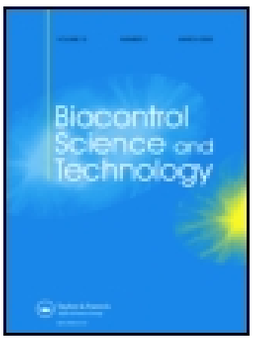

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RESEARCH ARTICLE



# *Beauveria bassiana* PSUB01 simultaneously displays biocontrol activity against *Lipaphis erysimi* (Kalt.) (Hemiptera: Aphididae) and promotes plant growth in Chinese kale under hydroponic growing conditions

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**ABSTRACT**

The mustard aphid, *Lipaphis erysimi*, is a significant agricultural pest, responsible for yield losses in several vegetable crops. A desire to reduce insecticide applications in green house vegetable production systems has led to increasing use of entomopathogenic fungi to manage pests, including aphids. In this study, we evaluated the efficacy of the entomopathogenic fungus, *Beauveria bassiana* PSUB01, for the management of *L. erysimi* in Chinese kale grown hydroponically. Drenching roots and spraying foliage with *B. bassiana* PSUB01 conidial suspension were used as inoculation methods. Tested conidia concentrations of *B. bassiana* PSUB01 ($10^8$ and $10^9$ conidia/ml) caused 87–89% mortality in *L. erysimi* after five days of application. The application of *B. bassiana* PSUB01 by root drenching colonised all treated parts (root, stem and leaf) with 64.0–96.0% colonisation (based on number of samples tested) in different part of tested plants during 7–21 days after inoculation. The application of *B. bassiana* PSUB01 on Chinese kale by either foliar spray or foliar spray + drenching of roots had reduced the fecundity of *L. erysimi* with very few progeny during 6–14 days of treatment. Also, application of *B. bassiana* PSUB01 by drenching of roots or by combination of foliar spray + drenching of roots enhanced plant growth parameters significantly except root length. These results suggest that *B. bassiana* PSUB01 could be included as part of an integrated pest management (IPM) plan for the management of *L. erysimi* under hydroponic production of Chinese kale and other vegetable crops.

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Entomopathogenic fungi; transient colonisation; vegetable crop; IPM

## Introduction

Insect pests are one of the most important factors limiting the healthy growth of crop plants. Aphids in particular are major insect pest of plants cultivated under greenhouse

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and field conditions worldwide (Blackman & Eastop, 2000). Aphids cause a direct economic impact through production losses, due to sucking of sap, weakening of plant structure, and contamination of plant leaves with exuvae, wax, and a sooty mold that grows on honeydew (Finch & Thompson, 1992). Additionally, aphids transmit viral plant pathogens that further affect plant health (Soomaro et al., 1992; Van Emden & Harrington, 2007).

The mustard aphid, *Lipaphis erysimi* (Kalt.) causes economic losses throughout the entire vegetable growing period worldwide (Gu et al., 1999). It attacks all parts of the plant except roots, sucking sap from the plant, and retards plant's growth. Aphids occur in large numbers on leaves and at the growing points of leafy vegetables (Blackman & Eastop, 2000). They tend to feed on the underside of leaves and damage plants by piercing and sucking sap from the attacked tissues, causing yellowing and curling of leaves (McKinley, 1992).

Chinese kale (*Brassica oleracea* var. *alboglabra*) is an original Chinese vegetable belonging to the *Brassicaceae* family and widespread in China and Southeast Asia (Sun et al., 2011). Chinese kale is an annual leafy vegetable crop grown under field and green house conditions globally due to rich source of antioxidant and anticarcinogenic compounds (Wang et al., 2017). In Chinese kale, aphids can cause 10-90% yield loss varying with level of infestation and the prevailing agro-climatic conditions (Bakhetia, 1983; Mpumi et al., 2020).

Chemical insecticides are the important for the efficient management of aphids in many parts of the world, but their indiscriminate use has enabled aphids to develop resistance against many chemical insecticides, resulting in increased aphid problems (Bass et al., 2014). Further, injudicious use of chemical insecticides has resulted in increased environmental pollution and harmful impact on human health and non-target organisms. For these reasons, entomopathogenic fungi are of interest as an alternative insect pest control agents. Fungal biopesticides based on the use of conidia, blastospores, or hyphae, alone or in combinations that cause infections and death in the target pest provide added benefit of horizontal transmission of infection to other individuals of the pest population through spores of mycosis cadavers (Kim, Jeong, et al., 2013; Shan & Feng, 2010; Thaochan & Ngampongsai, 2015, 2018).

Several species of fungi, *viz. Beauveria bassiana* (Quesada-Moraga et al., 2006), *Metarhizium anisopliae* (Shi & Feng, 2004; Wright et al., 2004), *Isaria* sp. (Shi & Feng, 2004), *Nomuraea rileyi* (Devi et al., 2003), and *Hirsutella* sp. (Choudhary et al., 2012), have been tested for the management of aphids and many other agriculturally important insect pests belonging to the Order Hemiptera. Among these species, some fungi are able to survive inside the plant system for at least some part of their life cycle, a quality that has been identified through a number of artificial inoculation techniques, including conidial drenching of the soil, injection of various plant parts, seed dressing, and foliar spray (Posada et al., 2007; Quesada-Moraga et al., 2006, 2014a; Schulz & Boyle, 2005). Colonisation patterns of entomopathogenic fungi inside the plants may be systemic or non-systemic, as well as long lasting or transient (Landa et al., 2013; Quesada-Moraga et al., 2014b). Fungal endophytes play an important role in the plant health by suppressing diseases or insect pests and enhancing growth, while in exchange they obtain food and shelter, and are able to reproduce and be distributed within the plant system (Garrido-Jurado et al., 2017; Resquín-Romero et al., 2016; Saikkonen et al., 2004).

