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Article

Integrating Trap Cropping and Entomopathogenic Nematode Foliar Sprays to Manage Diamondback Moth and Imported Cabbage Worm

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Abstract: Diamondback moth (DBM), *Plutella xylostella*, and imported cabbage worm (ICW), *Pieris rapae*, are destructive pests of crucifers worldwide. Although several insecticides are effective against ICW, pesticide management against DBM is challenged by insecticide resistant populations. The objective of this study was to explore the potential of integrating foliar sprays of the entomopathogenic nematode (EPN) *Steinernema feltiae* with trap cropping using kai choi (*Brassica juncea*) planted as an intercrop for the management of DBM and ICW. Four 2 × 2 (trap crop × EPN) factorial designed field trials were conducted with 2 trials on head cabbage (*Brassica oleraceae* var *capitata*) and 2 on kale (*Brassica oleraceae* var *acephala*). In the first head cabbage trial, trap cropping reduced DBM abundance by 46% and ICW abundance by 73%. Leaf damage by DBM and ICW were reduced by 45% and 33%, respectively. In the second head cabbage trial, DBM populations were reduced by 19% whereas ICW was reduced by 65%. No effects were observed on leaf damage. Trap cropping suppressed DBM abundance by 50% and DBM leaf damage by 19% in the first kale trial. No significant effects were observed on ICW. In the second kale trial, trap cropping reduced ICW leaf damage by 13%. In the first head cabbage trial, adding EPN foliar sprays further reduced DBM populations in plots with trap crops and ICW in plots without trap crops. In the second kale trial, EPNs suppressed DBM populations entirely. No effects from EPNs were observed in the second head cabbage trial or the first kale trial. It is concluded that trap cropping with kai choi did not improve the efficacy of EPN foliar sprays consistently. EPNs were most successful at suppressing DBM and ICW populations when the average pest pressure was below 0.5/plant whereas trap crops worked more effectively at insect populations above 0.5/plant. Although the use of trap cropping reduced pest abundance and leaf damage, the weight of head cabbage and kale was lower when planted 30 cm or closer to kai choi plants. This was resolved by leaving a distance of 60 cm between cash and trap crops. With further optimization, the use of trap cropping and EPN foliar sprays can be beneficial to an integrated pest management program to control DBM and ICW in cruciferous crops.

Keywords: *Brassica juncea*; *Steinernema feltiae*; integrated pest management; *Plutella xylostella*; *Pieris rapae*; head cabbage; kale

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1. Introduction

Farm scaping of agriculture ecosystems by intercropping, trap cropping, planting border crops or living mulch can aid in enhancing natural enemies of pests or provide a better niche to allow introduced biocontrol agents to perform more effectively [1]. This project aims to evaluate if intercropping trap crops could provide multiple benefits to manage insect pests in cruciferous cropping systems. Cabbage (*Brassica oleracea* var *capitata* and kale (*Brassica oleracea* var *acephala*) are plagued by diamondback moth (DBM) (*Plutella xylostella*), imported cabbage worm (ICW) (*Pieris rapae*), cabbage webworm (*Hellula rogatalis*), and cabbage looper (*Trichoplusia ni* (Hübner) [2]. Among these pests, DBM is considered the most damaging [3,4] inflicting up to 90% yield loss on cruciferous crops if unmanaged [4–6] followed by ICW with 71% [7] yield losses. Although several chemicals against ICW are still effective, pesticide management against DBM is challenged by the development of insecticide resistance against multiple pesticides [8–10]. The insecticide resistance management (IRM) program in Hawai'i recommends a complete 6-month insecticide rotation program with different mechanism of action groups being used every month [11] to control Bt and spinosad-resistant DBM. While conventional growers can mitigate resistance by doing this, organic growers have fewer pesticide options and are primarily limited to the rotation of only Bt and spinosad. In addition, due to the cryptic behavior of DBM hiding inside the spongy leaf tissue during its early instar stage, it is a challenge to rely on contact pesticides to kill the larvae effectively. Thus, it is imperative to identify other biocontrol agents and cultural practices to assist organic farmers in managing DBM infestations.

Entomopathogenic nematodes (EPNs) of the families Steinernematidae and Heterorhabditidae have been identified as biocontrol agents against insect pests as they are obligate parasites that kill insects with the help of mutualistic bacteria that inhabit the intestine of the infective juveniles (IJs) [12]. EPNs have been used with variable success for controlling DBM. Under laboratory conditions, *Steinernema thermophilum* caused 100% mortality of DBM within 48 h after inoculation [13]. The same EPN species applied at 1000 to 3000 IJs/mL along with an adjuvant (0.033% APSA80) as a foliar spray caused 35–46% mortality of DBM on head cabbage in the field [14]. Similarly, *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* caused 84% and 100% DBM mortality, respectively in the laboratory [15] whereas in a watercress field *S. carpocapsae* only achieved 41% control of DBM larvae [16].

Failure of EPNs to achieve effective control of DBM in the field could be due to environmental factors such as field desiccation, heat, and ultraviolet (UV) radiation [17]. Adding adjuvants to EPN sprays in the field has been found to improve [18] or not improve [19] EPN efficacy. A review by Somvanshi and Ganguly [20] suggested that EPNs should be one of the components in an integrated pest management (IPM) program against DBM. Perhaps integrating trap crops with foliar EPN applications could modify the field environment to optimize EPN survival and infection of DBM larvae.

Trap crops are plants grown close to a cash crop that can attract, retain, or intercept target pests to reduce or eliminate the pest damage on the cash crop [21]. The most tested trap crops against DBM are collard greens (*Brassica oleracea* var *acephala*), mustard (*Brassica juncea*), kale (*B. oleraceae* var *acephala*), and yellow rocket (*Barbarea vulgaris*) [22,23]. However, results can be contradictory. Indian mustard (*B. juncea*) was very effective as a trap crop for DBM in cabbage fields in Sweden and India [24,25] whereas it was not effective in reducing DBM populations on head cabbage in Hawaii and Texas [26,27].

A preliminary experiment conducted in Hawaii that compared five brassica crops including 'Joy choi' and 'Mei Ching' pak choi (*Brassica rapa* subsp. *chinensis*), 'Hirayama' kai choi (*Brassica juncea*), 'KK' head cabbage, and 'Starbor' kale for their attractiveness to DBM showed that DBM fed more on kai choi or mustard greens when interplanted with head cabbage [28]. Thus, this research aimed to evaluate the use of kai choi as a trap crop interplanted between two commonly grown cruciferous crops, head cabbage and kale in Hawaii to manage DBM and ICW.

The mechanisms for kai choi to function well as a trap crop for DBM and ICW could be multi-fold: (1) lower wax content on kai choi leaves than on head cabbage would improve the adhesiveness of DBM and ICW eggs thus attracting more egg laying on kai choi than on cabbage [29]; (2) higher larval predation on kai choi due to easier movement of predators on glossy (non-waxy) leaf surfaces [30], thus reducing larval survival rates; (3) higher glucosinolate sinigrin concentration in kai choi than head cabbage or kale is another factor that increases the feeding preference of DBM on this trap crop [31].

The specific objectives of this research were to integrate the use of an indigenous EPN isolated from Hawaii, *Steinernema feltiae* MG-14, as a biological control agent with kai choi as a trap crop to reduce DBM and ICW damage on head cabbage and kale in field settings. It is hypothesized that (1) kai choi as a trap crop would suppress the abundance and damage of DBM and ICW, (2) EPN foliar sprays would suppress the abundance and damage of DBM and ICW, and (3) a combination of trap crops and EPNs would produce an additive effect greater than each of these treatments alone. Hence, we selected to evaluate two main factors: trap cropping and EPN application and their combination on suppressive effects against DBM and ICW compared to an untreated control.

2. Materials and Methods

2.1. EPN Inoculum

Prior to the initiation of each field trial, EPN inoculum, *S. feltiae* MG 14, was prepared in the laboratory by inoculating 100 infected juveniles (IJs) per mealworm larvae (*Tenebrio molitor*) with 10 mealworm larvae per 100-mm-d Petri dish. Newly emerged IJs of *S. feltiae* were then harvested on White traps [32] and stored at 15 °C until use within 30 days after emergence.

2.2. Head Cabbage Field Trial

Two field trials were conducted, Trial I was on Oahu in Waialua, HI (21.56124° N, 158.1285° W), from 20 March to 10 May 2020, and Trial II was on Kauai in Kapaa, HI, USA (21.97656° N–159.7166° W) from 20 April to 10 June 2021. Six-week-old 'KY' head cabbage was transplanted at 30 cm spacing between plants in a row on a 90 cm wide planting bed. A 2 × 2 (trap crop × EPN) factorial designed experiment was installed to examine two key factors listed in the objectives. For plots designated as 'Trap Crop', all cabbage plants were interplanted with 'Hirayama' kai choi as a border crop. In "No Trap Crop' plots only head cabbage was planted. For treatments receiving EPNs (EPN+), cabbage was sprayed with *S. feltiae* MG-14 at 1000 IJs per plant (equivalent to 125 million IJs/ha) delivered through 8 mL of water suspension per plant and applied as described for each trial below mostly based on the economic threshold of DBM per plant (0.5/plant). Slight modifications between trials are described as below.

