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New Form of Strong Volatile Attractant for Flea Beetle (Phyllotreta Striolata) (Fab) Control in South Taiwan

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Abstract-The yellow sticky insect traps currently on the market are not effective in catching flea beetles, as they are non-specific for that pest. A color preference test was conducted on adult fleas, involving the use of 12 colored sticky plates in orange, blue, purple, black, pink, green, red, blue, yellow, brown, dark blue and white. An odor preference test was also conducted on adult fleas using several volatile chemical compounds, the best trapping effect being achieved using allyl-isothiocyanate (AIC), at 12.9-fold higher than the control group. Therefore, we further designed and developed a new form of powerful volatile spraying glue, Strong Volatile Chemical Yellow Glue 01 (SVCYG01), and multi-layer paper with AIC, in order to control the flea beetle, a crucifer-feeding pest. Our results showed that the attractive effect of 15% SVCYG01 was greatest, at 4.4-fold higher than the control. The caught ratios using 7.5%, 10% and 12.5% SVCYG01 were 1.6–2.3-fold higher than the control, while no significant differences were observed. A multi-layer paper trap using the volatile chemical AIC (1.2 ml) in combination with a conventional yellow sticky trap resulted in a 30.6-fold increase in the caught efficiency as compared with a conventional yellow sticky trap alone when tested in an organic carrot field against the flea beetle. The height of the sticky traps used to catch flea beetles was varied for comparison in the field, and a height of 0–10 cm above the vegetable surface resulted in a 5.1-fold increase in trapping efficiency as compared with traps set at 40–50 cm. The newly-developed efficient pest trap using SVCYG01 and multi-layer paper can protect cruciferous crops from key pests such as flea beetles and powder flies. It can also prevent farmers from inhaling pesticide during spraying and reduce the cost of purchasing pesticides.

Keywords- *Phyllotreta Striolata, Cruciferous Vegetables, Volatile Attractant, Yellow Sticky Trap*

I. INTRODUCTION

The flea beetle, Phyllotreta striolata (Fab), is one of the most important pests affecting crucifers worldwide. It has long been considered a serious pest, and the severity of damage to crucifer vegetables caused by the flea beetle is increasing in Taiwan [1]. The flea beetle mostly prefers host plants of the crucifer family [2,3], which includes rape, leaf mustard, ball or non-ball cabbage, Chinese cabbage, radish and others [4,5]. Flea beetle larvae feed on the roots, and adults feed on leaves, quickly wilting the plants and affecting vegetable crop yields. In general, most Taiwan farmers use pesticides to prevent and control flea beetle infestations. Feng et al. [6] reported that farmers found that pesticides carbaryl, malathion and mevinphos were not sufficiently effective by which to control flea beetles, and suspected that flea beetles may have a certain resistance in Taiwan. Liao et al. [7] reported the effects of some pesticides for the control of flea beetles. The best results were obtained by spraying with cartap; second best was a 60% efficacy for profenofos, followed by 38% for both emamectin benzoate (conc. 5.0%, SP) and carbofuran (40.64%). Carbaryl, emamectin benzoate (conc. 2.15%, EC) and abamectin had an effectiveness of 25–29%. The adverse results of long-term use of pesticides are resistance and residue issues. For several years, farmers in some countries have continued to report that the efficacies of government-recommended pesticides are unsatisfactory, which consequently results in the use of highly toxic pesticides such as carbofuran and phorate to control flea beetles. Flea beetles with resistance to cabofuran and cabaryl have also been reported [8]. Current control measures for the beetles, based on the application of broad-spectrum insecticides, are often ineffective due to difficulties in targeting beetles owing to their larvae's cryptic behavior under the ground, adult migration from surrounding areas, and the timing of attack.

