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Development of eco-friendly plant protecting agents using a food additive, sodium saccharin for fall webworms, *Hyphantria cunea* Drury

Nguyen Minh Anh Hoang[1], Yoo-Hee Kim[2], Hong Hyun Park[3*], and Young Ho Koh[1,2*]

[1]*Department of Bio-medical Gerontology, Hallym University Graduate School, Choocheon, Gangwon-do, Korea*
[2]*Ilsong Institute of Life Sciences, Hallym University, Seoul, Korea*
[3]*Plant Protection Division, National Institute of Agricultural Sciences, Wanju-gun, Jeollabuk-do, Korea*

Abstract

Currently, *Hyphantira cunea* Drury, the fall webworms (FWW), is one of the most severe insect pest for various landscaping trees and mulberry trees. In this study, we investigated whether FWW might be managed by Sodium saccharin (SAC) which is a food additive and not toxic to humans and environment. We found that FWW larvae treated with various concentrations of SAC showed dose-dependent delayed development and low survival rates. The lethal-concentration 50% of FWW larvae to SAC was 0.03 M. We also confirmed that SAC can be used to control FWW larvae attacking mulberry trees in the field. Compared to not-treated or tap-water-spraying control groups, SAC-spraying groups showed significantly higher mortality rates of FWW larvae (56.2%). Thus, SAC can be used for control FWW larvae in mulberry trees.

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Introduction

Food additives include various substances added to enhance the quality and preservation of food while the process of manufacturing, processing, by adding flavor and aroma, changing to more favorable color, and preventing oxidation and decomposition (MFDS, 2023b). Since food additives must provide benefits to consumers and have no health risks, only substances that have passed various toxicity tests and regulations on the necessity and the quality of use are permitted as food additives (MFDS, 2023b). As of 2019, a total of 618 items in 32 categories, including sweeteners and antioxidants, are permitted as food additives in Korea (MFDS, 2019).

Sodium saccharin (SAC) is included in the sweetener group among food additives. After being accidentally invented by Johns Hopkins University Professor Ramsen and his research associate Fehlberg in 1897, it has been widely used as an artificial sweetener (Okoduwa *et al.*, 2013). However, the use of SAC as an artificial food sweetener was banned after a problem was reported that bladder cancer occurred in rats administered with SAC in an experiment performed by the Canadian Environmental Protection Agency in 1977. After that, continuous research on the safety of SAC confirmed that

***Corresponding author.**
Young Ho Koh, Ph.D.
Ilsong Institute of Life Sciences, Hallym University, Seoul, Korea
Tel: + / FAX: +
E-mail: kohyh@hallym.ac.kr
Hong Hyun Park, Ph.D.
Plant Protection Division, National Institute of Agricultural Sciences, Wanju-gun, Jeollabuk-do, Korea
Tel: + / FAX: +
E-mail: honghyunpark@korea.kr

there was no toxicity to humans, and in 2010, the United State Environmental Protection Agency removed SAC from the list of harmful substances (EPCRA, 2023). The Republic of Korea also eliminated regulations on SAC in 2011, allowing SAC to be used in limited amounts only in permitted food (MFDS, 2023a). In addition, an acceptable daily intake of 5 mg/kg bw for SAC was determined by the WHO/FAO Joint Expert Committee on Food Additives (WHO, 1993).

In a previous study, we reported that SAC has an insecticidal effect on *Drosophila melanogaster* and *D. suzukii* (Osabutey *et al.*, 2021). We reported that *Drosophila* that ingested SAC did not develop normally due to problems with nutrient metabolisms, such as autophagy and unfolded protein response in the fat body. However, it has not been investigated whether SAC induces developmental abnormalities or is toxic to other pests yet, so we conducted to confirm whether SAC has an insecticidal effect on *Hyphantria cunea* Drury, fall webworms (FWW), which is the lepidopteran invasive pest and currently causes severe damaged to trees and crops.

FWW was first reported on a street tree in Itaewon, Seoul in 1958. Thus, it is assumed to have been introduced with US military cargo (Woo, 1961). According to the research results on host plants of FWW in 2012, FWW can attack 219 species of plants in 62 families (Kim and Kil, 2012). Hosts of the FWW include crops, vegetables, specialty crops, horticultural crops, and landscaping or street trees. In recent years, as the number of FWW that can survive after wintering increases due to global-warming, the occurrence of FWW has increased rapidly, emerging as a serious environmental problem (Park, 2022). In particular, when large numbers of FWW occur in landscaping trees or street trees in apartment complexes, parks, or amusement parks, it creates disgust for people and can bring about side effects such as skin rashes or allergic reactions caused by webs, caterpillar's hairs, or excretions secreted by FWW larvae (Bames, 2020). Currently, various types of plant protection agents are permitted for the control of FWW larvae that harm landscaping trees and street trees (RDA, 2020), but it is difficult to spray plant protection agents in areas where large numbers of the public gathers because of their toxicity. Therefore, there is an urgent need to develop new control techniques using substances that are not harmful to humans or the environment. We conducted this study to show that SAC toxic to FWW larvae and could be used for control FWW larvae at the field.