*B. bassiana* is one of the most important entomopathogenic fungi worldwide, infecting a wide range of insect species and being easy to isolate from different sources, including plant surfaces, soil and insects (Butt et al., 1994; Freed et al., 2011; Meyling & Eilenberg, 2006). However, the application of *B. bassiana* on host plants to promote growth and raise defense levels against insects is still rare in hydroponic system. Therefore, in the present study, we investigated the potential and endophytic properties of *B. bassiana* PSUB01 for the management of mustard aphid (*L. erysimi*), which is an important insect pest of Chinese kale and other vegetable crops cultivated in hydroponic systems around the world.

## Materials and methods

### Fungal strain and conidia preparation

*Beauveria bassiana* PSUB01 was obtained from the culture collection at the Pest Management Biotechnology and Plant Physiology Laboratory, Agricultural Innovation and Management Division, Faculty of Natural Resources, Prince of Songkla University, Songkhla, Thailand. The fungus was cultivated on Sabouraud dextrose agar and yeast extract (SDAY) (2.5 g/l peptone, 2.5 g/l yeast extract 10 g/l dextrose and 20 g/l agar) and incubated at 28 ± 2°C temp in complete darkness condition for 14 days. Aerial conidia were collected from 14-day-old cultures by adding 10 ml of 0.1% Tween 80 (vol/vol) (prepared with sterile distilled water) in culture plates and gentle scraping. This conidial suspension was filtered through three layers of cheese cloth, and then number of conidia was counted in the suspension with hemacytometer (Marienfeld-superior, Marien Field, Germany). Conidial concentrations from $10^6$ to $10^9$ conidia/ml were prepared by dilution with sterile 0.1%Tween 80 (vol/vol). Then, the germination rates of conidia were determined by plating of 100 µl aliquot of $10^3$ conidia/ml on SDAY and incubated at 28 ± 2°C temp in complete darkness condition for 24 h before each bioassay. Percent germination of conidia from test suspensions was determined after observations of conidia under Leica DM750 phase contrast microscope (Wetzlar, Germany). Conidia with a germ tube longer than its width were considered viable and only suspensions with 90% viable conidia were used for experiments.

### Insect preparation

*L. erysimi* were collected from the agricultural research field of Agricultural Innovation and Management Division, Faculty of Natural Resources, Prince of Songkla University, Thailand. Adults and nymphs of *L. erysimi* were reared on healthy mature Chinese kale in a hydroponic potted system (10 cm diameter and 15 cm height) by transferring with a camel-hair brush to host plants. The Chinese kale was supported by a polyethylene foam mat and 500 ml of nutrient solution per pot was continuously aerated. The nutrient solution in this study was prepared with the protocol mentioned by Boonnoi et al. (2017) with minor modification. The chemical composition of nutrient solution were Ca $(NO_3)_2$ 94.2 g, $KNO_3$ 44.9 g, $KH_2PO_4$ 16.3 g, MgSO4.7$H_2O$ 25.9 g, Fe-EDTA 1.6 g, Fe-EDHA 1.6 g and Nic spray (micronutrient fertilisers) 2.3 g. The hydroponic potted plants were maintained in a 50 × 50 × 50 cm mesh cage with 400 µm mesh fineness,

under ambient temperature (28 ± 2°C), 75 ± 3% RH and natural light. Different stages of *L. erysimi* were reared on the host plant for 5–6 generations. The newly hatched two days old wingless nymph of *L. erysimi* were used for further experiments.

### *Effects of conidia concentration of* B. bassiana *PSUB01 on* L. erysimi *mortality*

The 14 days old hydroponic potted Chinese kale were prepared as above. Ten wingless nymphs of *L. erysimi* were placed on the lower surface of the second youngest true leaf using a camel-hair brush and kept without any disturbance for 24 h to settle down the aphids. Ten potted plants with aphids were sprayed with one ml of each conidial suspension separately ($10^6$, $10^7$, $10^8$ and $10^9$ conidia/ml), while the control treatment was sprayed with sterile 0.1% Tween 80 (vol/vol), and plants were then covered with a mesh net with 400 µm mesh fineness (15 × 15 cm) to prevent the aphids from escaping. The experiment followed a completely randomised design with 10 replications. After treatment, 50 pots including control pots (10 pots per treatment and 10 pots in a control) were transferred to a 30 × 30 × 30 cm mesh cage (five pots/cage, a total of 10 cages) and was maintained under 28 ± 2°C temp, 75 ± 3% RH and natural light. At an interval of 24 h, the number of aphids were maintained by removing the new nymphs (progeny) from leaf of plants until death of all adult aphid (from the original inoculation with first or second instars nymphs). To confirm mycosis, all dead aphids were placed on a sterilised moist filter paper after surface sterilisation by dipping in 0.1% sodium hypochlorite (vol/vol), and then washed twice with sterile distilled water. Surface-sterilised dead aphids were then placed on moist filter paper, transferred to a Petri dish, incubated for 24–48 h and observed to determine any development of mycosis under a Leica S8APO stereo zoom microscope (Wetzlar, Germany).

### *Colonisation by* B. bassiana *PSUB01 of test plants*

Sixty Chinese kale plants in a hydroponic tray system (50 × 70 × 25 cm) were supported by a polyethylene foam mat. Each tray supported 10 plants with 5 L of continuously aerated nutrient solution. Three treatments were used in this experiment: (1) foliar spray of $10^7$ conidia/ml of *B. bassiana* PSUB01 suspension at 2 ml/plant, (2) drenching of roots with 500 ml/tray at the final concentration at $10^7$ conidia/ml, and (3) an untreated control. The treatments were applied seven days after seeding the trays. Following inoculation, the trays were maintained at 28 ± 2°C, 75 ± 3% RH and natural light. The determination of endophytic colonisation of *B. bassiana* PSUB01 in the plants was carried out by collecting roots, stems and leaves of five plants per treatment at 7, 14, 21, and 28 days post-inoculation. Roots, stems, and leaves were surface sterilised by running tap water, then submerged for two min in 0.5% sodium hypochlorite (vol/vol) for leaf, and stem tissues and for root tissues, in 1% sodium hypochlorite (vol/vol), followed by two min washing with 70% ethanol (vol/vol) and then two final washes with sterile distilled water. Plant materials were then allowed to surface-dry in a laminar flow hood for 1 h. Five each of leaf discs (5 × 5 mm), root and stem pieces (10 mm long) were cut using a sterile blade after surface-drying in a laminar flow hood for incubation on 9 cm Petri dishes with selective culture media [SDAY plus thiabendazole (0.5 g/l) and chloramphenicol (0.001%)] at 28 ± 2°C for 10 days (Fernandes et al.,