To monitor population levels, as well as to seek alternative means for pest management, both olfactory (AIC) and visual (colored trap) cues involved in host–plant selection have been studied [2,9,10]. However, the crucial components of the visual cues have still not been fully investigated. Adult flea beetles show a strong positive phototactic response. The action spectrum of phototaxis of dark-adapted beetles was measured at the minimal required light intensity between wavelengths of 300 nm and 600 nm. The changes in sensitivity of the photoreceptor influence the phototactic responses, and blue wavelengths were found to be more attractive than others [11]. In similar related reports, walking weevils were shown to respond to a mixture of 3-butenyl, 4-pentenyl, and 2-phenylethyl isothiocyanates in an olfactometer [12], and traps baited with a mixture of 3-butenyl, 4-pentenyl, 2-phenylethyl, and AIC were attractive to *Ceutorhynchus assimilis* [13]. Many factors may affect the catching of sap beetles, *Glischrochilus quadrisignatus* [14], and like trap design, the trap height influences beetle capture [15]. Synthetic host volatile chemicals increased the efficacy of trapping for management of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say): less insecticide (44%) was applied to plots bordered by attractant-treated trap crops [16]. For the above reasons, it is important to improve traps, and in order for the technique to be acceptable to growers, an easy-to-use alternative to the sticky trap is required for the protection of organic crucifer vegetables.

In the present study, our objective was to assess the efficacy of different colored traps, traps treated with glue containing AIC, trap height and trap designs used as bait to attract and kill flea beetles in the field. This study aimed to identify the most appropriate trap design for use as a new form of strong volatile attractant for control of the flea beetle.

II. MATERIALS AND METHODS

A. Test materials

First the test materials used in the study included bok choy, with different sika color sticky traps of a length and width of 20 × 15 cm; the control trap was provided by High Crown Enterprise Limited Company. The multi-layer paper patch (measuring 3.5 × 2.2 cm) was purchased from Chung Tai Shin Pyrethrum Company. An air quality detector (GT300-VC) was purchased from GAS TECH Co., LTD (Taiwan).

B. Chemicals and reagents

The Volatile chemicals and reagents used, including mustard oil, benzyl-isothiocyanate (BIC), ethyl-isothiocyanate (EIC) and allyl-isothiocyanate (AIC), were purchased from Sigma. Colorless glue was purchased from the Nan Pao Resins Group.

C. Color preference test for the flea beetle performed in a bok choy field

The twelve color traps used included black, red, pink, white, violet, blue, orange, grass green, green, yellow and brown sika paper sticky traps, measuring 20 × 15 cm, which were sprayed with colorless glue. The color traps were placed 5–7 m apart from one another and hung approximately 10 cm above the leaves of the crop. All color trap tests were replicated three times. The numbers of beetles in all three trap replicates were counted after 3 days.

D. Volatile chemical preference test for the flea beetle performed in a radish field

The preference test was carried out using several volatile chemicals, including BIC, EIC, AIC and mustard oil, which were dropped (at a volume of approximately 1.2 ml) on a patch separately, and combined with yellow sticky traps in order to catch flea beetles in a radish field. The volatile chemical traps were placed as described above.

E. Optimal adsorption materials and volatile chemical release rates for traps

1) *Experiment on the odor release value of allyl-isothiocyanate adsorbed by transparent soap.*

Transparent soap (80%), plus 10 or 5% AIC and 10 or 15% sunflower oil, was employed in this experiment. Chopped soap was dissolved into liquid form, after which the temperature was lowered, and the above-mentioned proportions of AIC and sunflower oil were added. After solidification, the test samples were placed into transparent closed boxes measuring 30 X 20 X 20 cm. An air quality detector (GT300-VC) was used to detect the odor release value (CIAQ: Composite Index of Air Quality) of the traps containing AIC after 0, 1, 2, 3, 4 and 5 days.