Materials and Methods

Chemicals

Food-grade sodium saccharin (SAC, $C_7H_4NNaO_3S$, molecular weight = 207.17, China Pingmei Shenma Group Kaifeng Xinghua Fine Chemical Co., LTD, Kaifeng, Henan, China) was dissolved in dH_2O or tap waters. SAC is allowed to used less than 1.2 g/Kg for health improvement functional food, chowing gums (MFDS, 2023a).

Insects

Hyphantria cunea Drury (Fall webworm, FWW) was collected from mulberry tree farms located at the campus of National Institute of Agricultural Sciences (NIAS), Wanju-gun, Jeollabuk-do, Republic of Korea from 2020 ~ 2023. The developmental stages of collected larvae were defined based on their head and body sizes and used for various assays. They were maintained with mulberry leaves before being used for assays.

SAC bioassays

Various concentrations of SAC were mixed with artificial diets for FWW. The content of the artificial diet for FWW larvae was wheat germ (Sigma, W0125), casein as the protein (Sigma, C6554), (30g/kg (modified amount)); saccharose as the carbohydrate (Sigma, S1888), (30g/kg (modified amount), torula yeast (Sigma, Y4625), vitamin mixture (Sigma, V-1007), salt mixture (Sigma, W1374), cholesterol (Sigma, C 2044), sorbic acid (Sigma, S1626), methyl paraben (Sigma, H3647), linseed oil (Sigma, L3026), agar (DaeJung Chemical and Metals Co., Siheung, Korea) and water (Yanar, 2016). 2.0 M SAC solution was made and mixed with FWW artificial diet. FWW artificial diets were boiled by using a microwave oven and then appropriate SAC solutions were added and mixed well. The final concentrations of SAC in artificial diets were 0.4 M, 0.08 M, and 0.016 M each. Ten 2^{nd} instar larvae were inoculated on a petri-dish containing FWW artificial diets with appropriate concentration of SAC. All experiments were replicated five times. The weight of larvae alive were measure at Day 7. The lethal-concentration 50% (LC50) of SAC for FWW larvae was calculated by the Probit analysis using the Excel program (Microsoft Corp. Redmond, WA, USA) as previously published (Kim et al., 2018).

SAC field bioassays

Two-year-old mulberry trees were planted in pots on the campus of the NIAS to create a mulberry tree bioassay field.

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Fifty FWW larvae collected from mulberry trees in the NIAS campus were inoculated on a branch of mulberry trees. On the next day after inoculation, an untreated control group, a tap-water-spraying group, and a SAC-spraying group were set up. The SAC-spraying group was prepared by dissolving 2.0 g of SAC in 100 ml of tap water (0.096 M SAC) and sprayed on five mulberry tree pots using a mist sprayer. The concentration of SAC for the mulberry tree field bioassays were determined based on the laboratory bioassay result which was 0.04 M SAC for LD50 of FWW. Since there were more variables at the filed than the laboratory, 2.4 times higher concentration of SAC was used. The untreated control group received no treatment after inoculating 50 FWW larvae in five mulberry-tree pots. The tap-water-spraying control group was sprayed 100 ml of tap water on five mulberry trees using the same kinds of mist-sprayer used for spraying SAC. Inoculated FWW larvae were protected by mesh coverings to prevent attack by birds and other predators. The number of surviving FWW larvae in the control and spraying groups was counted two days and 7 days after spraying with tap water or SAC.

Statistical analysis

One-way Analysis of Variance (ANOVA) and two-way ANOVA with repetitions were performed by using Excel (Microsoft Corp.). The statistical significance of differences between samples was analyzed by a Tukey's honestly significant difference post hoc analysis using Excel (Microsoft). Significant differences were determined at $P < 0.05$.