In Trial I, kai choi was planted at 30 cm between plants and planted 15 cm away from head cabbage (Figure 1A). Each plot had 5 cabbage plants. The field trial was surrounded by more than 4.5 m of bare fallow areas during the trial. For treatments receiving EPNs (EPN+), plants were sprayed with *S. feltiae* MG-14 at 1000 IJs per plant (equivalent to 125 million IJs/ha) delivered through 8 mL of water suspension per plant the 4th week after transplanting. The spray solution contained Barricade® gel (Barricade International, Inc., Hobe Sound, FL, USA) as an anti-desiccant and PABA (Sigma Chemical, Steinheim, Germany) as a UV protectant. EPN-plots did not receive the foliar spray. Each treatment had 4 replications. All treatment plots were irrigated, fertilized, and occasionally sprayed with Bt as needed following commercial farming practices. The percentage of leaves with DBM or ICW damage on each plant were recorded from 4 plants per plot weekly from transplanting to harvesting, at 7-week intervals. The abundance of insect eggs, larvae, pupae, and adult stages of DBM and ICW per plant were also recorded from 4 plants per plot weekly.

The second trial (Trial II) was conducted from 20 April to 10 June 2021, in Kauai with slight modifications. Six-week-old 'KY' head cabbage was transplanted at 30 cm spacing between plants in a row on a 90 cm wide planting bed (Figure 1B). Each plot had 16 cabbage plants surrounded by 8 kai choi planted 30 cm away from head cabbage on both sides. The plots designated as EPN+ received *S. feltiae* MG-14 at 1000 IJs/plant (125 million IJs/ha) the 2nd, 4th and 6th week after transplanting. The spray solution contained Oroboost® (Oro Agri, Inc. 2788 S. Maple Ave., Fresno, CA 93725, USA) as an adjuvant at 4.5 mL/L of nematode suspension.

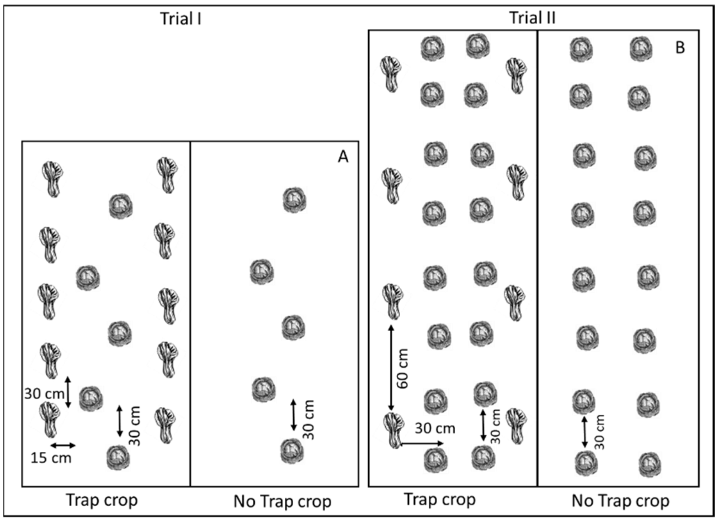

Figure 1. Kai choi trap crops around head cabbage in (**A**) Trial I and (**B**) Trial II.

In this trial, rating of level of DBM or ICW damage was based on a scale of 0–4 where 0 = no damage, 1 = 1–10% damage, 2 = 11–25% damage, 3 = 26–50% damage, and 4 = 51–100% damage (Figure 2).

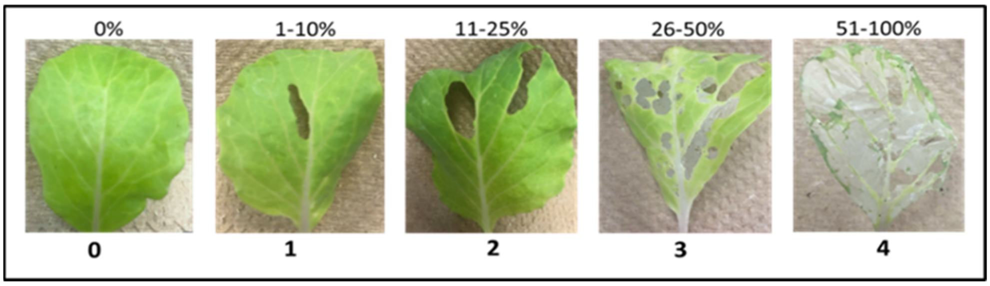

Figure 2. Damage index scale of 0–4 caused by diamondback moth or imported cabbage worm on a cabbage leaf.

Unlike Trial I, the insect count and damage were recorded from 4 plants per plot bi-weekly from transplanting to harvesting over a 7-week cropping period. Head cabbage weight from each plant was recorded at harvest.

2.3. Kale Field Trial

Two field trials were conducted in Waianae, HI on Oahu (21°27′13.2″ N 158°09′03.8″ W). The first trial (Trial I) was conducted from 13 May to 24 June 2020 to evaluate the effects of trap crops and EPN sprays on curly kale against DBM and ICW. The trials were arranged in a 2 × 2 (trap crop × EPN) factorial design with 4 replicated plots. Seven-week-old kale seedlings were transplanted in two rows per plot at 30 cm spacing between plants and between rows (Figure 3A). Each plot was 1.2 × 1.8 m^2 surrounded by 2 rows of kai choi seedlings transplanted at the same time. A total of 16 plots with 12 kale plants per plot were established. For 'Trap Crop' plots, 'Hirayama' kai choi was planted at the edge of the plot with 30 cm between plants and 30 cm from the kale plants. For the plots designated as EPN+, kale plants were sprayed with water suspension containing 1.6 mL/L of Oroboost® (Oro Agri, Inc. 2788 S. Maple Ave., Fresno, CA, USA) with *S. feltiae* MG-14 applied at 1000 IJs/plant (125 million IJs/ha) once on the 3rd week after initiation of the trial. All the treatment plots were irrigated, fertilized, and occasionally sprayed with Bt as needed following commercial farming practices.

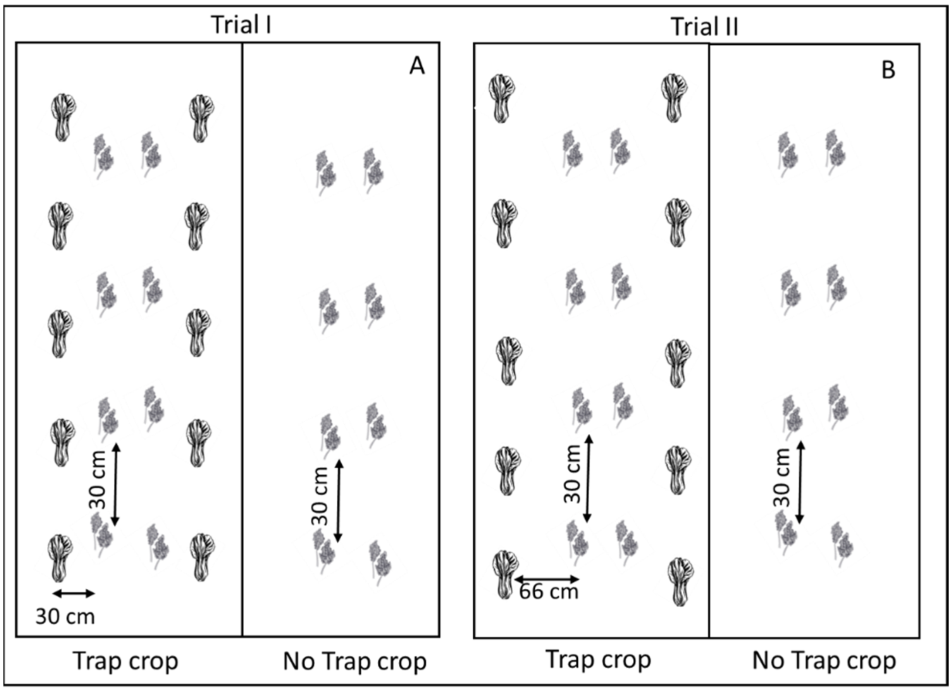

Figure 3. Kai choi as  trap crops around kale crop in  (**A**) Trial I and (**B**) Trial II.

Four randomly selected plants were recorded from each plot for (1) percentage of leaves with DBM or ICW damage, and (2) abundance of eggs, larvae, pupae, and adult stages of DBM and ICW per plant weekly for 5 weeks. Kale leaves were harvested weekly starting from the third to fifth weeks after transplanting.

A second field trial (Trial II) was conducted from 22 June to 16 July 2020 in a kale field next to Trial I. Slight modifications from Trial I were performed whereby plot size was reduced to 1.2×0.9 m^2 with 8 kale plants per plot. Kai choi was planted 66 cm away from the kale planting rows. For EPN+ plots, the kale plants received a foliar spray of *S. feltiae* at 125 million IJ/ha using Oroboost® as an adjuvant like in Trial I except that it was applied on the first week of data collection. Both kale and kai choi in all treatment plots were irrigated, fertilized, and occasionally sprayed with Bt as needed following commercial farming practices. Insect counts and damage data were collected over 5 weeks from 4 plants per plot. Kale was harvested from the 3rd to 5th week after initiation of Trial II.

