Influence of treatment interval between eco-friendly agricultural materials and *Beauveria bassiana* GHA on sweet potato whitefly control

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Abstract

For the effective control of pests and plant diseases during crop cultivation, farmers simultaneously or sequentially spray various eco-friendly agricultural materials (EFAM) and microbial control agents on the same fields. There is little research that examines the compatibility of these EFAMs with entomopathogenic fungi and the influence of EFAMs on the control efficacy of mycopesticides. In this research, the influence of 3 fungicides and 4 insecticides, which were made from EFAMs, on mycelial growth, germination and virulence of *Beauveria bassiana* on sweet potato whitefly (*Bemisia tabaci*) was studied. The germination rate of *B. bassiana* was inhibited by Eunhasu®, Whalwhasan®, Chameleon® and Jinsamiplus®. The mycelial growth was also inhibited by all of the tested EFAMs. The results also showed that all of the insecticides tested, except Jinsamiplus®, showed synergistic additive effects with *B. bassiana*, regardless of pre-treatment time, in controlling sweet potato whitefly. However, the mortality of *B. tabaci* with *B. bassiana* and 3 EFAM fungicides was lower than or similar to the mortality with *B. bassiana* alone.

Key words: *Beauveria bassiana*, eco-friendly agricultural material, entomopathogenic fungi, compatibility, sweet potato whitefly
As biocontrol agents, entomopathogenic fungi have been widely used for the control of insect pests in many countries [1]. Beauveria bassiana, the most important and common type of entomopathogenic fungus, has been successfully used to control many different kinds of forest and agricultural pests [2-6]. This fungus is usually preferred as a model for the study of entomopathogenic fungi and the biological control of pests [7, 8]. The primary reasons for interest in B. bassiana include its wide host range [9], availability of abundant strains [10], safety towards non-target organisms [11], and its capacity for in vitro mass-culture [12].

However, there are some factors that can cause the failure of B. bassiana in agro-ecosystems. For example, many agrochemicals are harmful to entomopathogenic fungi, especially some fungicides with broad spectrum activity that are routinely applied for the control of plant diseases [13]. It was reported that some fungicides and pesticides may antagonize the potential insecticidal activity and efficiency of B. bassiana [14-16]. These chemicals may also disrupt the natural epizootics of B. bassiana [17]. Therefore, sometimes the utilization of B. bassiana in forestry and agricultural production is limited because of the undesirable interference from some fungicides and pesticides [16].

Conversely, some reports show that compatible products could be associated with entomopathogenic fungi, decreasing the amount of pesticides and fungicides required, and in some cases, increasing the control efficiency and minimizing the risks of pest resistance and environmental contamination that are associated with those chemicals [18, 19]. Therefore, it is very important to take into account the compatibility of all products sprayed on forests and crops when integrated pest management (IPM) strategies are being devised, and consideration should be given to using them during seasons when the effects on natural control agents are minimized and avoiding the use of the most toxic strategies [20]. The sensitivity or tolerance of a candidate entomopathogenic isolate for the fungicide and
pesticide must be determined before its simultaneous use in IPM programs.

Many studies have focused on the effects of chemical fungicides and pesticides on entomopathogenic fungi [21, 22], although many eco-friendly EFAMs have been used commercially for crop protection [23-26]. In addition, when farmers spray control agents on their farms, they want to routinely mix and spray several control agents together to reduce the cost of application and to widen the range of treatments provided by a single application. Fungicides, insecticides and/or microbial control agents can be sprayed sequentially on a farm to control various insect pests and diseases. The aim of this study was to determine the influence of seven different commercial fungicides and pesticides, which were made from EFAMs and registered to use tomato the mycelial growth, germination, and virulence of the B. bassiana GHA isolate on sweet potato whitefly (Bemisia tabacì) which is the most serious pest in tomato. Our detailed research concerning the compatibility of different EFAMs with B. bassiana should help in choosing the optimal combination to improve the efficiency of IPM and to achieve a higher level of sustainability and reliability within the integrated crop protection program.