2) *Experiment on the odor release value of allyl-isothiocyanate at varying concentrations adsorbed by a multi-layer paper patch.*

The multi-layer paper patch was prepared using multi-layer paper, and was treated with certain proportions of AIC and sunflower oil at 0.4, 0.6, 0.8, 1.0 and 1.2 cc, respectively. The total odor index (CIAQ) of the traps containing AIC were detected after being kept in closed boxes (measuring 30 × 30 × 30 cm) for 0, 1, 2, 3, 4 and 5 days.

F. Proportion test of strong insect attractant formula

1) *The caught effect of the multi-layer paper patch was measured following addition of allyl-isothiocyanate and 12.5–15% strong volatile chemical yellow glue (SVCYG01) spray to a plate to catch flea beetles.*

1.2 ml of AIC were added to the multi-layer paper pasted on yellow sticky paper. A transparent colorless plastic sheet measuring 20 X 15 cm was sprayed with 5% strong volatile chemical glue without yellow dye. 12.5% and 15% SVCYG01 were separately sprayed evenly on a colorless plastic plate (20 × 15 cm), and the trap samples were placed in an organic net room with bok choy. After three days, the number of flea beetles in each trap was recorded, and the differences in the numbers of fleas between treatments were compared using Duncan's statistical analysis.

2) *Experiment on the trapping effect of SVCYG01 with allyl-isothiocyanate at various concentrations to catch flea beetles.*

The AIC formula was mixed with a uniform yellow dye, adhesive glue and an organic solvent, to final concentrations of 7.5, 10, 12.5 and 15%. A propellant (LP) was poured into the aluminum can using a high-pressure method. A white sika plate (20 × 15 cm) was sprayed evenly with the mixture, and the plates were spaced 7–8 meters apart in an organic net room with bok choy. After three days, the numbers of flea beetles caught by the traps were recorded, and the differences in the numbers of flea beetles caught between treatments were compared using Duncan's multiple range statistical analysis in order to determine the attractive effects of different concentrations of AIC.

G. Test of trap height for catching flea beetles in a bok choy field

The effect of trap height on the catching of flea beetles was examined using a strong volatile spraying glue trap. Tests were conducted in an organic net room, in which traps were hung from support cables, and in a bok choy field, three trap heights (0–10, 20–30 and 40–50 cm) being tested. Traps were collected after 3 days. Three replicates were used in all trials, and treatments were completely randomized. Trap data were analyzed and compared using Duncan's multiple range test.

III. RESULTS AND DISCUSSION

A. *Color preference test for the flea beetle performed in a bok choy field.*

This experiment was conducted using 12 different colored sticky plates of orange, blue, purple, black, pink, green, red, blue, yellow, brown, dark blue and white. The results are presented in Figure 1. It was found that the number of insects caught could be divided into three levels: yellow (198) was the best; the middle level included green (139.3) and grass green (115.3); while the rest included orange, blue, purple, black, pink, red, brown, dark blue and white, at only 7–50.3. According to a previous report [13], most insects demonstrate a preference for yellow, but the combined response of some insects to the visual and perceptual effects of color cannot be explained only by light wavelengths visible to humans. In fact, insects' preferences for color may vary depending on the background of the experiment, plant species, pest species, sex, color, and photometric factors [15]. In general, yellow is more attractive to insects, but this may be affected by differences in the vicinity of the test field, and so in some cases, white and light green sticky traps may be more effective. The results of this study showed that the flea beetle's preferred color is yellow, followed by green and grass green. Our results were consistent with a previous report [17] that pointed out that fleas have a preference for yellow sticky plates as compared with other colors. Therefore, yellow sticky traps can be used as a method by which to kill large numbers of flea beetles in a field and to monitor the density of the flea beetle population in order to assess whether it should be immediately controlled using chemical pesticides.