2010). The efficiency of the surface sterilisation procedure was assessed by making imprints of surface sterilised plant tissue and plating the final rinse water (Schulz et al., 1998). Incubated plates with plant materials were checked every two days for 10 days to record the fungal growth on the plant materials. Fungal growth on plated plant materials were confirmed as *B. bassiana* based on growth, colony morphology, and microscopic examination of conidia. Percent colonisation of different plant parts by the respective inoculated fungus was calculated following the Petrini and Fisher (1987). The presence of other bacteria or fungi on the incubated plates was occasionally observed, but not scored in our study.

### *Effects of* B. bassiana *PSUB01 on aphid fecundity*

Hydroponic potted Chinese kale at seven days after seeding were individually treated with conidial suspension of *B. bassiana* PSUB01at $10^7$ conidia/ml in a hydroponic potted system (10 cm diameter and 15 cm height). The Chinese kale was supported by a polyethylene foam mat in 500 ml of nutrient solution with continuous aeration. A final solution of $10^7$ conidia/ml of *B. bassiana* PSUB01 was used to drench the roots (50 ml/plant), as a foliar spray (2 ml/plant) or a combined foliar spray (2 ml/plant) + drenching (50 ml/plant). Control plants were sprayed with sterile distilled water (2 ml/plant). The experiment followed a completely randomised design with five replications. Five wingless nymphs of *L. erysimi* with mixed age (two day olds after hatched) were placed on the lower surface of the second youngest true leaf of 14 days old Chinese kale using a camel-hair brush. Individual leaves were then covered with a mesh net as described in the previous experiments. Following inoculation, the pots were maintained at 28 ± 2°C, 75 ± 3% RH and natural light. The number of aphids on individual leaves was recorded 2, 4, 6, 8, 10, 12 and 14 days after initial placement of aphids on Chinese kale leaf (16, 18, 20, 22, 24, 26 and 28 days after planting). The new nymphs were removed after each count, and the experiment was terminated on the 14th day of observation.

### *The effects of* B. bassiana *PSUB01 on plant growth*

Two sets of 40 Chinese kale plants (infested and un-infested with aphids) were constructed in a hydroponic tray system (50 × 70 × 25 cm), supported by a polyethylene foam mat. Each tray supported 10 plants with five liters of continuously aerated nutrient solution. At seven days after seeding, a final conidial suspension of *B. bassiana* PSUB01 at $10^7$ conidia/ml was applied as a root drench (500 ml), foliar spray (2 ml/plant), and foliar spray (2 ml/plant) + drenching (500 ml). The control plant was treated with water only. Following inoculation, the trays were maintained at 28 ± 2°C, 75 ± 3% RH and natural light. For the first set of aphid infestations, two day olds 10 *L. erysimi* nymphs were placed on the lower surface of the second youngest true leaf of each 14-day-old Chinese kale plant using a camel-hair brush. The second set was kept without any aphids. The experiment followed a completely randomised design with 10 replications. Plant growth parameters, including shoot height and root length were measured at 7, 14, 21, 28, 35, and 42 days after treatment. Finally, fresh and dry weights were measured at 42 days after treatment.

### *Statistical analysis*

Prior to statistical analyses, all data were tested fornormality and homogeneity of the variance. Probit analysis was used to calculate values of the lethal time ($LT_{50}$ and $LT_{90}$) for each concentration. The percent cumulative mortality, lethal timeof aphids infected with *B. bassiana* PSUB01, and fresh and dry weights (of plant) were subjected to one-way ANOVA and Tukey's Honestly Significant Difference test ($\alpha = 0.05$) to compare means. Arcsine transformations were carried out before analysis for the cumulative percentage mortality analysis. Kaplan-Meier survival analysis was used to determine the average survival time (AST) and compared with a long-rank test. A repeated-measures ANOVA was performed with time as a repeated factor to test for root length (plant), shoot height (plant) anddifferent number of progeny of aphids between treatments. The SPSS 17.0 program for Windows was used for all statistical analysis (SPSS, 2008).

## Results

### *Effective conidial concentration of* B. bassiana *PSUB01 on killing* L. erysimi

All conidial concentrations of *B. bassiana* PSUB01 caused mortality of *L. erysimi,* of varying levels (Figure 1). The concentrations $10^8$ and $10^9$ conidia/ml were highly effective against *L. erysimi,* with over 50% mortality by the third day after treatment. Mortality further increased with time and 87–89% morality was recorded in *L. erysimi* on the fifth day after treatment with $10^8$ and $10^9$ conidia/ml, whereas only 6% mortality was recorded from the untreated control (Figure 1). On the other hand, the conidial concentrations at $10^7$ and $10^6$ conidia/ml showed 52.0% and 15.0% mortality, respectively in aphids on the fifth day after treatment.