MATERIALS AND METHODS

Fungus

The commercial isolate of B. bassiana GHA (Mycotech Corp., Butte, MT), which is registered in North America for the control of whitefly, mealybug, aphids and thrips, was used in our experiments. The isolate was grown for 10 days on potato dextrose agar (PDA, Difco, Sparks, MD, USA) at 25±1°C. The conidia were harvested from the medium by adding a sterilized Tween 80 solution (0.01%) and scrubbing the surface with a glass bar. Then, the conidial suspension was filtered through sterilized cheesecloth. To produce a homogeneous suspension, the conidia suspensions were vortexed for 1 min, and the
concentration of conidia suspensions was determined using a microscope and a haemocytometer (Sigma-Aldrich, St. Louis, MO, USA). Finally, conidial suspensions of different concentrations were made to assess their effect on germination, mycelial growth and the bioassay with EFAMs.

**Eco-friendly agricultural materials**

Three fungicides and four insecticides, which were made from EFAMs using tomato, were studied in our experiment. Information on trade name, manufacturer and active ingredient of these products is shown in Table 1, and all of them were used before their expiration date (less than one-year-old). All fungicides and insecticides were dissolved and homogenized in sterilized 0.01% aqueous Tween 80. Three different concentrations (0.5X, 1X and 2X the recommended dose) were used to evaluate spore germination and mycelial growth.

**Effects of different eco-friendly agricultural materials on conidial germination**

To assess the influence of EFAMs on conidial germination of *B. bassiana* GHA, seven commercial EFAM products were tested. Conidial suspensions were diluted in the EFAM solutions of three different concentrations to obtain a final concentration of $10^5$ conidia/ml. The mixtures were vortexed for 1 min to produce a homogeneous solution.

A total of 10 ml of each sample was incubated for various durations (0.5, 2 and 4 h) at 25°C. It will be called as "tank mixing time" later for the incubation time of conidia with a EFAM. Spore suspensions without EFAMs were used as controls. After treatment, 10 μl of conidial suspension was dropped onto the surface of a water agar (WA) plate (30 mm in diameter) and was incubated for 16 hours at 25°C. In order to stop and stain fungal growth, lactophenol cotton blue was dropped on the WA plate. The germination rate was assessed
using a microscope at 400× magnification by randomly observing 100 conidia. The conidia were considered germinated if their germ tubes were equal to or greater than the conidial width.

**Effects of different eco-friendly agricultural materials on mycelial growth**

The influence of EFAMs on mycelial growth of *B. bassiana* GHA was also evaluated with three different concentrations of each EFAM. After an incubation period of 0.5 to 4 h at 25°C, a 5 μl sample of each mixed solution with a final conidial concentration of $10^7$ conidia/ml was point-inoculated onto the center of a WA plate (90 mm in diameter). These plates were then incubated in the dark for 7 days at 25°C. The conidial suspensions without EFAMs were used as controls. The mycelial growth was measured with a digital micrometer caliper (SparkFun Electronics, Niwot, Colorado, USA) based on the colony diameter of each treatment after cultivation. The width of the colony growth at the widest and the narrowest points was averaged to obtain the diameter [27].

**Effects of treatment interval between eco-friendly agricultural materials and *B. bassiana* GHA for sweet potato whitefly control**

To investigate the effects of the treatment interval between EFAMs and *B. bassiana* GHA for pest control, bioassays were performed using sweet potato whitefly adults which are a key pest in tomato. Before applying the conidial suspension of *B. bassiana* GHA, seven different EFAM solutions of recommended doses were sprayed on 15-day-old cucumber seedlings. Then, the sprayed seedlings were kept in a greenhouse for various durations (0, 4 and 7 days) until they were treated with *B. bassiana* GHA (Fig. 1).

The approach of the bioassay was slightly modified from that of Zhu and Kim [28]. A 2 ml conidial suspension of *B. bassiana* GHA ($10^8$ conidia/ml) was applied to each treated
cucumber seedling, and the controls were treated with 0.01% Tween 80 solution. After air
drying at room temperature for 1 h, each cucumber seedling was placed in a small plastic
box (10 cm diameter and 15 cm height) with a ventilation hole covered with nylon mesh (3
cm diameter) on the lid. Twenty whitefly adults (30-day-old) were placed in each box using
a mini pump (MP-2, Sibata Scientific Technology Ltd, Japan) and were maintained at 25°C
with a photoperiod of L:D 16:8. The mortality of sweet potato whiteflies was recorded daily
for 6 days. Three seedlings were used in each treatment. The entire assay was repeated on
three different dates.