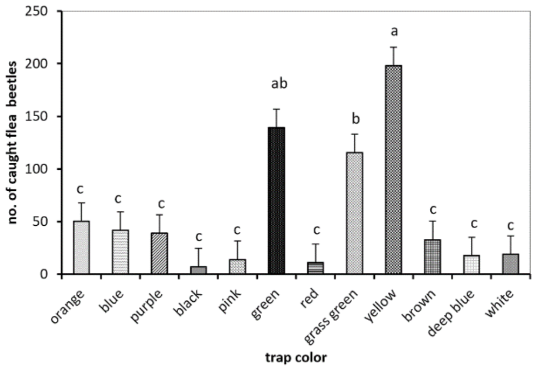

Figure 1. Comparison of the caught effects of different colored sticky traps for adult flea beetles

B. *Volatile chemical preference test for the flea beetle performed in a radish field.*

The results showed that of the volatile chemical compounds examined in this study, AIC exhibited the best trapping effect, at 12.9-fold higher than the control group, followed by EIC and mustard oil, at 4.8-fold and 3.4-fold higher than the blank group, respectively. BIC exhibited a 1.20-fold increase as compared with the control group (Table 1).

TABLE I. TRAPPING EFFECTS OF SEVERAL VOLATILE CHEMICALS USED IN YELLOW STICKY TRAPS TO CATCH FLEA BEETLES.[1]

Volatile chemical	Average trapped flea beetle no.	Total trapped flea beetle no.	Trapped ratio
Blank	8.33 ± 7.77[1]a[2]	25	1.0
Mustard oil	28.67 ± 5.03ab	86	3.4
Benzyl-isothiocyanate	10.00 ± 7.81a	30	1.2
Ethyl-isothiocyanate	40.00 ± 17.35b	120	4.8
Allyl-isothiocyanate	107.33 ± 21.73c	322	12.9

[1] Values are presented as means ± SD.
[2] Means within a column followed by the same letter were not significantly different (*P*>0.05; Duncan's multiple range test).

Prokopy and Owens [18] and Liao [19] pointed out that the addition of isothiocyanate to yellow sticky traps can increase the number of flea beetles trapped. Cruciferous vegetables include a wide range of species, such as the common cabbage, cauliflower, radish and other edible plants. The above-mentioned vegetables are also known as the mustard family, and white and black mustard seeds contain a considerable amount of mustard oil [20]. Reports [19,20] have indicated that certain components of mustard oil have trapping effects on flea beetles, and its main component is isothiocyanic acid propylene ester, a volatile and irritative agent. Other pest species such as the northern false chinch bug, *Nysins niger* [22], and the cabbage seed weevil, C*eutorhynchus assinilis* [12] also have an olfactory attraction to isothiocyanate propene, which demonstrates that isothiocyanate has a similar trapping effect on different insect species.

C. *Tests of optimal adsorption materials and volatile chemical release rates for traps.*

1) *Experiment on the odor release rate of AIC adsorbed by transparent soap.*

An odor detector was used to detect the release rates of 10 and 5% AIC adsorbed into a transparent soap base after 0–5 days (Table 2), which were 183.3, 144.7, 109.3, 112.3, 129.3, 130 and 184, 145, 120.7, 114.7, 122.3, 135 mg/M3, respectively. The results showed that AIC mixed with a sunflower oil fusion soap base had a strong odor on the first day, which was then much lower after the second day, consistent with the actual trapping results obtained in the field.

2) *Experiment on the odor release rates of allyl-isothiocyanate at different volumes adsorbed by a multi-layer paper patch.*

The odor release rates of AIC at different volumes adsorbed by a multi-layer paper patch after 0 – 5 days are presented in Table 3.

After the multi-layer paper patch had adsorbed 90% sunflower oil and 10% AIC at different volumes, the odor emission rates were measured after 0–5 days (Table 3), and the results showed a large decrease in odor, from 158.7–170.0 on day 0 to 87.0–88.7 on day 2. However, comparing the volatile chemical-laced multi-layer paper patch with the soap base, which had odor release values of 183.3 – 184 on day 0, reducing to 109.3–120.7 on day 2, and the odor, was stored in the latter for a longer duration, but the method is not as simple for farmers to employ. Therefore, convenient use and long-lasting attract effects must be considerations during the R&D process of products.