Based on the Kaplan-Meier survival analysis, *L. erysimi* treated with *B. bassiana* PSUB01 at $10^9$ conidia/ml showed the lowest average survival time (AST), i.e. 2.71 ± 0.12 days, significantly different from the other conidial concentrations ($F_{4,45} = 59.01$, $p < 0.01$) (Table 1). The

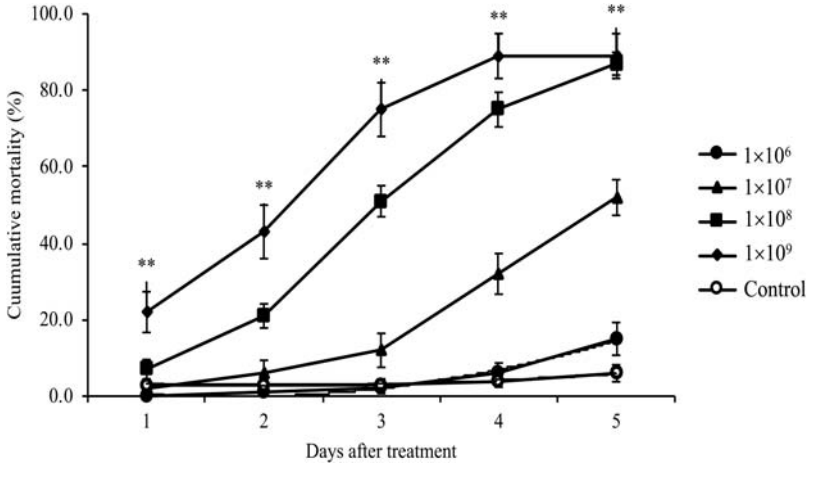


**Figure 1.** Mean (±SE) cumulative mortality of the adult mustard aphid [*Lipaphis erysimi* (Kalt.)] infected with *Beauveria bassiana* PSUB01 in different spore concentrations under laboratory conditions.

**Table 1.** Kaplan–Meier survival analysis of adult mustard aphid *L. erysimi* infected with *B. bassiana* PSUB01 under laboratory conditions on Chinese kale.

| Conidial concentration (spore/ml) | Average survival time (AST) (day) (mean ± SE)[a] | 95% confidence interval | |
|---|---|---|---|
| | | Lower | Upper |
| $1 \times 10^9$ | 2.71 ± 0.12a | 2.46 | 2.95 |
| $1 \times 10^8$ | 3.46 ± 0.12b | 3.22 | 3.69 |
| $1 \times 10^7$ | 4.48 ± 0.09c | 4.29 | 4.66 |
| $1 \times 10^6$ | 4.91 ± 0.04c | 4.83 | 4.99 |
| Control | 4.87 ± 0.06c | 4.73 | 5.00 |

[a]There were 10 replicates per treatment, with 10 individuals per replicate. Average Survival Time (AST) limited to 5 days. Means within columns with the same letter are not significantly different ($p < 0.05$) according to the log-rank test.

**Table 2.** Mean (±SE) lethal time ($LT_{50}$ and $LT_{90}$) in relation to conidial concentration of *B. bassiana* PSUB01 used to infect adult *L. erysimi* (Kalt.), under laboratory conditions on Chinese kale.

| Conidial concentration (spore/ml) | Lethal time (day)[a] | |
|---|---|---|
| | $LT_{50}$ | $LT_{90}$ |
| $1 \times 10^9$ | 2.39 ± 0.31a | 4.06 ± 0.53a |
| $1 \times 10^8$ | 3.13 ± 0.15b | 4.85 ± 0.24a |
| $1 \times 10^7$ | 6.05 ± 0.25c | 6.67 ± 0.35b |
| $1 \times 10^6$ | 6.05 ± 0.23c | 7.90 ± 0.56b |

[a]Data followed by the same letter in the same column are not significantly different by Tukey's HSD test ($p < 0.01$).

concentrations at $10^7$ and $10^6$ conidia/ml showed an AST range from 4.48 to 4.91 days, not significantly different from the control treatment (4.87 ± 0.06 days) (Table 1).

The $LT_{50}$ of *L. erysimi* treated with *B. bassiana* PSUB01 concentration of $10^9$ conidia/ml was 2.39 ± 0.31 days, significantly different from $10^8$ conidia/ml with an $LT_{50}$ of 3.13 ± 0.15 days ($F_{3,33} = 38.79$, $p < 0.01$) (Table 2). The concentrations at $10^7$ and $10^6$ conidia/ml had similar $LT_{50}$ values of 6.05 ± 0.25 and 6.05 ± 0.23 days, respectively. The $LT_{90}$ with $10^8$ and $10^9$ conidia/ml ranged from 4.06–4.85 days and was significantly different from the concentrations at $10^6$ and $10^7$ conidia/ml ($F_{3,33} = 13.98$, $p < 0.01$) (Table 2).

## *Colonisation of* B. bassiana *PSUB01 in host plant*

All treated plant by foliar spray and root drenching with *B. bassiana* PSUB01 were colonised by *B. bassiana* PSUB01 but varied level of colonisation by *B. bassiana* PSUB01 were observed on plant parts. Percent colonisation of *B. bassiana* PSUB01 on different plant parts (leaf, stem, and root) varied significantly among the application method at 7 to 28 day post inoculation (dpi) (Table 3). The highest colonisation percentage of *B. bassiana* was recorded in different plant parts via root drenching (Table 3 and Figure 2). Overall, a decline in colonisation percentage of inoculated plants was observed over time regardless of the application methods, although the lowest percentage of colonisation (less than 16.0%) was observed via foliar application, after 14 and 21 dpi, and no colonisation was found at 28 dpi (Table 3). In the control treatment, no colonisation of *B. bassiana* PSUB01 was found in all of tested periods and parts of plant.

