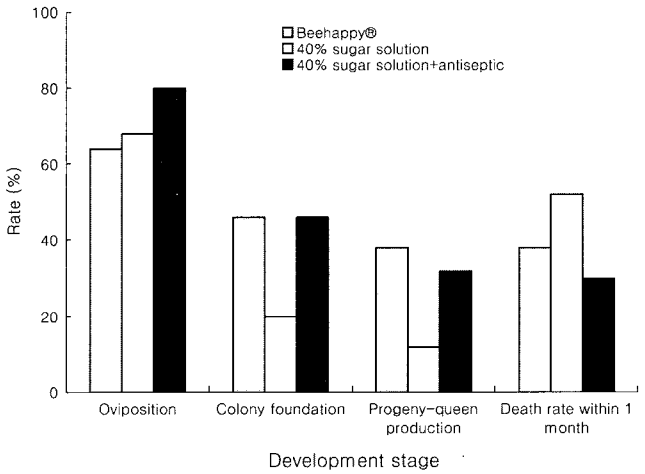


**Fig. 2.** Comparison of colony development of queen of *B. terrestris* at kinds of nectar solution as carbohydrate source. For the statistical analysis, a Chi-square test was used for each developmental stage: $x^2=3.345$, $p>0.05$ (N. S.) for oviposition; $x^2=9.631$, $p<0.05$ for colony foundation; $x^2=9.331$, $p<0.05$ for progeny-queen production; and $x^2=5.167$, $p<0.05$ (N. S.) for death rate of one month. Fifty queens were allotted for each experiment.

happy® was 38.0% and this value was also 1.2–3.2 fold higher than that of 40% sugar solution and added in antiseptic on it. There was statistically differences in the progeny-queen production rate of *B. terrestris* queen at kinds of nectar at $p < 0.05$ by Chi-square test ($x^2 = 9.331$, df = 2, $p = 0.009$). In case of the death rate within one month, the 40% sugar solution added in antiseptic was 30.0%, which was 1.3–1.7 fold lower than that of the Beehappy® and the 40% sugar solution. There was no significant difference in the death rate within one month ($x^2 = 5.167$, df = 2, $p = 0.076$) (Fig. 2).

Table 3 shows the duration up to preoviposition, colony foundation, and adult emergence from *B. terrestris* queen at kinds of nectar solution. The duration up to preoviposition at the 40% sugar solution was 6.8 days, which was only 1.3–1.4 days shorter than that of the 40% sugar solution added in antiseptic and the Beehappy® (F = 0.66, df = 2, 102, $p = 0.519$). The duration up to colony foundation was 58.7 days. It was 5.0–5.6 days longer in the 40% sugar solution than that of the 40% sugar solution added in antiseptic and the Beehappy®. But the colony foundation period was not affected by kinds of nectar solution (F = 1.89, df = 2, 51, $p = 0.162$). The period up to first worker emergence of the 40% sugar solution was 28.9 days, which was 2.0–2.4 days longer than that of the Beehappy® and the 40% sugar solution added in antiseptic. The period of first worker emergence was not statistical difference (F = 0.97, df = 2, 64, $p = 0.386$). The period up to first male emergence was short at the 40% sugar solution and prolonged in the order of the 40% sugar solution added in antiseptic and the 50% sugar solution. However there was no statistical difference between them (F = 0.31, df = 2, 54, $p = 0.732$). The period of queens emergence at the 40% sugar solution was 85.4 days, which was 5.7–16.8 days longer than that of the Beehappy® and the 40% sugar solution added antiseptic. There was significant difference in the period of queen emergence (F = 5.42, df = 2, 39, $p = 0.009$).