2.4. Statistical Analysis

Data from each field trial were checked for normality using Proc Univariate in Statistical Analytical Software (SAS) version 9.4 (SAS Institute Inc., Cary, NC, USA). Insect counts were normalized using log10 (x + 1) whenever needed before analysis of variance (ANOVA). Insect counts and damage data from cash crops in each trial were subjected to repeated measures ANOVA using Proc GLM in SAS with sampling dates blocked within treatments. If no significant interaction between treatment and sampling date occurred, data across sampling dates were pooled for ANOVA, whereas if significant interaction occurred, data were analyzed by date. All data were subjected to 2×2 (Trap crop $\times$ EPN) factorial analysis of variance (ANOVA) in a randomized complete block design using SAS. To examine the short-term effect of EPNs, the reduction in DBM and ICW abundance 1 week after EPN application were compared among treatments. To further understand the feeding preference of DBM and ICW, abundance and damage of DBM and ICW on cabbage and kai choi within the trap crop plots were compared using one-way ANOVA. Means were separated by the Waller-Duncan k-ratio (k = 100) *t*-test wherever appropriate, but only true means were presented. Similarly, yield data were also subjected to 2 × 2 factorial ANOVA and Waller-Duncan k-ratio (k = 100) *t*-test when appropriate.

3. Results

3.1. Head Cabbage Field Trial

In Trial I, based on the 2 × 2 ANOVA with sampling dates blocked within treatments, interaction between trap crop and EPN effects were observed for number of DBM ($F (1, 333) = 6.5$, $p < 0.01$) but not DBM damage, nor ICW number and damage ($p > 0.05$). However, the interaction between treatment (trap crop × EPN) and date was not significant for number of DBM but were significant for DBM damage, ICW number, and ICW damage. Thus, the means of DBM were presented by trap crop × EPN on each sampling date (Figure 4A), whereas the means of DBM damage, ICW number, and ICW damage were presented for trap crop by date (Figure 4B–D). Since EPNs were only expected to be effective after an EPN spray, comparison between EPN treatments was only conducted on the changes before and after the EPN spray on 23 May 2020. Interaction between trap crop × EPN was significant for changes in DBM abundance after EPN application (Figure 5A), but no significant difference between EPN treatments was observed regardless of T or NT (Figure 5B). Significant interaction between trap crop and EPN also occurred in changes of ICW populations after EPN application ($F (1, 56) = 8.93$, $p < 0.01$) where EPN only reduced ICW abundance in the NT plots ($F (1, 56) = 8.93$, $p < 0.01$).

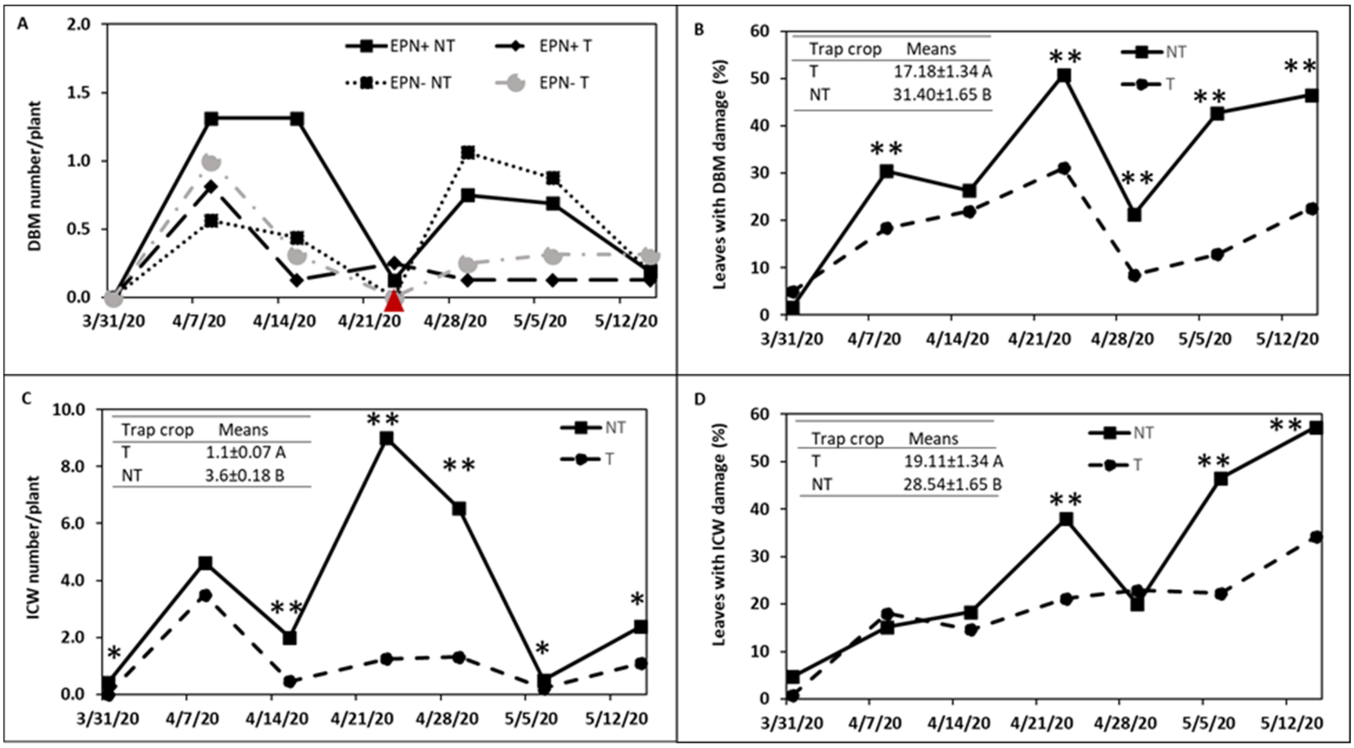

Figure 4. Effect of kai-choi as trap crop and entomopathogenic nematodes (EPN) on the reduction of (**A**) Abundance of diamondback moth (DBM) after EPN application; (**B**) Percentage of leaves with DBM damage; (**C**) Abundance of imported cabbageworm (ICW); (**D**) Percentage of leaves with ICW damage on head cabbage in Trial I. T = trap crop, NT = no trap crop. Δ Indicates EPN application. * and ** indicate significant differences between trap crop (T) and no trap crop (NT) at $p \leq 0.05$ and 0.01, respectively. Means followed by the same letters are not different based on analysis of variance.

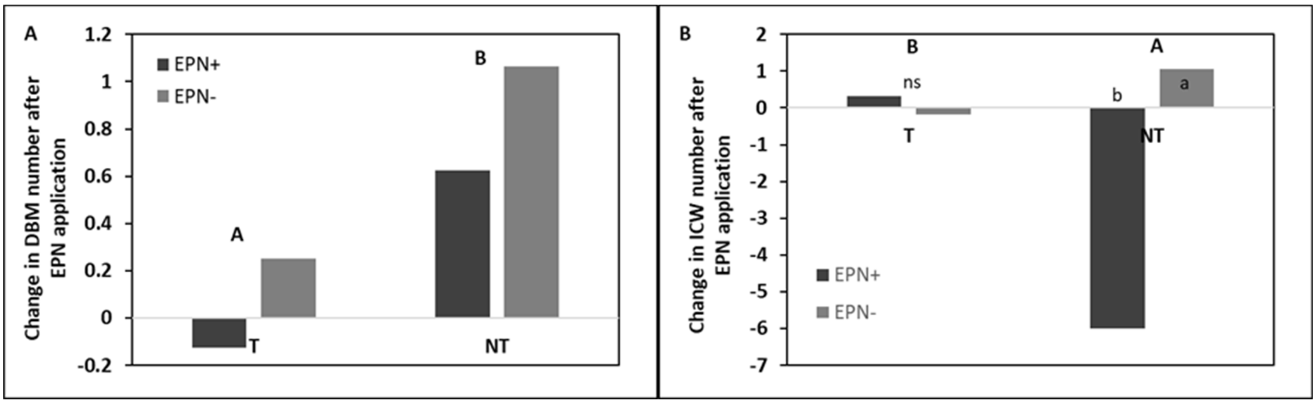

Figure 5. Effect of entomopathogenic nematodes (EPN) on the reduction in number of (**A**) Diamondback moth (DBM); (**B**) Imported cabbageworm (ICW) before and one week after EPN application in trap crop (T) and no trap crop (NT) plots on head cabbage in Trial I. Means (n = 16) of trap crop treatments or EPN treatments followed by the same letter were not different according to analysis of variance. ns = no significant difference between EPN+ and EPN−.

Planting of kai choi as a trap crop in Trial I suppressed the total abundance of DBM (F (1, 333) = 13.13, $p \leq 0.01$; Figure 4A) regardless of EPN treatment. While no significant effects of EPN application on DBM abundance was observed in the repeated measure analysis, a slight reduction in DBM number was observed 1 week after the application of EPNs on 23 April 2020 ($p > 0.05$; Figure 4A), but only in the trap crop treatment. On the other hand, the EPN treatment slightly increased DBM numbers when no trap crop was planted ($p > 0.05$; Figure 4A).