**Table 3.** Percent colonisation of different plant parts (leaf, stem, and root) of Chinese kale with different application of fungal entomopathogen, *B. bassiana* PSUB001 at 7, 14, 21 and 28 days post inoculation (dpi).

| | 7 dpi | | | 14 dpi | | | 21 dpi | | | 28 dpi | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Applications | Leaf | Stem | Root | Leaf | Stem | Root | Leaf | Stem | Root | Leaf | Stem | Root |
| Drenching | 68.0 ± 8.0a | 88.0 ± 8.0a | 96.0 ± 4.0a | 64.0 ± 11.7a | 88.0 ± 4.9a | 80.0 ± 6.3a | 68.0 ± 4.9a | 80.0 ± 6.3a | 76.0 ± 4.0a | 40.0 ± 8.9a | 44.0 ± 7.5a | 64.0 ± 7.5a |
| Foliar spray | 16.0 ± 7.5b | 8.0 ± 4.9b | 4.0 ± 4.0b | 16.0 ± 4.0b | 12.0 ± 4.9b | 8.0 ± 4.9b | 4.0 ± 4.0b | 12.0 ± 4.9b | 0.0 ± 0.0b | 0.0 ± 0.0b | 0.0 ± 0.0b | 0.0 ± 0.0b |
| Control | 0.0 ± 0.0b | 0.0 ± 0.0b | 0.0 ± 0.0b | 0.0 ± 0.0b | 0.0 ± 0.0b | 0.0 ± 0.0b | 0.0 ± 0.0b | 0.0 ± 0.0b | 0.0 ± 0.0b | 0.0 ± 0.0b | 0.0 ± 0.0b | 0.0 ± 0.0b |

For each part, means (±SE) followed by the same letter within the same sampling date are not significantly different at $p > 0.05$ by Tukey's HSD test.

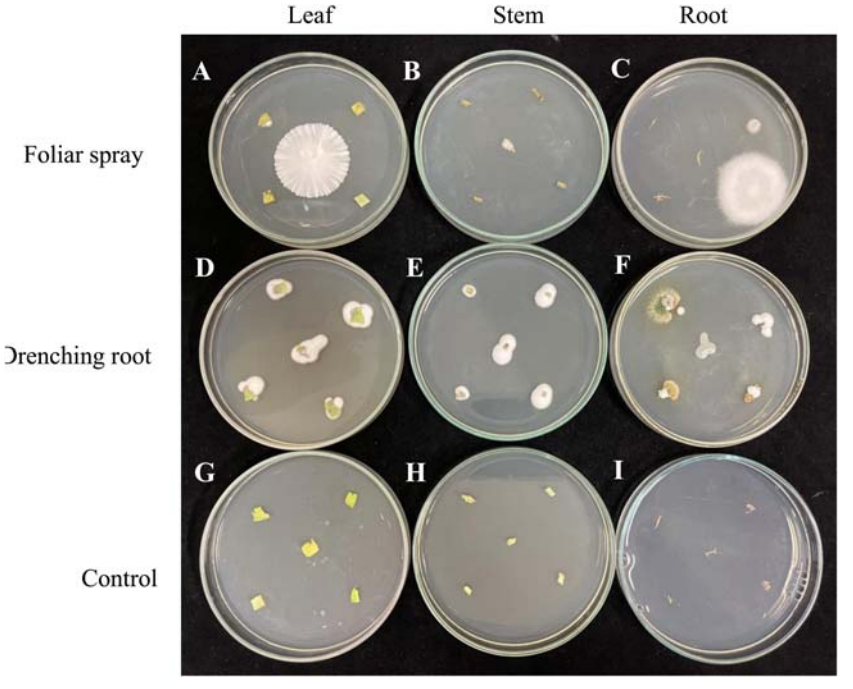


**Figure 2.** The endophytic colonisation of entomopathogenic fungus, *Beauveria bassiana* PSUB01, in the leaves, stems and roots of Chinese kale after foliar spray treatment with the fungus suspension (A, B and C) and drenching root (D, E and F) 28 days after inoculation. The control was untreated (G, H and I).

### *Effects of* B. bassiana *PSUB01 on aphid fecundity*

The application of *B. bassiana* PSUB01 on Chinese kale in a hydroponic system reduced the fecundity of mustard aphid. For all main effects and associated interactions were significantly different (Table 4). The application of *B. bassiana* PSUB01 by foliar spray and foliar spray + drenching showed lower numbers of aphid progeny from day 6th after treatment (20 days after seeding) ($F_{3,16} = 7.96$, $p < 0.01$) until the 14th day after treatment (28 days after seeding) ($F_{3,16} = 8.44$, $p < 0.01$), which were significantly different from the control treatment. The number of new aphid progeny after foliar spray and foliar spray + drenching treatments after 14 days of treatment (28 days after seeding) were 8.60 ± 0.51 and 12.60 ± 2.60 aphids/leaf, respectively, while 37.80 ± 7.44 aphids/leaf was found for the control treatment (Table 5). Application of *B. bassiana* PSUB01 via root drenching had no effect on aphid numbers, which were not significantly different from the control (Table 5).

### *Effects of* B. bassiana *PSUB01 on plant growth*

Application of *B. bassiana* PSUB01 to Chinese kale in a hydroponic system enhanced the growth of treated plants. Shoot height of Chinese kale was significantly enhanced for aphid-infested plants by applications of *B. bassiana* PSUB01 with root drenching and

**Table 4.** Repeated-measures ANOVA parameters for number of progeny of mustard aphid, *L. erysimi* on Chinese kale treated with *B. bassiana* PSUB01 by drenching roots, foliar spray, foliar spray combined with drenching root, and an untreated control.

| | Number of progeny | | |
|---|---|---|---|
| | df | *F* | *p* |
| *Between-subjects factors* | | | |
| Application | 3 | 8.003 | 0.002 |
| *Within-subjects factors* | | | |
| Time | 6 | 58.250 | 0.000 |
| Time*Application | 18 | 5.131 | 0.000 |

f-

**Table 5.** Mean (±SE) count number of progeny of *L. erysimi* on Chinese kale treated with *B. bassiana* PSUB01 by drenching roots, foliar spray, foliar spray combined with drenching root, and an untreated control.