**Statistical analysis**

The conidial germination, mycelial growth and percent mortality of whitefly were
analyzed using SAS software version 9.2 (SAS Institute Inc, Cary, NC, USA). In our study,
two-way analysis of variance (Proc GLM) was used to compare germination rate and
mycelial growth of *B. bassiana* GHA with eco-friendly agricultural materials at first. The
categories compared were EFAM, concentration, tank-mixing time and the interaction
between EFAM and tank-mixing time, the interaction between EFAM and concentration,
the interaction between tank-mixing time and concentration and between EFAM, tank-
mixing time and concentration. After finding the influence of EFAM on *B. bassiana* GHA,
analysis of variance (Proc GLM) was used to compare the germination rate and mycelial
growth of *B. bassiana* GHA. Analysis of variance (Proc GLM) was also used to compare
the mortality of whitefly with GHA plus eco-friendly agricultural material pre-treated at
different days. Means were separated using Fisher’s protected least significant difference
(LSD) at $P = 0.05$. All of the experiments were conducted using triplicate samples for each
treatment, and all experiments were repeated three different times.
RESULTS

Influence of eco-friendly agricultural materials on conidial germination of *B. bassiana*

The effects of the various EFAMs of different concentrations on the conidial germination of *B. bassiana* are shown in Fig. 2A. In untreated Petri dishes, mean conidial germination was nearly 92% in 16 h. According to two-way analysis, each EFAM, concentration, and tank-mixing time showed significant difference. The interaction between EFAM and concentration was significantly different (P < 0.0014), but the interaction between EFAM and tank-mixing time (P = 0.0892), the interaction between tank-mixing time and concentration (P = 9551) and the interaction between EFAM, tank-mixing time and concentration (P = 0.9990) was not significantly different (Table 2). All fungicides tested, including Eunhasu®, Whalwhasan® and Chameleon®, and the insecticide Jinsamiplus® significantly inhibited the germination rate of the fungus at all concentrations for tank mixing time of 0.5 h compared with control (0.5X, F = 94.38; P < 0.0001; 1X, F = 60.97; P < 0.0001; 2X, F= 51.7; P < 0.0001). A mixture of Japanese apricot + ginkgo nut extracts (Chameleon®) and a mixture of Japanese honeysuckle + Jeffersonia + Korean pasque flower extracts (Eunhasu®) elicited a decrease in the germination percentage with increasing concentrations. However, the extract of yellow sophora (Eungbaksa® and Allcatch®), which is used as an insecticide, had no significant impact on conidial germination relative to the control, with 90% and 91% germination, respectively. Although not significant, *B. bassiana* treated with bead tree + yellow sophora extract (Bogumeco®) showed a slightly higher mean germination rate (96%) than the control. All fungicides tested inhibited germination of *B. bassiana* and the inhibition rate of Chameleon and Eunhasu was higher with increasing concentration.

However, the results also indicated that when the tank mixing time increased from 0.5 h to 2 or 4 h, spore germination increased with a mixture of spore suspension and each EFAM and control (only spore suspension without EFAM) all EFAMs tested increased spore germination.
germination when the tank mixing time increased (Table 3). For example, the mean germination rates of B. bassiana mixed with Eunhasu® increased from 24% with 0.5 h tank mixing to 42% with 4 h tank mixing (F = 3.07; P = 0.0761). Whalwhasan® showed the same trend, with germination increasing from 46% to 73% (F = 11.08; P = 0.0011). Eungbaksa® (F = 8.93 P = 0.0032), Jinsamiplus® (F = 4.21; P = 0.0371) and Allcatch® (F = 3.89; P = 0.0452) also significantly increased the rate of spore germination with increased tank-mixing time. Although there was not significantly interact between EFAM and tank-mixing time, germination rate of each fungicide or insecticide increased with increased tank-mixing time.

Influence of eco-friendly agricultural materials on mycelial growth of B. bassiana