Planting of a kai choi trap crop reduced the percentage of leaves with DBM damage (F (1, 333) = 13.6, $p \leq 0.01$) by 45% compared to no trap crop on head cabbage (Figure 4B). Trap crop also reduced the abundance (F (1, 333) = 140.54, $p \leq 0.01$) and leaf damage of ICW (F (1, 333) = 30.8, $p \leq 0.01$) by 69% and 33%, respectively (Figure 4C,D). Soon after EPN application, though not significant, EPN+ was showing a trend in reducing DBM only in the T ($p > 0.05$) (Figure 5A). In contrary, EPN only reduced ICW number in the NT but not in the T plot (F (1, 333) = 8.64, $p \leq 0.05$; Figure 5B).

When comparing kai choi and cabbage within the trap crop plots, the abundance of ICW (F(1, 420) = 88.43, $p \leq 0.01$) and DBM (F(1, 420) = 41.02, $p \leq 0.01$) were higher on the head cabbage than the kai choi (Figure 6A,B), but feeding damage of ICW (F(1, 420) = 65.27, $p \leq 0.01$) and DBM (F(1, 484) = 145.74, $p \leq 0.01$) were more severe on kai choi compared to head cabbage (Figure 6C,D). No interaction between crop type and date was observed throughout the trial, thus only means of the main factor was presented.

In terms of cabbage yield, no interaction between trap cropping and EPN effect was observed. Thus, the data for main effect on cabbage yield were pooled. Since EPN effect was not significant, only means of trap cropping were presented. Planting of kai choi as a trap crop 15 cm away from the cabbage row reduced the head cabbage weight (F (1, 60) = 77.11, $p \leq 0.01$) by 41% compared to cabbage in no trap crop plots (Figure 7).

In Trial II, no interaction between trap crop and EPN was observed for any of the parameters measured, thus only means of the main treatments were presented. It is encouraging that planting kai choi as a trap crop suppressed 19% of DBM abundance (F (1, 180) = 3.83, $p \leq 0.05$) and 65% of ICW abundance (F (1, 180) = 15.79, $p \leq 0.05$) on cabbage (Figure 8A,C). However, trap cropping did not reduce the percentage of leaves with DBM or ICW damage ($p > 0.05$) in this trial (Figure 8B,D). EPN treatment also did not affect the number and damage of both ICW and DBM ($p > 0.05$, data not shown).

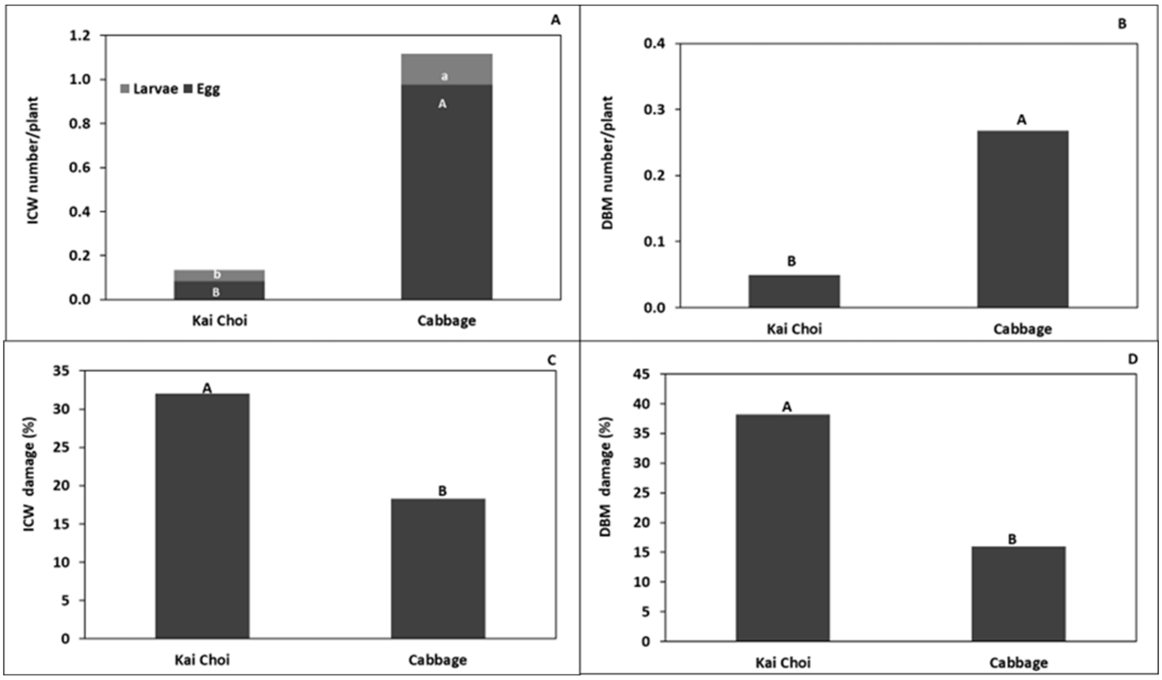

Figure 6. Abundance of (**A**) Imported cabbage worm (ICW) larvae and eggs; (**B**) Diamondback moth (DBM larvae); Percentage of leaf damage by (**C**) ICW or (**D**) DBM on kai choi and head cabbage. Columns (n = 224) followed by the same letter are not different based on analysis of variance.

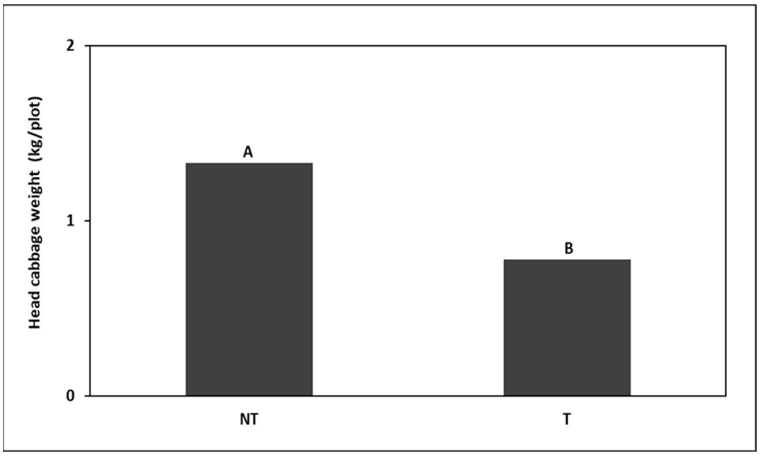

Figure 7. Effect of trap cropping on the productivity of head cabbage. T = trap crop, NT = no trap crop in the Waialua Trial (Trial I). Means followed by the same letters are not different based on analysis of variance.

In Cabbage Trial II, when comparing the abundance and damage of ICW between cabbage and kai choi within the trap crop treatment, ICW numbers (larvae and eggs) (F (1, 181) = 32.16, $p \leq 0.05$), ICW damage (F (1, 420) = 59.63, $p \leq 0.05$), and DBM damage (F (1, 181) = 23.79, $p \leq 0.05$) were higher on cabbage than on kai choi (Figure 9A,C,D). However, DBM numbers (F (1, 181) = 1.38, $p \leq 0.05$) were similar on cabbage and kai choi (Figure 9B).