Results

SAC wad toxic to the FWWs

In order to investigate the effect of SAC on the growth and survival of FWW larvae, we investigated the survival rate of FWW larvae for 7 days after inoculating FWW larvae to its artificial diet containing various concentrations of SAC (Fig. 1). We found that the sensitivity of FWW larvae to SAC increased in a dosage-dependent manner. FWW larvae raised on the its artificial diet containing 0.4 M SAC were 90% dead after 1 day and 100% dead after 2 days. In addition, 70% of FWW larvae raised on its artificial diet with 0.08 M SAC died after 1 day, 78% after 2 days, 80% after 3 days, and no more deaths by 7 days. While FWW larvae cultured on its artificial diet containing 0.016M SAC, 10% died after the 1st day, 22% after the 2nd day, 28% after the 3rd day, 32% after the 4th day, and 34% after the 5th day. And then, there was no additional dead larva until 7 days. In addition, 22.5% of FWW larvae raised on its normal artificial diet died on the 1st day, after 25% died on the 2nd day no more larva died. When we performed a two-way ANOVA with repetitions with survival analysis data of FWW larvae treated with various concentration of SAC, there were a significant interaction between the concentration of SAC and periods of exposure of FWW larvae to SAC ($F_{(14, 96)} = 9.0152$, $P = 3.0 \times 10^{-12}$). Our two-way ANOVA analysis results suggested that the concentrations of SAC have a statistically significant effect on the death of FWW larvae ($F_{(2, 96)} = 420.23$, $P = 3.3 \times 10^{-48}$). In addition, our statistical analysis results also suggested that the treatment periods also have a statistically significant effect on the death of FWW larvae ($F_{(7, 96)} = 94.171$, $P = 3.8 \times 10^{-40}$). Calculated LC_{50} of SAC to FWW larvae based on our SAC bioassay data at Day 7 with a Probit analysis was 0.04 M.

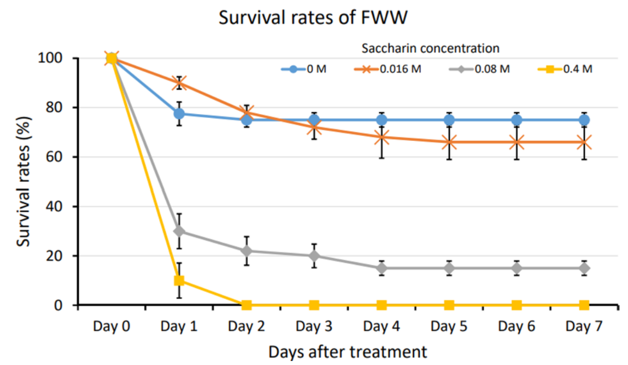

Fig. 1. Saccharin susceptibility of Fall webworm (FWW) were dose-dependently increased. A two-way ANOVA was conducted to investigate the effect of SAC concentration and exposure periods to FWW survivals. Our analysis revealed that there was a significant interaction on the survival of larvae between the concentration of SAC and exposure periods ($F_{(14, 96)} = 9.0152$, $P = 3.0 \times 10^{-12}$). The concentration of SAC had a statistically significant effect on survival of FWW larvae ($P = 3.3 \times 10^{-48}$). In addition, the exposure periods of FWW to SAC had a statistically effect on their susceptibility ($P = 3.8 \times 10^{-40}$).

We also measure the body weights of FWW larvae alive at Day 7 (Fig. 2). The body weight of FWW larvae raised on an artificial diet without SAC was 0.024 ± 0.0011 g, and that of FWW larvae raised on an artificial diet containing 0.016 M SAC was 0.022 ± 0.00089 g, showing no significant difference. However, the weight of FWW larvae reared on a diet containing 0.08 M SAC significantly decreased to 0.012 ± 0.00055 g. When

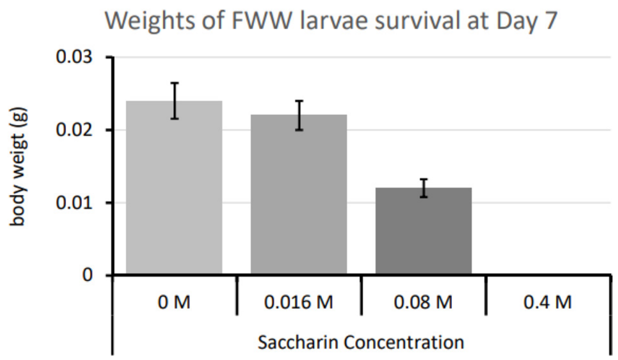

Fig. 2. The body weights of FWW larvae survival at Day 7 after exposure to various concentration of SAC. There was a statistically significant difference in body weights between FWW larvae exposure to various concentration of SAC ($F_{(3, 16)}$ = 42.087, P = 8.1 × 10^{-8})

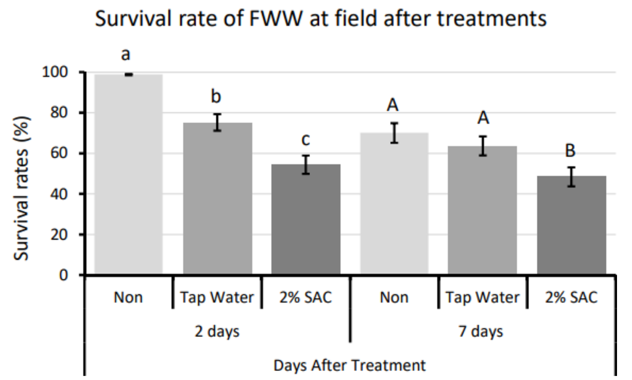

Fig. 3. SAC had an insecticidal effect on FWW larvae inoculated on mulberry trees. Compared to the non-treated control group and the tap water-treated control group, a clear insecticidal effect was observed at two-days and seven-days after treatment in the SAC-treated group ($F_{(2, 24)}$ = 6.302909, P = 0.006309). However, there was no statistical difference according to the date after treatment group ($F_{(1, 24)}$ = 4.142219, P = 0.53013). And the treatment method and observation days did not affect each other (P = 0.454588).