The effects of different EFAMs on the average radial growth of B. bassiana are presented in Fig. 2B. According to two-way analysis, each EFAM, concentration, and tank-mixing time showed significant difference. The interaction between EFAM and tank-mixing time (P = 0.0008), the interaction between EFAM and concentration (P < 0.0001), and the interaction between EFAM, tank-mixing time and concentration (P = 0.0222) was significantly different, but the interaction between tank-mixing time and concentration (P = 1858) was not significantly different (Table2). After 7 days of incubation, the mean mycelial growth of isolate GHA was significantly affected by all EFAMs at all tested concentrations (0.5X, F = 11.16; P < 0.0001: 1X, F = 15.41; P < 0.0001: 2X, F= 40.22; P < 0.0001). Eunhasu® (F = 11.31; P < 0.0001), Allcatch® (F = 2.66; P = 0.1046) and Jinsamyiplus® (F = 4.92; P = 0.0241) caused a greater reduction in radial growth with increasing concentrations. Various concentrations of Eunhasu® were found to be toxic and caused different levels of inhibition of radial growth, with a reduction of 22.5, 34.9 and 49.5% over the control. The lowest level of inhibition (12.6%) was observed for Bogumeco® when it was used at 0.5X the recommended dose. All EFAMs tested inhibited mycelia growth of the
GHA. Eunhasu®, Allcatch® and Jinsamyiplus® showed greater inhibition of mycelia growth according to increasing concentration.

We also found that mixing time did significantly affect mycelial growth of *B. bassiana* for most EFAMs (Table 4). However, mycelial growth exhibited a decreasing trend with increased mixing time, although it was not always significantly different. This was the case, for example, with Eunhasu® (F = 3.07; P = 0.0761), Allcatch® (F = 0.03; P = 0.9744) and Jinsamyiplus® (F = 4.57; P = 0.0282), whereas the decrease in mycelial growth with Whalwhasan® was significant (F = 4.57; P = 0.0282).

**Influence of treatment interval between eco-friendly agricultural materials and *B. bassiana* GHA for sweet potato whitefly control**

The results showed that the average mortality of the control (insects sprayed with 0.01% Tween 80) was less than 5% after 6 days. Whiteflies began to die 1 day after exposure to the conidial suspension in most treatments (Fig. 3 and 4).

In this study, *B. bassiana* applied alone caused an average mortality of 65% at the concentrations of 1×10⁸ conidia/ml after 6 days. Compared with the solitary application of *B. bassiana*, the percent mortality of *B. tabaci* treated with both EFAMs (insecticides) and *B. bassiana* was much higher for all EFAMs (insecticides), except Jinsamyiplus®. For Eungbaksa®, Allcatch® and Bogumeco®, there were significant differences in the mortalities from the application of *B. bassiana* alone and the combinations of these EFAMs (insecticides) with the fungus. These results suggested that these EFAMs (insecticides) showed additive/synergistic effects with *B. bassiana* GHA in the control of sweet potato whitefly (Fig. 4). The results, depicted in Fig. 3, also showed that the mortalities of *B. tabaci* treated with fungus and EFAMs (fungicides) were lower or similar when compared to the treatments with *B. bassiana* alone. It was determined that Chameleon® and Eunhasu® inhibited the infection
capacity of *B. bassiana* in the bioassay at three different treatment intervals. This was especially true for Chameleon®, as the mortality caused by the combination of this EFAM with *B. bassiana* ranged between 45% and 25%, which was much lower than the application of the fungus alone (65%). *B. bassiana* GHA with insecticides tested except Jinsamyiplus® had additive/synergistic effect to control sweet potato whiteflies, but the fungus with fungicides tested showed antagonistic effect to control the whitefly.

**DISCUSSION**

The present study clearly showed that different EFAMs have variable effects on the growth and germination of *B. bassiana* and its ability to infect sweet potato whitefly.

It is well known that conidial germination is one of the most important steps of the fungal infection process for entomopathogenic fungi [29]. Therefore, the germination tests carried out in our study allowed for the evaluation of the effects of EFAMs on this process. Previous reports indicated that the treatment of entomopathogenic fungal spores with biological and chemical products may remove the mucous layer that covers the spores or may neutralize the electrostatic charge on the spore surface, thus affecting the process of substrate recognition and the transduction of signals that initiate germination [29]. The inhibition of conidial germination and mycelial growth can also be related to the presence of biological and chemical products, which may block fungal metabolic functions [30].

The negative impacts of fungicides and pesticides on entomopathogenic fungi in vitro are widespread [31]. In this study, the conidial germination of *B. bassiana* GHA isolate was strongly influenced by all three fungicides, which were made from EFAMs, with a fungistatic effect observed at 1X and 2X the recommended doses. Whalwhasan® and Eunhasu® also markedly inhibited the germination of *B. bassiana* at half of their recommended doses.
Chameleon® was the only fungicide tested that did not significantly inhibit the germination of B. bassiana at a reduced dose. The mycelial growth of B. bassiana was also strongly restricted by these three fungicides. Most of the insecticides that were used in this study did not have pronounced negative impacts on the spore germination of B. bassiana. The exception to this was Jinsamiplus®. The mean mycelial growth of B. bassiana was significantly affected by all insecticides tested. Our study also revealed that the 2 and 4 h tank mixing increased spore germination and did not influence mycelial growth for most of the EFAMs. However, Eunhasu® negatively affected the mycelial growth of B. bassiana.