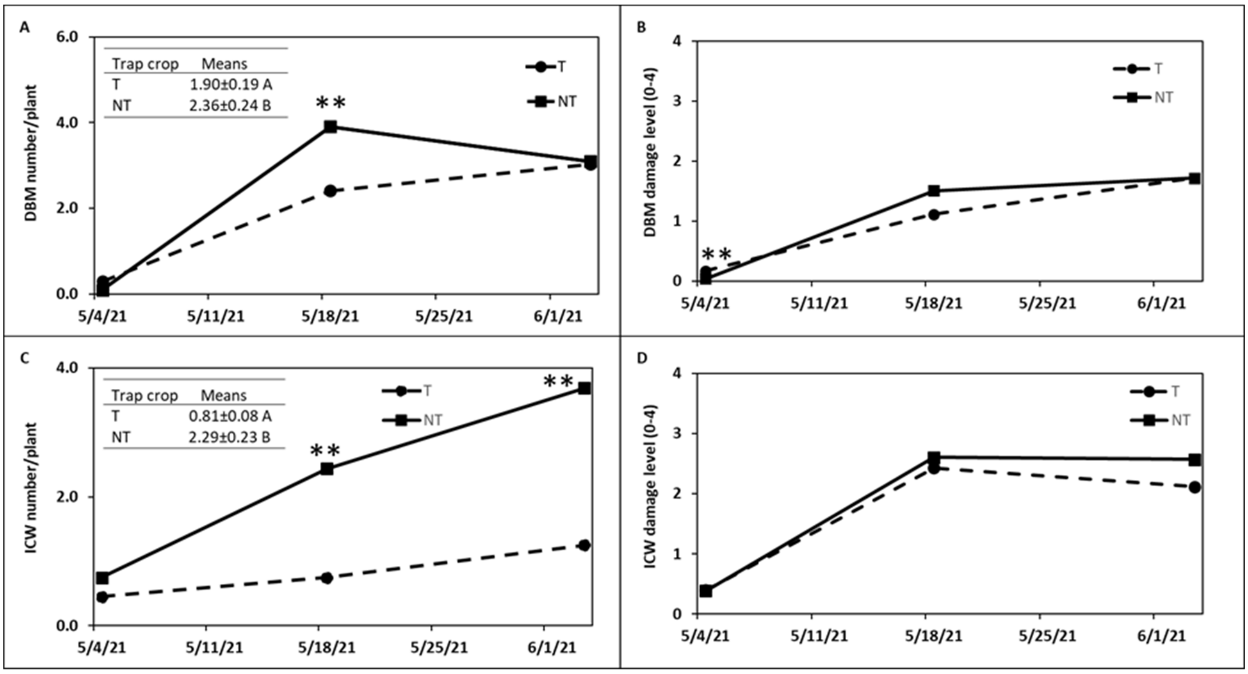

Figure 8. Effect of kai choi as a trap crop on (**A**) Abundance of DBM; (**B**) Percentage of leaves with DBM damage; (**C**) Abundance of ICW; (**D**) Percentage of leaves with ICW on head cabbage in Trial II. ** indicate significant difference between trap crop (T) and no trap crop (NT) at $p \leq 0.05$ and 0.01, respectively. Means followed by the same letters are not different based on analysis of variance.

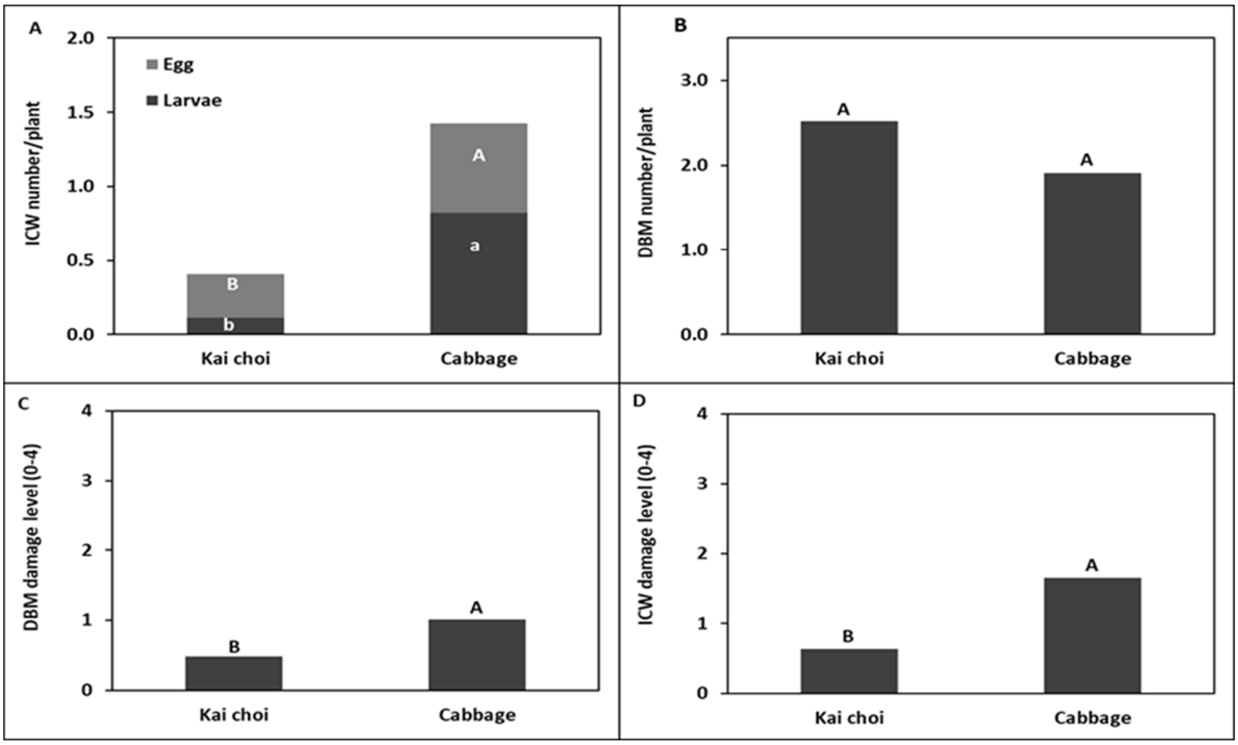

Figure 9. Abundance of (**A**) Imported cabbage worm (ICW) larvae and eggs; (**B**) Diamondback moth (DBM) larvae; (**C**) Percentage of ICW; (**D**) DBM leaf damage on kai choi and head cabbage in Trial II (Kauai Trial). Columns (n = 96) followed by the same letter in a graph are not different based on analysis of variance.

In terms of cabbage yield, neither trap cropping and EPN, nor their interaction affected the yield in this trial (Figure 10).

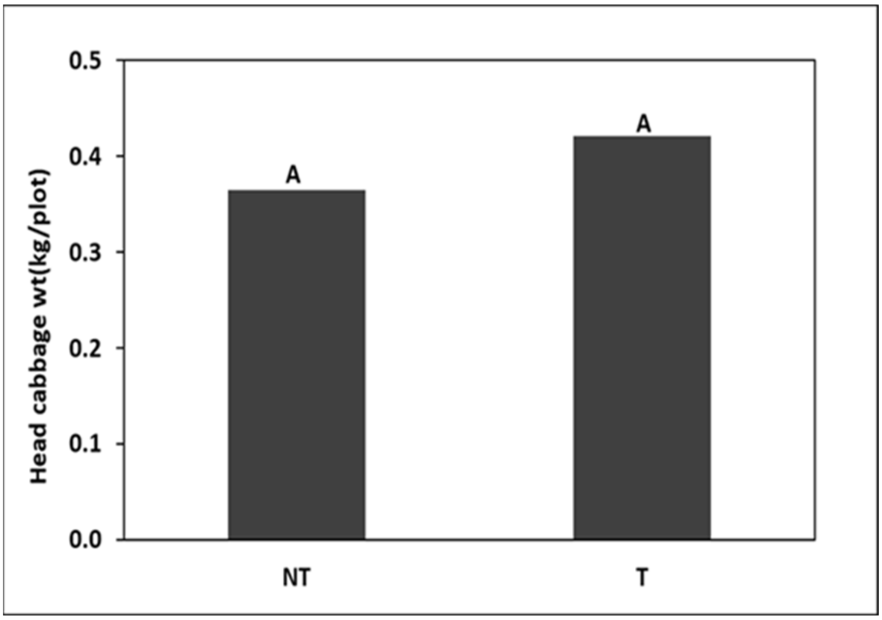

Figure 10. Effect of trap cropping on head cabbage yield in Cabbage Trial II. T = trap crop, NT = no trap crop. Means followed by the same letters are not different based on analysis of variance.

3.2. Kale Field Trial

In Kale Trial I, based on the 2 × 2 ANOVA, no interaction between trap crop and EPN effects was observed for any of the parameters measured, thus only means of the main treatments were presented. It is encouraging that planting of kai choi as a trap crop suppressed 50% of DBM abundance (F (1, 237) = 8.68, $p \leq 0.05$), mostly dominated by larva and pupal stages, and 19% of leaves with DBM damage (F (1, 237) = 11.54, $p \leq 0.01$) on kale (Figure 11A,B). However, the trap cropping effect was not significant ($p > 0.05$) for ICW number and percent of leaves with DBM damage ($p > 0.05$) in Trial I of kale (Figure 11C,D). On the other hand, EPNs did not affect the number and damage of both ICW and DBM ($p > 0.05$), thus data were not shown.

When comparing the abundance and damage of ICW between kale and kai choi within the trap crop treatment, ICW numbers (larvae and eggs) (F (1, 420) = 38.42, $p \leq 0.01$) and ICW damage (F (1, 420) = 4.61, $p \leq 0.01$) were both higher on kale than on kai choi. No interaction between crop type and date was observed, thus only means of the crop type were presented (Figure 12). Similarly, DBM abundance (larvae and pupae), was also higher on kale than on kai choi (F (1, 420) = 12.12, $p \leq 0.01$) but DBM damage (F (1, 420) = 44.61, $p \leq 0.01$) was higher on kai choi than on kale.

In terms of kale yield in Kale Trial I, ANOVA showed no interaction between trap cropping and EPN, and no significant effect from EPN applications. Thus, only means of trap cropping was shown. Trap cropping reduced kale weight in Trial I by 24% (Figure 13A) even though the leaf numbers were not different (Figure 13B).