| | Average cumulative number of progeny (aphid/leaf)[a] (Two days interval count from 14 days after treatment) (days) | | | | | | |
|---|---|---|---|---|---|---|---|
| Treatment | 16 | 18 | 20 | 22 | 24 | 26 | 28 |
| Drenching roots | 8.20 ± 2.91a | 18.80 ± 3.96ab | 21.20 ± 3.83ab | 22.60 ± 4.01ab | 23.20 ± 4.28ab | 23.80 ± 4.04ab | 26.00 ± 4.64ab |
| Foliar spray | 1.20 ± 0.49ab | 4.80 ± 0.73c | 6.60 ± 0.68c | 8.00 ± 0.89b | 8.40 ± 0.68b | 8.60 ± 0.51b | 8.60 ± 0.51b |
| Foliar spray + drenching | 1.00 ± 1.00b | 8.60 ± 2.56bc | 10.80 ± 2.46bc | 12.00 ± 2.43b | 12.20 ± 2.52b | 12.40 ± 2.62b | 12.60 ± 2.60b |
| Untreated control | 5.20 ± 1.93ab | 26.80 ± 4.96a | 29.00 ± 5.52a | 30.40 ± 5.91a | 31.40 ± 6.28a | 32.00 ± 6.25a | 37.80 ± 7.44a |

[a]There were 5 replicates per treatment. Means within columns with the same letter are not significantly different by Tukey's HSD test ($p < 0.01$).

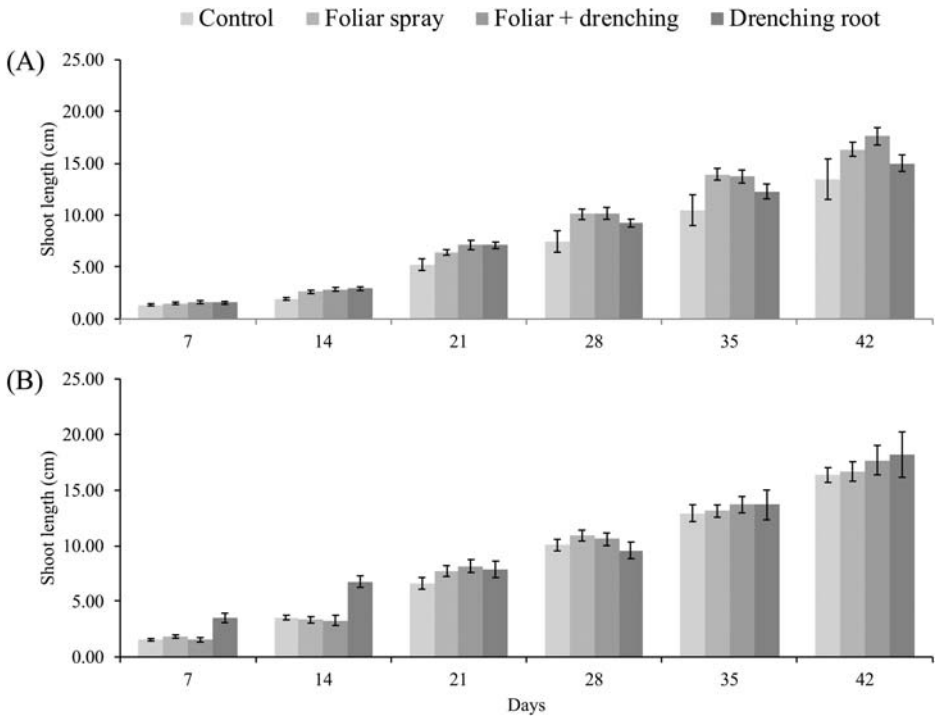


**Figure 3.** Mean (±SE) of shoot height of Chinese kale plant in a hydroponic system with (A) and without (B) mustard aphid [*Lipaphis erysimi* (Kalt.)] infestations. *Beauveria bassiana* PSUB01 treatments were conducted by drenching root, foliar spray, or foliar spray + drenching root in the hydroponic system, plus an untreated control.

oliar spray + drenching on days 14 ($F_{3,36} = 7.95$, $p < 0.01$) and 21 after treatment ($F_{3,36} = 4.57$, $p < 0.01$). Shoot height on days 7, 28, 35 and 42 post-treatment were not different from the untreated control (Figure 3).

Root length of Chinese kale infested with aphids, on the other hand, did not differ significantly from the un-infested control treatment after application of *B. bassiana* PSUB01 (Figure 4). The root length of Chinese kale was significantly greater under aphid free conditions in all application methods of *B. bassiana* PSUB01 on days 7 ($F_{3,36} = 13.66$, $p < 0.01$) and 14 ($F_{3,36} = 5.40$, $p < 0.01$) compared to control plants. Also, root length of plants treated by foliar spray + drenching was significantly different from the control 21 days after treatment ($F_{3,36} = 3.49$, $p < 0.01$). However, 28–42 days after treatment, root length did not differ significantly between treated and control plants (Figure 4).

Forty two days after treatment, for plants not infested with aphids, there were no differences in either fresh or dry weight of Chinese kale between *B. bassiana* treated plants and the controls ($F_{7,72} = 2.07$, $p > 0.05$) (Figure 5). In contrast, on aphid-infested plants all the applications of the fungal entomopathogen, plant growth was enhanced, with significantly greater fresh and dry weights compared to the control plants ($F_{7,72} = 2.85$, $p < 0.05$) (Figure 5).