According to Dillon and Charnley (1990), a prolonged period of soaking of spores in distilled water accelerated an initial pre-swelling phase of germination, spherical growth and germ-tube formation dependent on an exogenous carbon source. We guess the GHA spores survived in the a suspension mixture of both including EFAM and spores was activated by the water and the germination rate increased with tank mixing time.

The repeated application of these EFAMs, which were fungistatic (retarding the development of mycelia while in contact with the chemical) and fungicidal (inhibiting the germination of fungal spores) in vitro, over a short period would result in reduced populations of entomopathogenic fungi. Because the fungi in all of the treatments ultimately grew (as evidenced by observation of colony development), this indicates that the effects of the EFAMs were fungistatic, rather than fungicidal, for B. bassiana.

For entomopathogenic fungi, earlier studies have shown that varying levels of susceptibility to fungicides depend upon growth stage [15]. Moorhouse et al. (1992) reported that the impact of some pesticides and fungicides were more pronounced on mycelial growth than conidial germination of M. anisopliae [33]. Bruck (2009) also found that the mycelial growth of entomopathogenic fungi was more sensitive than conidial germination to fungicides [31]. Similarly, our results demonstrated that these EFAMs had less of a negative
impact on the spore germination of *B. bassiana* than on mycelial growth, which is in agreement with Moorhouse et al. (1992) and Bruck (2009).

The potential inhibitory effects of EFAMs on the germination and mycelial growth of entomopathogenic fungi may vary among taxa and strains. Although the EFAMs tested in vitro in this study caused a reduction in germination and mycelial growth in relation to the control, the effects under greenhouse or field conditions may differ because factors such as temperature, UV and humidity may impair the exposure of the fungus to the EFAM compounds.

Some researchers reported little correlation between in vitro laboratory studies and in situ applications of insecticides and fungicides on entomopathogenic fungi [33]. Chandler et al. (2005) found *M. anisopliae* to be compatible with several fungicides under greenhouse conditions for the control of *Delia radicum* (L.), although in vitro laboratory studies found that they were inhibitory to fungal growth [34]. There were some indications that reduced germination of entomopathogenic fungi in vitro may be linked to a reduction in their rate of infection, but the relationship was not significant [33]. In our bioassay with sweet potato whitefly, the effects of the EFAM fungicides (Chameleon® and Eunhasu®), which significantly reduced the efficiency of *B. bassiana*, were fungistatic and fungicidal in vitro and required short reapplication intervals (4 and 7 days, respectively) to obtain a similar control efficacy to the *B. bassiana* GHA treatment alone. Whalwhasan® was the only fungicide of the EFAMs tested where the reapplication interval did not have any effect on infection rate of *B. bassiana*. The EFAM insecticides, Eungbaksa®, Allcatch®, Jinsamyiplus®, and Bogumeco®, showed synergistic-additive effects with *B. bassiana* GHA in the control of sweet potato whitefly even though these insecticides were not enrolled to control *B. tabaci*.

From the results obtained in the present research, we suggest that among EFAMs, fungicides such as Chameleon®, Whalwhasan® and Eunhasu®, inhibited spore germination
and mycelia growth of *B. bassiana* GHA. These fungicides showed less control efficacy against sweet potato whitefly when the fungus treated at the same day or 4 or 7 days after EFAM treatment. The extract of yellow sophora (*Eungbaksa*® and *Allcatch*®), and the mixture of bead tree + yellow sophora (*Bogumeco*®), did not inhibited spore germination of GHA isolate and are appropriate insecticides for use in integrated pest management programs against *B. tabaci* in combination with *B. bassiana*. Further research under field conditions should be conducted in order to confirm the compatibility of EFAMs and entomopathogenic fungi within an IPM strategy.

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Table 1. List of eco-friendly agricultural materials used in this study.