TABLE II. ODOR RELEASE VALUES (MG/M^3) OF 10 AND 5% ALLYL-ISOTHIOCYANATE IN A SOAP BASE FOR 0 TO 5 DAYS.

Attractant formula/days	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
80% soap base +10% Allyl-isothiocyanate +10% sunflower oil	183.3 ± 1.2[1]a[2]	144.7 ± 3.8b	109.3 ± 10.2c	112.3 ± 3.1c	129.3 ± 5.5c	130 ± 15.1c
80% soap base +5% Allyl-isothiocyanate +15% sunflower oil	184 ± 3.6a	145 ± 5.6b	120.7 ± 10.4c	114.7 ± 8.1c	122.3 ± 2.3c	135 ± 0c

[1] Values are presented as means ± SD.
[2] Means within a column followed by the same letter were not significantly different (P>0.05; Duncan's multiple range test).

TABLE III. ODOR RELEASE RATES (MG/M3) OF ALLYL-ISOTHIOCYANATE AT DIFFERENT VOLUMES ADSORBED BY A MULTI-LAYER PAPER PATCH AFTER 0–5 DAYS.

Volume/days	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
0.4 ml	158.7 ± 7.1[1]a[2]	101.0 ± 0b	87.0 ± 0c	87.0 ± 0c	83.3 ± 1.5c	75.6 ± 1.2c
0.6 ml	163.7 ± 1.5a	104.3 ± 0.6b	88.0 ± 0c	87.7 ± 0.6c	83.7 ± 0.6c	76.3 ± 0.6c
0.8 ml	165.3 ± 3.8a	103.0 ± 1.0b	87.7 ± 0.6c	88.0 ± 0c	84.7 ± 0.6c	76.0 ± 0c
1.0 ml	166.0 ± 1.7a	107 ± 1.7b	88.0 ± 0c	88.0 ± 0c	85.0 ± 0c	76.3 ± 0.6c
1.2 ml	170.0 ± 1.7a	111.7 ± 0.6b	88.7 ± 0.6c	88.0 ± 0c	85.0 ± 0c	76.7 ± 0.6c

[1] Values are presented as means ± SD.
[2] Means within a column followed by the same letter were not significantly different (P>0.05; Duncan's multiple range test).

D. Proportion testing of the strong insect attractant formula

1) *Caught effects of the allyl-isothiocyanate-adsorbed multi-layer paper patch on yellow sticky paper and the 12.5–15% strong volatile chemical yellow glue (SVCYG01) spray on plates for the control of flea beetles.*

Multi-layer paper with adsorbed AIC on commercial yellow sticky paper (21.5 × 15 cm) and the 12.5–15% strong volatile chemical yellow glue (SVCYG01) sprayed on colorless plates were used to catch flea beetles (Figure 2.). The experimental results showed that the attractive effect of 15% SVCYG01 was high, at 4.4-fold higher than the control. The caught ratios of 12.5% SVCYG01 and the multi-layer volatile sticky trap were 3.2-fold and 3.3-fold higher than the control. However, the transparent plate sprayed with 5% SVCYG01 has a caught ratio 0.8-fold lower than that of the control.

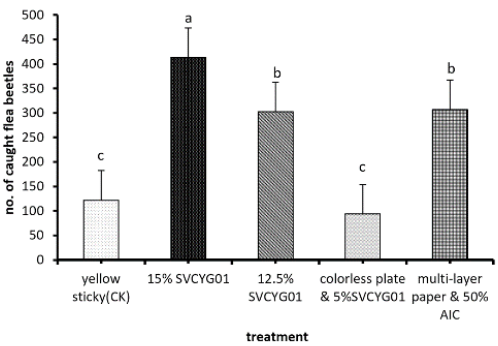

Figure 2. Caught effects of the allyl-isothiocyanate-adsorbed multi-layer paper patch on yellow sticky paper and 12.5–15% strong volatile chemical yellow glue sprayed on plates to control flea beetles.