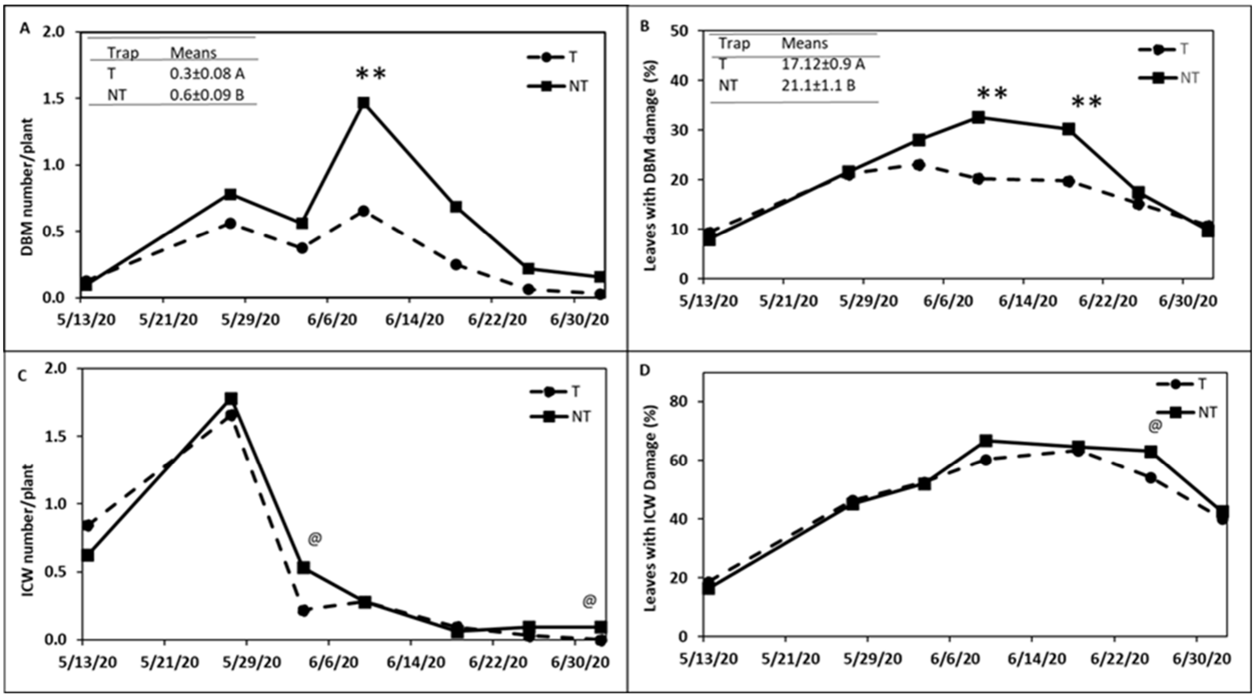

Figure 11. Effects of kai choi as a trap crop on (**A**) Abundance of diamondback moth (DBM); (**B**) Percentage of leaves with DBM damage; (**C**) Abundance of imported cabbageworm (ICW); (**D**) Percentage of leaves with ICW damage in Kale Trial I. ** indicate significant difference between trap crop (T) and no trap crop (NT) at $p \leq 0.05$ and 0.01, respectively. @ indicates differences between EPN+ and EPN− at $p \leq 0.10$ level. Means followed by the same letters are not different based on analysis of variance.

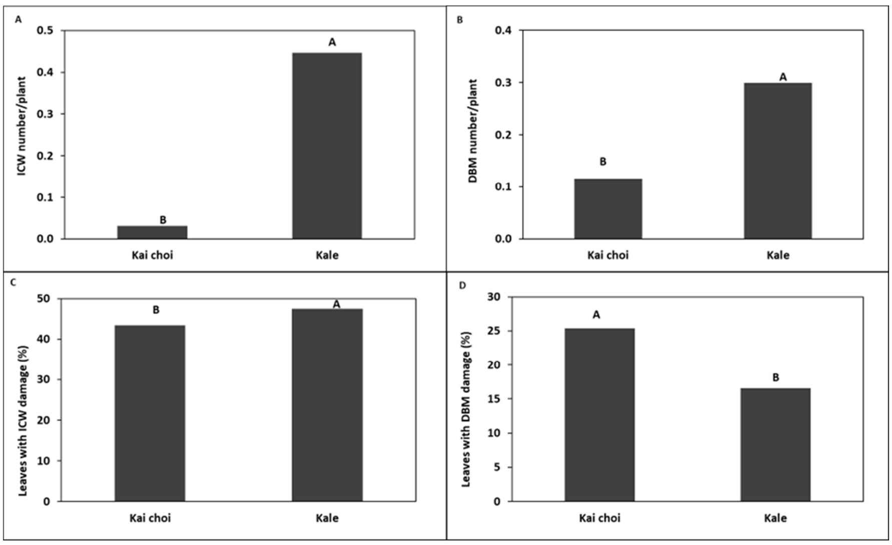

Figure 12. Abundance of (**A**) Imported cabbageworm (ICW) larvae and eggs; (**B**) Diamondback moth (DBM) (larvae and pupae); (**C**) Percentage of ICW leaf damage; (**D**) DBM leaf damage on kai choi and kale in Trial I. Means followed by the same letters in a graph are not different based on analysis of variance.

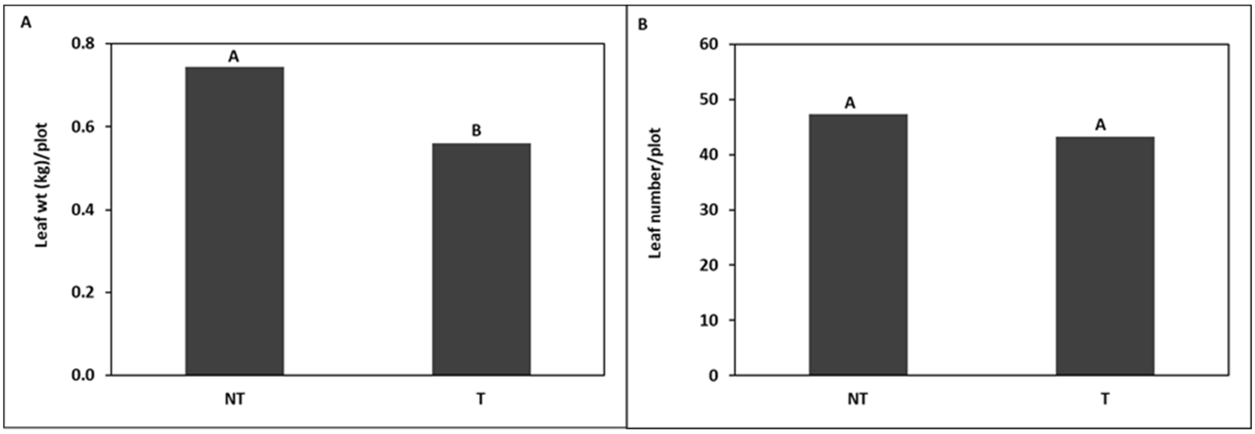

Figure 13. Effect of trap cropping on kale (**A**) leaf weight; (**B**) leaf number in Kale Trial I. Means followed by the same letters are not different based on analysis of variance.

In Kale Trial II, no interaction between trap crop and EPN was observed for all parameters (Figure 14). However, an opposite trend from Trial I occurred. Encouragingly, one week after EPN application, EPNs suppressed DBM numbers by 100% (F (1, 237) = 19.14, $p \leq 0.05$) regardless of trap crop treatment (Figure 15), but no effect was observed on ICW in Trial II. Trap cropping did not affect DBM abundance, DBM damage and ICW abundance. However, trap cropping reduced ICW damage (F (1, 237) = 7.83, $p \leq 0.05$) by 13% (Figure 14D).