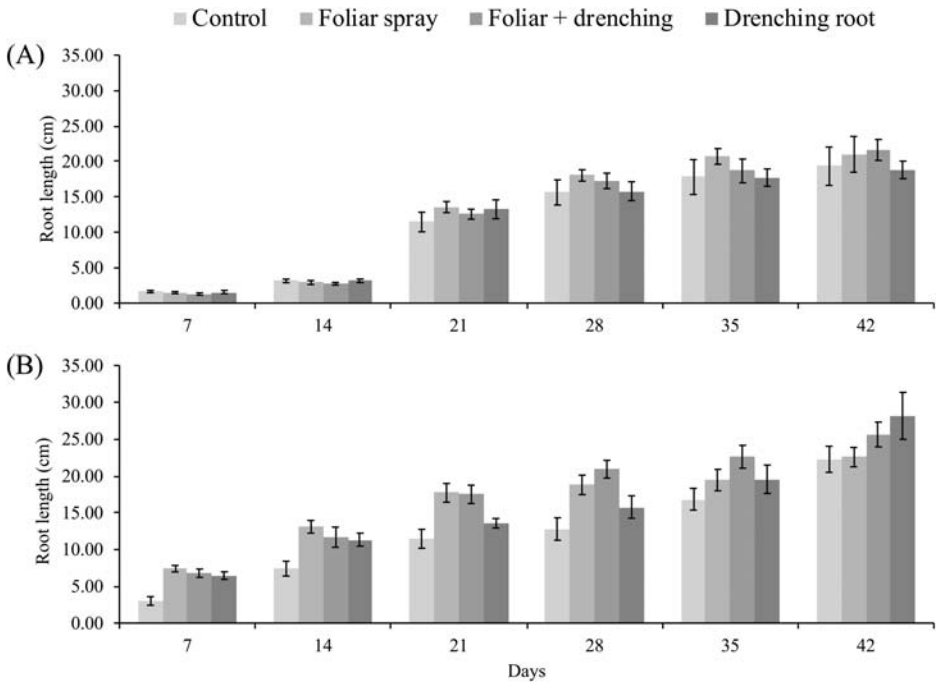


**Figure 4.** Mean (±SE) of root length of Chinese kale plant in a hydroponic system with (A) and without (B) mustard aphid [*Lipaphis erysimi* (Kalt.)] infestations. *Beauveria bassiana* PSUB01 treatments were administered through drenching root, foliar spray, or foliar spray + drenching root in the hydroponic system, plus an untreated control.

## Discussion

Previous studies have demonstrated that propagules such as conidia, blastospores, hyphae or culture filtrates of *B. bassiana* are pathogenic to aphids (Gurulingappa et al., 2011; Kim et al., 2010; Kim, Je, et al., 2013; Shrestha et al., 2015). Different conidial concentrations of *B. bassiana* PSUB01 were tested against *L. erysimi* and the various conidial concentrations of *B. bassiana* PSUB01 significantly affected aphid populations in different treatments. The concentrations of *B. bassiana* PSUB01 at $10^8$–$10^9$ conidia/ml were the most effective among the concentrations tested, with comparatively low values of AST, $LT_{50}$ and $LT_{90}$. These findings indicate that the effectiveness of *B. bassiana* PSUB01 against *L. erysimi* depends upon conidial concentration and duration of infection. This has also been supported by high mortality in cabbage aphid, *Brevicoryne brassicae* L., with the application of increased conidial concentrations of entomopathogenic fungi and increasing exposure time (Asi et al., 2009). Other researchers have also suggested that mortality in target insects with fungal infection is dependent on concentration (dose), duration of exposure and the temperature following application (Ansari et al., 2004; Liu et al., 2002; Wright et al., 2005).

The entomopathogenic fungal strain, *B. bassiana* PSUB01, used in the present study was able to colonise different plant parts, such as roots, stems and leaves, of Chinese kale under hydroponic system, irrespective of the mode of application. Previously, successful colonisation by entomopathogenic fungi in several plants was observed with

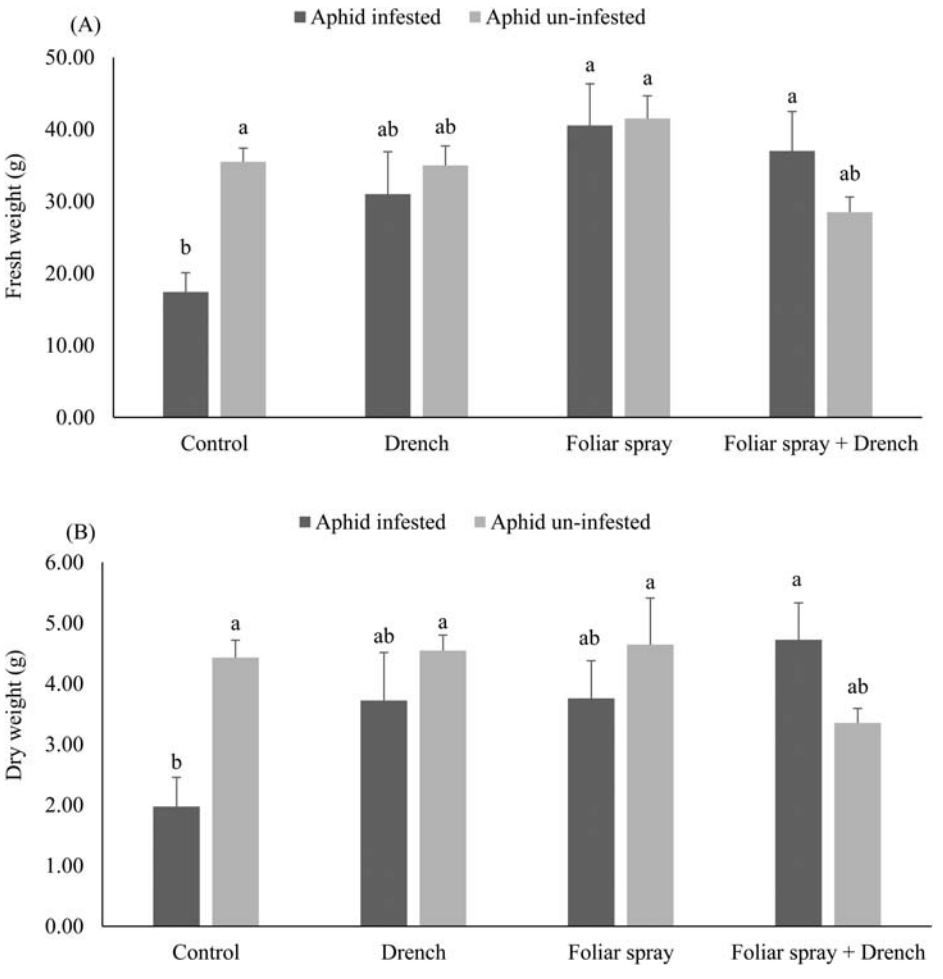


**Figure 5.** Mean (±SE) of fresh (A) and dry (B) weight of Chinese kale plant cultivated in a hydroponic system with and without infestation by mustard aphid [*Lipaphis erysimi* (Kalt.)]. The *Beauveria bassiana* PSUB01 treatments were made by drenching, foliar spray, and foliar spray + drenching in the hydroponic system. The control was not treated. The mustard aphid was introduced on day 14 after treatments. Bars with similar letter did not differ significantly by Tukey's HSD test ($p < 0.05$).