2) *Caught effects of volatile chemical yellow glue at different concentrations for the control of flea beetles in the field.*

The trapping effects of different concentrations of SVCYG01, i.e., 7.5%, 10%, 12.5% and 15%, to catch flea beetles in the field (Figure 3) were measured, and the results showed that the attractive effect of 15% SVCYG01 was high, at 4.4-fold higher than the control (CK). The caught ratios resulting from the use of 7.5%, 10% and 12.5% SVCYG01 were 1.6 – 2.3-fold higher than that of the control, although no significant differences were observed.

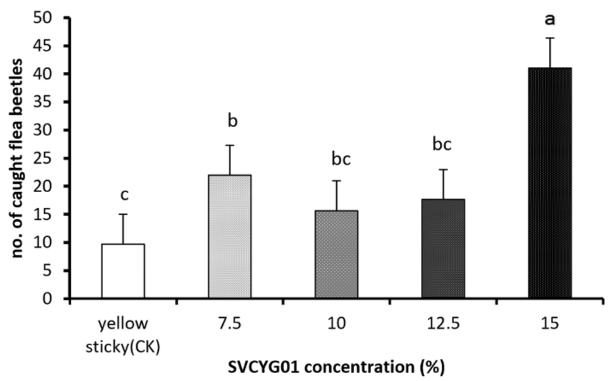

Figure 3. The trapping effects of different SVCYG01 concentrations to catch flea beetles in the field.

E. Experiment on the trapping effects of a powerful multi-layer paper patch trap and a strong volatile chemical yellow glue sprayed on a plastic bottle to attract flea beetles in the field.

1) *Comparison of the trapping effects of the multi-layer paper patch with a strong odor and commercial yellow sticky paper for catching flea beetles in the field.*

A powerful multi-layer paper patch containing 1.2 ml AIC on commercial yellow sticky paper was compared with the commercial yellow sticky paper only (blank) in terms of the trapping effect of flea beetles in an organic radish field (Figure 4). The test results showed a powerful trapping ability of the multi-layer paper patch adhered to yellow sticky paper, which was 30.6-fold higher than that of the control group.

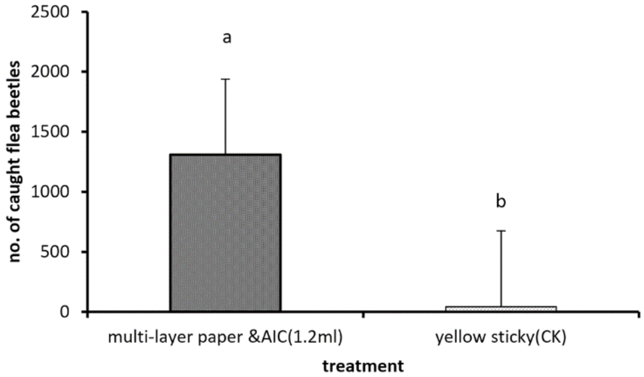

Figure 4. Comparison of the trapping effects of a multi-layer paper patch with a strong odor and commercially-available yellow sticky paper to catch flea beetles in the field.

2) *Trapping effects of SVCYG01 sprayed on a plastic bottle for attracting flea beetles in the field.*

The effects of SVCYG01 sprayed on a plastic bottle or pearl board placed in a vegetable field to catch flea beetles were measured (Figure 5), and the test results showed that the yellow spraying glue containing AIC quickly attracted flea beetles in the field, resulting in great reduction in the number of flea beetles. This will effectively reduce the vegetable damage rate and decrease the mating and breeding rates of adult flea beetles. This method can also be combined with other comprehensive prevention methods for the control of flea beetles in an integrated pest management strategy. Therefore, the use of pesticides can be reduced, and hence the occurrence of pesticide residues will decrease.