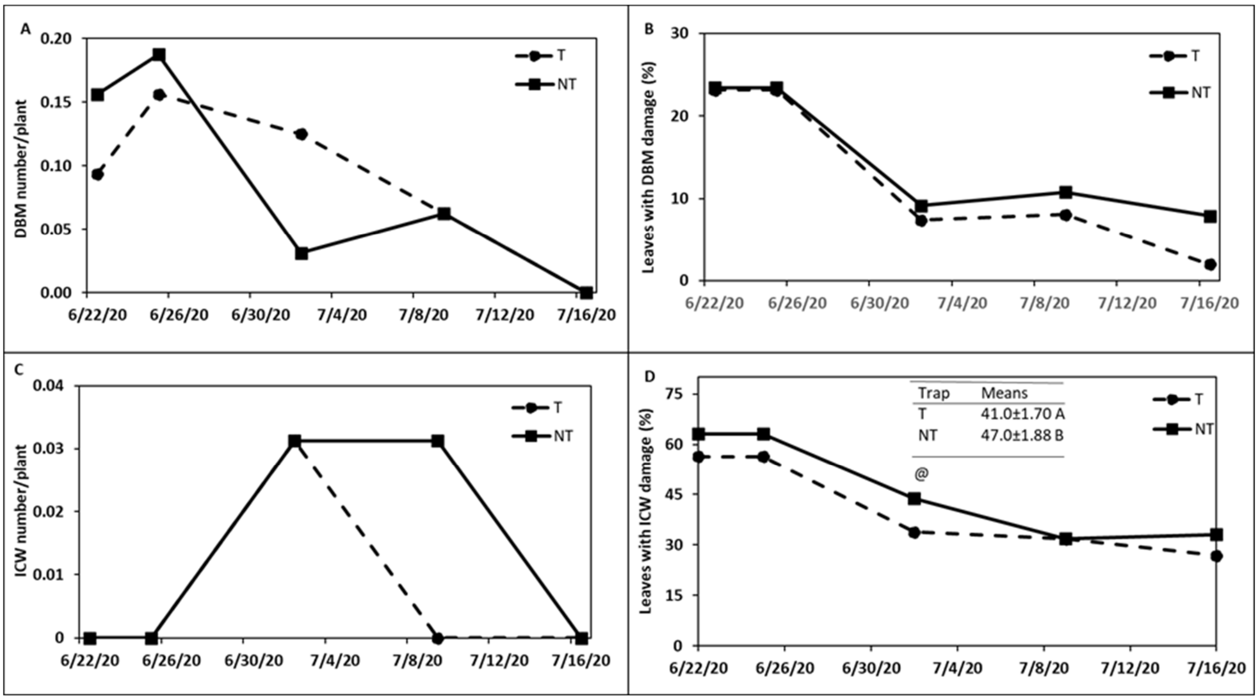

Figure 14. Effects of kai choi as a trap crop on (**A**) Abundance of DBM; (**B**) Percentage of leaves with DBM damage; (**C**) Abundance of ICW; (**D**) Percentage of leaves with ICW damage on kale in Trial II. T = Trap crop, NT = No trap crop. Means followed by the same letter are not different based on analysis of variance. @ indicates differences between EPN+ and EPN− at $p \leq 0.10$ level.

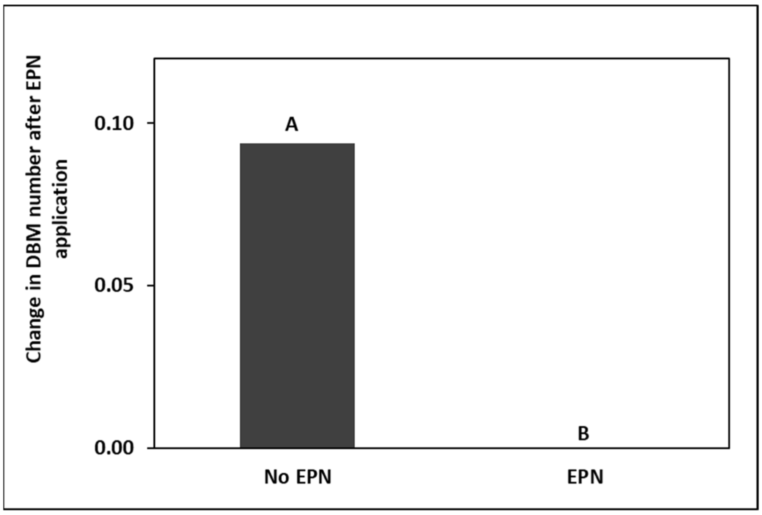

Figure 15. Effects of EPN on abundance of diamondback moth (DBM) in Kale Trial II. Means followed by the same letters in a graph are not different based on analysis of variance.

When comparing kale and kai choi within the trap crop plots, ICW population (F (1, 300) = 37.42, $p \leq 0.01$) and damage F (1, 300) = 17.10, $p \leq 0.01$) were both higher on kai choi than kale (Figure 16A,C). Similarly, DBM numbers F (1, 300) = 20.55, $p \leq 0.01$) and DBM damage (F (1, 300) = 11.23, $p \leq 0.01$) were also higher on kai choi compared to kale (Figure 16B,D). No interaction between crop type and date was observed, thus only means of the crop type were presented.

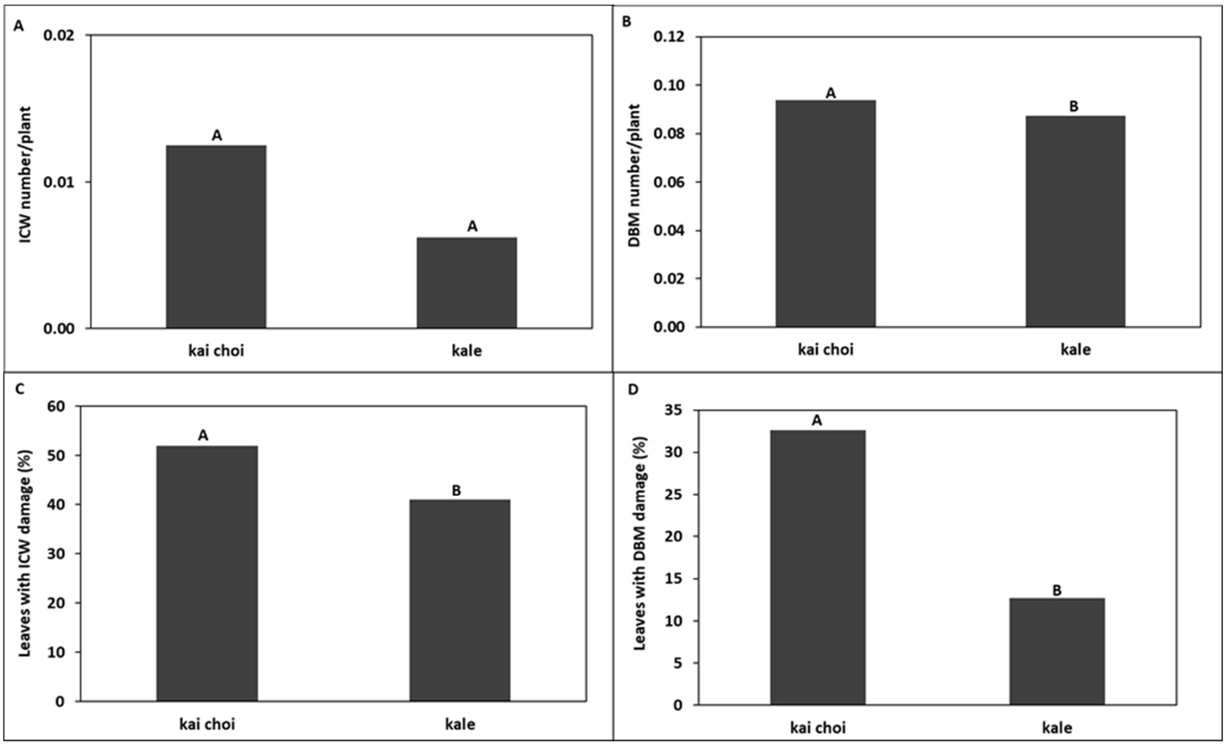

Figure 16. Abundance of (**A**) ICW larvae; (**B**) DBM larvae; (**C**) ICW leaf damage; (**D**) DBM leaf damage on kai choi and kale in Trial II. Means followed by the same letters in a graph are not different based on analysis of variance.

In terms of kale yield, ANOVA showed no interaction between trap cropping and EPN, and no significant effect from EPNs was observed. Both kale weight and leaf numbers (Figure 17A,B) were not affected by trap cropping in Kale Trial II.

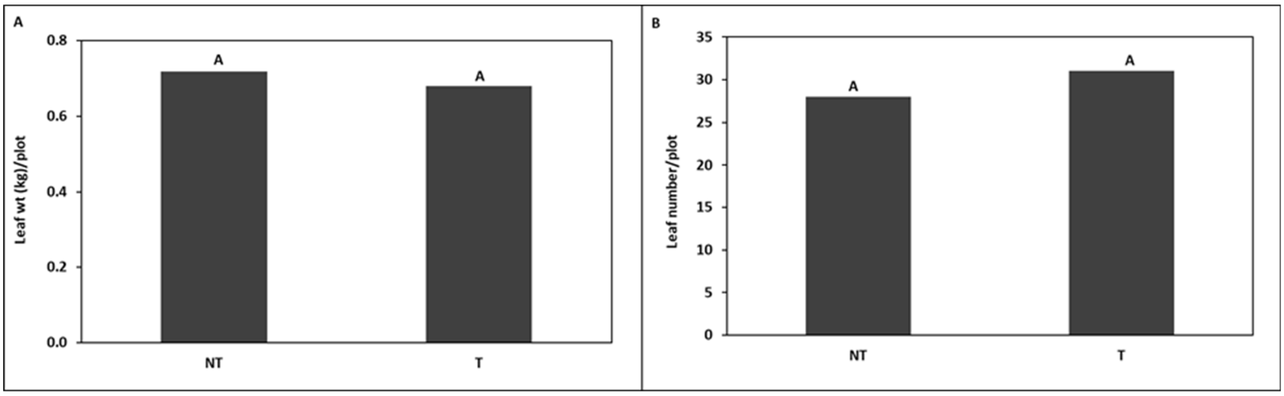

Figure 17. Effect of trap cropping on (**A**) leaf weight; and (**B**) number of leaves of kale in Trial II. Means followed by the same letters in a graph are not different based on analysis of variance.