The number of adults produced from *B. terrestris* queen at kinds of nectar solution was surveyed with more than 50 workers emerged colonies (Table 4). The numbers of worker produced at the 40% sugar solution added in antiseptic was 146.6 ± 42.0, which was 19.5 – 34.9 numbers more than that of the Beehappy® and the 40% sugar solution. But there was no statistical difference between them at $p < 0.05$ (F = 2.82, df = 2, 50, $p = 0.069$). In case of the number of males produced at kinds of nectar solution, the 40% sugar solution added in antiseptic was 220.8 ± 96.8 numbers and these values were 31.4–45.2 numbers more than that of the Beehappy® and the 40% sugar solution

**Table 3.** Duration up to preoviposition, colony foundation, and adult emergence from queen of *B. terrestris* at kinds of nectar solution as carbohydrate source

| Kinds of nectar solution | n[a] | Preoviposition (days)[b] | n[a] | Colony Foundation (days)[b] | First adult emergence (days)[b] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | n[a] | Worker | n[a] | Male | n[a] | Queen |
| Beehappy® | 31 | 8.2±6.3 | 23 | 53.7±7.0 | 25 | 26.5±5.1 | 21 | 65.5±17.5 | 19 | 79.7±11.3 a |
| 40% sugar solution | 34 | 6.8±4.9 | 10 | 58.7±9.4 | 15 | 28.9±6.1 | 10 | 60.8±15.5 | 5 | 85.4±20.2 a |
| 40% sugar solution + antiseptic | 40 | 8.1±5.8 | 21 | 53.1±8.0 | 27 | 26.9±5.1 | 26 | 63.2±14.7 | 16 | 68.6± 9.8 b |

[a]n means the number of colony surveyed.
[b]The figures stand for means ± SD. Means followed by different letters in the same column are significantly different at $p < 0.05$ by Tukey's pairwise comparison test.

**Table 4.** Number of adults produced from queen of *B. terrestris* at kinds of nectar solution as carbohydrate source

| Kinds of nectar solution | Number of adults produced | | | | | | n[a] | Longevity of foundation queen (days)[b] |
|---|---|---|---|---|---|---|---|---|
| | n[a] | Worker[b] | n[a] | Male[b] | n[a] | Queen[b] | | |
| Beehappy® | 22 | 127.1±36.2 | 22 | 175.6±70.4 | 19 | 33.7±30.2 a | 23 | 111.5±19.2 |
| 40% sugar solution | 10 | 111.7±45.0 | 10 | 189.4±71.8 | 5 | 19.4±21.3 a | 16 | 103.4±29.6 |
| 40% sugar solution + antiseptic | 21 | 146.6±42.0 | 21 | 220.8±96.8 | 17 | 77.4±42.1 b | 26 | 106.9±25.8 |

[a]n means the number of colony surveyed.
[b]The figures stand for means ± SD. Means followed by different letters in the same column are significantly different at $p < 0.05$ by Tukey's pairwise comparison test.

without antiseptic. However there was no statistical difference at kinds of nectar at $p < 0.05$ ($F = 1.66$, $df = 2, 50$, $p = 2.000$). And also, the number of queens produced of the 40% sugar solution added antiseptic was 77.4 ± 42.1, which corresponded to 2.3 – 4.0 fold of that of the Beehappy® and the 40% sugar solution. There was statistically significant difference between them at kinds of nectar solution ($F = 9.19$, $df = 2, 38$, $p = 0.001$). The longevity of foundation queens of Beehappy® was 111.5 ± 19.2 days, which is 4.6 – 8.1 days longer than those of the other regimes though there was no significant difference at kinds of nectar solution ($F = 2.53$, $df = 2, 48$, $p = 0.090$).

Above results showed that the 40% sugar solution added in antiseptic and the Beehappy®, sugar solution imported from Kopport company was about equal in the rates of oviposition, colony foundation and progeny-queen production of bumblebee. But the number of adults produced was 1.2 – 3.0 fold higher the 40% sugar solution added antiseptic than that of the Beehappy®. Therefore, the 40% sugar solution added in antiseptic was more effective than the Beehappy® in the oviposition, death rate within 1 month, and number of aduts produced.

The mechanisms, which antiseptic affects colony development of bumblebee are not yet known. Further experiments can clearly this cause. In rearing of *B. terrestris*, to prevent Nosema multiplication, Fumidil power at 2 g/l was added to the sugar solution during periods of 14 days alternating with two-week intervals without the antibiotic (Tasei, 1994).

In conclusion, the present results indicate that the 40% sugar solution was more effective than the 50% sugar solution and the 40% sugar solution added in antiseptic was the most effective in colony development and mass rearing of bumblebee.

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