F. Comparative test of the trapping effect of the height of the sticky trap for the control of flea beetles in the field.

Comparison of the trapping effects of 15% SVCYG01-sprayed plates placed at heights from the vegetable leaf of 0–10 cm, 20 – 30 cm and 40 – 50 cm to catch flea beetles (Figure 6). The test results showed that the trapping effect at a height of 0 – 10 cm from the vegetable leaf surface was 5.1-fold that at 40 – 50cm, the number of fleas caught decreasing with the height of the trap. We therefore recommend the use of a trap plate hanging to approximately 10 cm higher than the vegetable leaf, which has the best trapping effect.

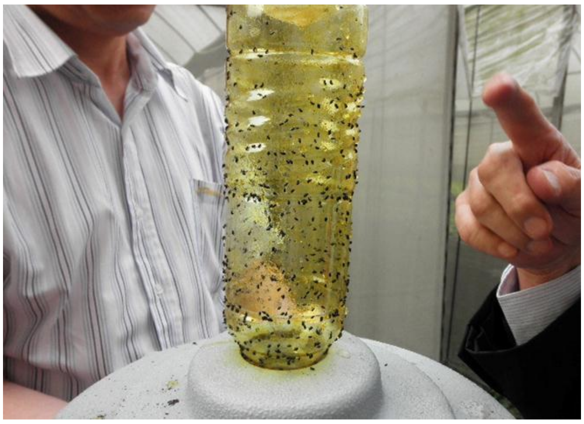

Figure 5. Strong volatile chemical yellow glue sprayed on a plastic bottle for attracting flea beetles in the field.

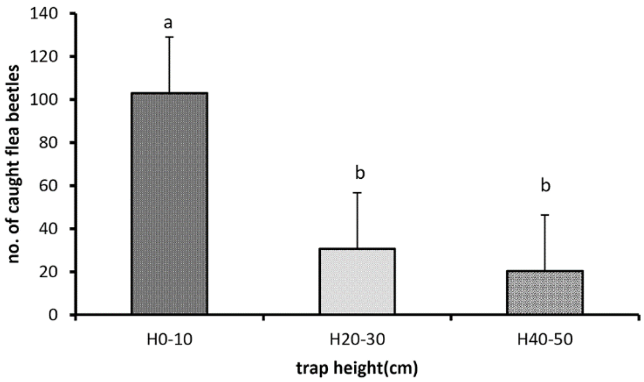

Figure 6. Comparison of the trapping effect of the sticky plate trap at different heights above the vegetable leaf level to catch flea beetles in the field.

IV. CONCLUSION

In this study, a powerful odor attractant patch and a powerful odor attractant spray glue product were applied for the prevention and control of an important pest that attacks vegetables. Significantly greater numbers of trapped adult flea beetles were observed on the odor attractant-treated rows than on untreated rows of bok choy in a trapping experiment. The attraction to the volatile chemical trap appeared to diminish after 2 days in the field; however, further research into volatile chemical release rates and types of dispenser and adsorbent materials could improve this situation. Martel *et al.* [16] pointed out that the use of odor attractants to trap pests can reduce pesticide use by 44%. The results of this study demonstrated the potential for use of a strong odor attractant patch and strong odor attractant spray glue for the control of pests such as flea beetles and white powder flies, resulting in significant reduction in the use of pesticides and the risk of pesticide residues, and providing infinite value in terms of ecological protection. In addition, according to a market survey, no similar product is currently available on the market in Taiwan. Therefore, this research and development of a strong odor attractant patch and powerful odor attractant spray glue for the control of flea beetles is unique and original. It is hoped that these products can be developed into high-quality green non-pesticide insect control materials for use by organic farmers and general farmers in order to increase the industrial income and reduce the use of pesticides to ensure consumer safety.

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