different modes of application (Biswas et al., 2012; Gurulingappa et al., 2010; Jaber & Araj, 2018; Quesada-Moraga et al., 2009; Tefera & Vidal, 2009). Entire plants have been colonised following a single foliar spray of entomopathogenic fungi (Garrido-Jurado et al., 2017; Resquín-Romero et al., 2016; Wei et al., 2020). Similar results were found in the present study, where a single application of *B. bassiana* PSUB01 by root drenching helped in the colonisation of the applied fungal strain in all plant parts of the Chinese kale under hydroponic system for 28 days after inoculation. This indicated transient movement as well as colonisation of the applied fungal strain within the plant in non-treated tissues. Earlier studies have also found the presence of *B. bassiana* in the space between parenchymal cells as a site of profuse colonisation (Garrido-Jurado et al., 2017; Jia et al., 2013; Landa et al., 2013). Localised colonisation of *B. bassiana* PSUB01 was found to be lower with foliar application than that of the root drenching

method, due to the poor efficiency of fungal penetration into leaf tissues, and possibly due to the low density of stomata, leaf surface structure, and specific cuticular components (Mantzoukas & Eliopoulos, 2020; Muvea et al., 2014; Posada et al., 2007).

Moreover, the application of *B. bassiana* PSUB01 conidia on Chinese kale in a hydroponic system reduced the reproduction and fecundity of *L. erysimi*. The population density of *L. erysimi* after application of *B. bassiana* PSUB01 conidia with foliar spray and foliar spray with roots drenching was significantly lower than in the roots drenching and control at 4–14 days after treatment. In our study, the reduction in reproduction and fecundity of these insect might be affected by the sublethal conidial colonisation developed from the foliar application. Similarly, wheat treated with *B. bassiana* at sublethal concentrations showed reduced feeding and fecundity in *Eurygaster integriceps* (Trissi et al., 2019). In another study, cotton leaves colonised by *B. bassiana* resulted in reduced reproduction in *A. gossypii* Glover (Gurulingappa et al., 2011). Meanwhile, reduced fecundity was observed in the 1st and 2nd generation *Myzus persicae* (Sulzer) that were fed on sweet potato plants, colonised with a combination of the entomopathogenic fungi *B. bassiana* and *M. brunneum* (Jaber & Araj, 2018). The sublethal effects of an entomopathogenic fungus can be demonstrated by a change in fecundity, longevity and fitness of the insect (Midthassel et al., 2016). Contrarily, in our study, a single application of *B. bassiana* PSUB01 on Chinese kale through root drenching showed no direct impact on the reproduction and fecundity of insects on treated plants. This may be due to the low level of colonised conidia, which gradually decreased over time in the host plant with no adverse effects on the insect (Russo et al., 2015).

Our study demonstrated enhanced plant height and root length in treatments with the application of entomopathogenic fungus, *B. bassiana* PSUB01, in Chinese kale, without dramatic differences between plants with or without aphid infestations. However, the fresh and dry weights of Chinese kale with aphid infestation were significantly different from the control. Many studies have reported that endophytic or transient fungal entomopathogens can reduce the impact of abiotic and biotic stress, which is reflected in increased plant vigour as well as safeguard from plant feeding insects (Gao et al., 2010; Jaber & Araj, 2018; Jaber & Enkerli, 2016, 2017; Jaber & Ownley, 2018; Lopez & Sword, 2015).

Endophytic entomopathogenic fungi can elicit induced systemic resistance (ISR) in host plants, causing reduction of disease severity, improvement in plant stress tolerance, and augment the defense against insect pests (Hartley & Gange, 2009; Mantzoukas & Eliopoulos, 2020; Yadav & Yadav, 2017). The improvement of plant responses via endophytic entomopathogenic fungi happens through either ISR or the production of compounds such as insecticides, antifungals, herbicides and antivirals during multiple biocide activities. The results of these processes enhance plant growth (Jaber & Enkerli, 2017), plant nutrition (Qiu et al., 2014; Skinner, Gouli, Frank, Parker, & Kim, 2012), root development (Vega et al., 2008), and relief from abiotic stresses such as salinity (Jaber & Ownley, 2018) or iron deficiency (Vega, 2018). The successful colonisation of cotton plants by *B. bassiana* resulted in increased plant biomass and reduced pest survival (Lopez & Sword, 2015). The application of entomopathogens improved plant health and yield as well as reduction in wireworm damage in wheat (Reddy et al., 2014).

In our study, both foliar application of conidia and the combination of a conidial foliar spray together with a root drench promoted growth (as both fresh and dry weight) of

Chinese kale with aphid infestations. Moreover, application of *B. bassiana* to host plant of consumable food is considered to be safe for human health (Hu et al., 2015; Zimmermann, 2007). Therefore, we recommend the use of the entomopathogenic fungus, *B. bassiana* PSUB01, as a component of integrated pest management (IPM) programs for control of mustard aphid, *L. erysimi,* in Chinese kale or in other vegetable crops when cultivated in a hydroponic system.

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## Disclosure statement

No potential conflict of interest was reported by the author(s).

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