the body weight changes according to various concentrations of SAC treatments were compared by the one-way ANOVA, it was found that 0.08 M SAC significantly reduced the body weight of FWW larvae and 0.4 M SAC was enough to killed FWW larvae ($F_{(3, 16)}$ = 42.087, P = 8.1 × 10^{-8}).

SAC had an insecticidal effect on FWW larvae even in the field

A field experiment was conducted to determine whether SAC could be used for the control of FWW larvae on trees. In order to compare the insecticidal effect of SAC on FWW larvae, an untreated control group and a tap water-spraying control group were set up at the field. Two days after spraying-treatments, survival rates of FWW larvae with tap water or SAC solution was investigated. The FWW larvae's survival rate of the untreated control group was 99.8 ± 0.36% while that of tap water sprayed group was 75.2 ± 4.06%. In contrast, the survival rate of the SAC-sprayed group was 54.4 ± 5.50%, significantly lower than that of two control groups (Fig. 3). In seven days after treatments, the survival rate of FWW larvae in the non-treated control group was 70.0 ± 4.80% while that of FWW larvae in the tap-water spraying group was 62.8 ± 4.67%. In contrast, the survival rate of FWW larvae in the SAC spraying group was 48.4 ± 4.69% (Figure 3).

When the survival rate of FWW larvae on the control and SAC-spraying groups were analyzed by a two-way ANOVA with repetitions, a clear insecticidal effect was observed at two-days and seven-days after treatment in the SAC-spraying group ($F_{(2, 24)}$ = 6.303, P = 0.006309). However, there was no statistical difference according to the date after treatment group ($F_{(1, 24)}$ = 4.142, P = 0.53013). In addition, the treatment methods and observation days did not affect each other (($F_{(1, 24)}$ = 0.815, P = 0.454588). Taken together, it was clear that SAC-spraying also has an insecticidal effect against FWW larvae that feed on mulberry trees.

Discussion

The occurrence of the FWW is increasing due to the increase in average annual temperature caused by global warming (Park, 2022). In addition, as the numbers of landscaping or street trees that can be hosts of FWW in residential and park areas increases, the damages caused by the FWW larvae is getting more and more serious and manifested every year (Aptnew, 2019). Currently, there are various types of plant protection agents approved for the control of FWW larvae infected on landscaping trees, but it is dangerous to spray the large amounts of plant protection agents frequently in areas where humans frequently gather or pass by walk, such as apartment complexes and parks. Therefore, the most important contribution of this study is to show that SAC, a food additive proven to be harmless to humans, has the potential to be used as a control agent of FWW larvae. FWW larvae exposed to SAC showed higher insecticidal rates compared to the control group in both laboratory and filed tests (Figs. 1 ~ 3, Table 1 ~ 3). However, our data suggested that there is a disadvantage in that a high concentration of SAC must be sprayed if FWW larvae were controlled with only SAC. In a previous study, we proved that SAC was toxic to *D. suzukii* and *melanogaster* (Osabutey *et al.*, 2021). We also showed that when SAC was treated together with spinosad, which is used for the control of *D. suzukii* in the field (Kirst, 2010), insecticidal activity of SAC and spinosad combined treatment showed equal or superior insecticidal activity compared to single treatments (Osabutey *et al.*, 2021). Several organic farming materials such as pyrethrin, wood vinegar, paraffin oil, and azadirachtin were reported to have toxicity to FWW larvae (Park, 2019). Therefore, because there is a difference in toxicity mechanism between SAC and other organic farming materials, we speculated that there may be synergistic insecticidal effects if they are used together.

Mulberry trees, one of FWW's preferred hosts, were used as test plants for FWW larvae in the field test of this study (Fig. 3). Currently, various types of plant protection agents are approved for the control of FWW larvae that attack mulberry trees for mulberry fruit cultivation, but there are no plant protection agents approved for the control of FWW larvae for mulberry trees used for silkworm feed for sericulture (RDA, 2023). Therefore, after confirming that silkworms are not toxic to SAC, SAC can be used to control pests including FWW larvae on mulberry trees for silkworm feed. In this study, we have provided evidence that SAC, which is non-toxic to humans, can control FWW larvae, which are gradually increasing in their host range, occurrence area, and numbers.

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