4. Discussion

4.1. Trap Cropping

All four field trials (2 cabbage and 2 kale) partially supported the hypothesis that planting kai choi as a trap crop next to the head cabbage or kale would reduce the population densities and damage of DBM and ICW on both cash crops. This result is similar to that reported by Srinivasan and Moorthy [25] where planting 15 rows of head cabbage with two border rows of mustard (*B. juncea*) reduced DBM population densities on cabbage in India. Åsman [24] also reported increased DBM oviposition on cabbage surrounded by mustard compared to cabbage monoculture in the field. Similar results were also reported where Indian mustard planted 60 cm away from cabbage as a border crop had lower abundance and damage of DBM on cabbage compared to cabbage planted with other non-host border crops and no border control [33]. However, cabbage field trials in Texas [27] and Hawaii [26] showed no effect of trap crop on larval densities between monoculture cabbage and cabbage with Indian mustard as a trap crop when the distance between the mustard border and the cabbage was spanning from 180 cm and 60–120 cm in the trials conducted by Bender et al. [27] and Luther et al. [26], respectively. These ambiguous results of trap cropping could be due to differences in the distance between trap crop and cash crop, or the population densities of DBM or ICW on the cash crop. There is a trend that trap crops are more effective if the population densities of DBM or ICW were $\geq$0.5/plant. For example, kai choi reduced the abundance and damage of DBM and ICW on cabbage in the Waialua cabbage trial (Trial I) where there were 4 ICW/plant and about 0.5 DBM/plant. Similarly, head cabbage Kauai trial (Trial II) also showed a positive effect from kai choi as a trap crop in suppressing DBM and ICW when their population was >2/plant. However, kai choi as a trap crop did not reduce the ICW population in Trial I of kale because the ICW abundance was <0.5/plant but did reduce DBM abundance and damage where DBM was >0.5/plant. In the Kale Trial II, trap cropping did not suppress DBM because the average abundance of DBM was <0.5/plant. Even though trap cropping slightly reduced ICW damage in Kale Trial II where the ICW abundance was only <0.01/plant, the reduction was only 13% which was minimal. Banks and Ekbom [34] also concluded that the success of the trap cropping system relies on the abundance of target pests in the field. Nevertheless, our results suggested the promising effects of using kai choi as a trap crop for reducing DBM and ICW, especially when the pest density is high.

Current results also suggested that the distance between the trap crop and cash crop could affect the efficacy of the trap crop. Though the field trials conducted were not designed to determine the optimal distance between the trap crop and cash crop for kai choi to be effectively trapping DBM, it can be concluded that the distance should be at least 60 cm to avoid competition with cash crop growth. At 60 cm between kai choi and kale, DBM abundance can still be reduced by a kai choi trap crop. Future studies are needed to determine how far kai choi trap crops can be intercropped with kale or head cabbage beyond 60 cm and still be effective.

Although Bender et al. [27] and Luther et al. [26] concluded that the use of Indian mustard as a trap crop for cabbage failed to reduce DBM abundance when planted 100–200 cm or 60–180 cm away from cabbage, host feeding preferences of DBM and ICW could also affect the efficacy of trap cropping. Charleston and Kfir [35] found that the number of DBM larvae was the same or lower on the Indian mustard plants compared to other cruciferous crops such as cabbage, cauliflower, broccoli, and Chinese cabbage in a study conducted in South Africa. Results of the current field trials suggest that kai choi is a better trap crop in a cabbage field than in a kale field when DBM populations are intermediate (>0.5/plant but lower than 2/plant). Kai choi reduced 45% of DBM damage and 33% of ICW damage in a cabbage field whereas in a kale field, it only reduced DBM damage by 19% in Trial I or ICW damage by 13% in Trial II. Differential host preferences of DBM and ICW may be a consequence of leaf wax content which could influence oviposition of these insects. In general, glossy leaves such as kai choi have a reduced wax load [36,37] which improves the adhesiveness of eggs [29] but reduces larval survival due to increased predation on DBM. This is because predators are known to move easily on glossy leaf surfaces [30]. This explained why kai choi works well as a trap crop with cabbage that has low glossiness. However, current kale trials showed that DBM preferred to feed on kai choi more than kale. Glucosinolate sinigrin concentration is another factor that increases the feeding preference and behavior of DBM [31]. Therefore, more DBM damage was observed to be on kai choi (with higher sinigrin) than head cabbage and kale.

As the DBM larvae are more prone to predation on leaves with high glossiness (e.g., kai choi) suggested by Eigenbrode et al. [30] explained why less DBM abundance was observed on kai choi (higher glossiness) compared to kale and cabbage. Among these three crops, head cabbage has the least glossiness, thus kai choi works better as a trap crop in cabbage fields than in kale fields. However, this did not hold true on Kauai (Cabbage Trial II) where population densities of DBM was higher and perhaps different natural enemies were present in the field.

Despite a positive result of using kai choi as a trap crop to reduce DBM and ICW damage on the cash crop when population densities of these pests are higher than 0.5/plant, planting of trap crops could exhibit a resource competition effect on cash crop yield depending on the planting distance between the trap crop and the cash crop. Up to 41% yield loss of head cabbage was found when seedlings were planted 15 cm away from the kai choi, and 24% of kale yield loss was observed in Kale Trial I when seedlings were planted 30 cm from the kai choi. However, no yield loss was found in Trial II of kale when seedlings were planted 66 cm from the kai choi in addition to the reduction of ICW damage on kale. Hasheela et al. [33] reported the highest marketable yield of cabbage when it was planted 60 cm away from the mustard row. Hence, more research is needed to determine the fine line between planting distance to avoid competition while functioning effectively as a trap crop.

4.2. Effect of EPNs

The current research partially supported the hypothesis that foliar applications of EPNs can suppress ICW and DBM. In the current project, *S. feltiae* applied once at 1000 IJs/plant, which is equivalent to the commercial recommended rate of 125 million IJ/ha, suppressed abundance of ICW in no trap crop plots whereas it only reduced DBM abundance in the trap crop plots for the cabbage in Cabbage Trial I (with maximum DBM populations in the untreated control of <1.5/plant). Amazingly, EPNs suppressed 100% of DBM in Kale Trial II at 1 week after EPN application. Baur et al. [16] reported effective control of DBM in a watercress field when they applied a high dosage of *S. carpocapsae* at 5 billion IJs/ha as foliar spray and achieved 41% control of DBM larvae. Suppression of *S. feltiae* in Kale Trial II was surprisingly impressive, achieving 100% reduction of DBM larvae. One of the reasons for high efficacy of EPNs in this trial could be due to low abundance of DBM in Kale Trial II (0.15 DBM/plant). On the other hand, lack of effect of *S. feltiae* against ICW in Kale Trial II is simply due to the lack of ICW visits (0.01/plant) to the kale. EPNs did not work on Cabbage Trial II when DBM populations per plant were reaching >2/plant in the untreated control (NT and EPN−).

The current application rate of *S. feltiae* in the field trials here (125 million IJs/ha) is within the recommended rate in commercial EPN products. Failure of *S. feltiae* to suppress DBM when DBM populations were high could be resolved by better protection of EPNs using different adjuvants. Current research used Oroboost® (alcohol ethoxylate), a registered material for use in organic agriculture. Acar and Sipes [38] reported the importance of using a UV protectant and anti-desiccant to improve the efficacy of *S. feltiae*. Future research should examine other formulations of adjuvants compatible for organic food crop production to improve the EPNs performance against DBM.

5. Conclusions

Our study demonstrated the potential of using 'Hirayama' kai choi (*Brassica juncea*) as a trap crop in reducing DBM and ICW populations in the field, although the effectiveness of trap cropping was influenced by the population of target pests in the field. Preferably high pest densities in the field are conducive to management by trap cropping.

This study also demonstrated that EPN foliar applications occasionally provide very effective control of DBM or ICW when their population densities were below the economic threshold. However, EPN did not provide consistent suppression against DBM on cabbage or kale. Nonetheless, EPNs could still be a viable tool to be added into the pesticide rotation program for organic farmers because it is less likely for DBM to develop resistance against EPNs. EPN foliar applications at 125 million IJs/ha are effective against ICW when population pressure is below 0.5/plant. It is vital to maintain DBM populations below 0.5/plant for cabbage and kale to be economically profitable. Organic farmers could achieve this by rotating Bt, spinosad, and *B. bassiana* if insecticide resistant DBM populations were not present in their field. Having EPNs in the pesticide rotation program would reduce the frequency of Bt and spinosad applications which will improve the DBM management program. It is disappointing that the integration of trap crops with EPNs did not improve the suppression against DBM and ICW consistently. Hence, instead of combining the two strategies to manage the pests, improving the efficacy of each of the treatments alone would be more helpful. Moreover, using a high dose of EPNs (2.5 billion IJs/ha) as suggested by many publications along with appropriate adjuvants might work better when the DBM population is high.

Our study shows that planting kai choi as a trap crop provided a consistent reduction in damage from both DBM and ICW without compromising the yield of kale when they were planted at least 60 cm away from kai choi. This suggests that maintaining a proper distance between the trap crop and cash crop is as important as reducing the pest infestation to avoid the competition between the plants. Additionally, future research should focus on finding the effective distance required to maintain between the trap crop and cash crop to effectively manage the pest and get optimum yield of cash crops. Our study also shows that kai choi as a trap crop works better for cabbage than for kale in terms of reducing DBM infestation. Hence, future research should explore more effective trap crops for kale against DBM and ICW.

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Conflicts of Interest: The authors declare no conflict of interest.

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