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<th>Active ingredients</th>
<th>Products® (Company)</th>
<th>Recommended concentration (ppm)</th>
<th>Categories</th>
</tr>
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<tr>
<td>Japanese apricot + ginkgo nut</td>
<td>Chameleon® (Chobi Co.)</td>
<td>1,400</td>
<td>Fungicide</td>
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<tr>
<td>Japanese honeysuckle + Jeffersonia + Korean pasque flower</td>
<td>Whalwhasan® (Jin-young Co.)</td>
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<td>Japanese honeysuckle + Jeffersonia + Korean pasque flower</td>
<td>Eunhasu® (Chobi Co.)</td>
<td>500</td>
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<tr>
<td>Yellow sophora</td>
<td>Eungbaks® (Pioneer Co.)</td>
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<td>Insecticide</td>
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<tr>
<td>Yellow sophora</td>
<td>Allcatch® (Nambo Co.)</td>
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<td>Yellow sophora + derris</td>
<td>Jinsamiplus® (KoreaBio Co.)</td>
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<tr>
<td>Bead tree + yellow sophora</td>
<td>Bogumeco® (Kyungnong Co.)</td>
<td>1,000</td>
<td>Insecticide</td>
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</table>
Table 2. ANOVA tables for the germination rate and mycelial growth of *B. bassiana* GHA with different concentrations and tank-mixing times of eco-friendly agricultural materials.

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<tr>
<th>Source</th>
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<td>EFAM</td>
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<td>5646.08</td>
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<td>2114.34</td>
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<td>194.93</td>
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<tr>
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<td>210.10</td>
<td>17.51</td>
<td>5.63</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Tank-mixing time* concentrations</td>
<td>4</td>
<td>19.35</td>
<td>4.83</td>
<td>1.56</td>
<td>0.1858</td>
</tr>
<tr>
<td>EFAM<em>tank-mixing time</em>concentrations</td>
<td>24</td>
<td>127.45</td>
<td>5.31</td>
<td>1.71</td>
<td>0.0222</td>
</tr>
</tbody>
</table>
Table 3. Effect of tank mixing time of eco-friendly agricultural materials of recommended concentrations on spore germination of *B. bassiana* GHA.

<table>
<thead>
<tr>
<th>Eco-friendly agricultural materials</th>
<th>Germination rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tank mixing time (h) with conidia of <em>B. bassiana</em> GHA and eco-friendly agricultural materials</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>Control</td>
<td>90.4±2.2 A</td>
</tr>
<tr>
<td>Chameleon® (Japanese apricot + ginkgo nut)</td>
<td>77.1±4.1 B</td>
</tr>
<tr>
<td>Whalwhasan® (Sulphur)</td>
<td>45.8±3.2 B</td>
</tr>
<tr>
<td>Eunhasu® (Japanese honeysuckle + Jeffersonia + Korean pasque flower)</td>
<td>23.8±6.9 A</td>
</tr>
<tr>
<td>Eungbaksu® (Yellow sophora)</td>
<td>85.7±1.1 B</td>
</tr>
<tr>
<td>Jinsamiplus® (Yellow sophora + derris)</td>
<td>69.5±7.6 B</td>
</tr>
<tr>
<td>Allcatch® (Yellow sophora)</td>
<td>90.2±0.7 B</td>
</tr>
<tr>
<td>Boguneco® (Bead tree + yellow sophora)</td>
<td>93.1±2.9 B</td>
</tr>
</tbody>
</table>

* Means with the same letters in each EFAM are not significantly different (P < 0.05, LSD
Table 4. Effect of tank mixing time of eco-friendly agricultural materials of recommended concentration on mycelial growth of *B. bassiana* GHA.

<table>
<thead>
<tr>
<th>Eco-friendly agricultural materials</th>
<th>Mycelial growth (mm)</th>
<th>Tank mixing time (h) with conidia of <em>B. bassiana</em> GHA and eco-friendly agricultural materials</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>Control</td>
<td>24.4±0.7 A</td>
<td>23.3±0.8 A</td>
</tr>
<tr>
<td>Chameleon® (Japanese apricot + ginkgo nut)</td>
<td>19.2±0.4 A</td>
<td>17.8±0.6 A</td>
</tr>
<tr>
<td>Whalwhasan® (Sulphur)</td>
<td>21.7±0.3 A</td>
<td>20.3±0.4 AB</td>
</tr>
<tr>
<td>Eunhasu® (Japanese honeysuckle + Jeffersonia + Korean pasque flower)</td>
<td>16.9±0.4 A</td>
<td>15.7±0.6 AB</td>
</tr>
<tr>
<td>Eungbaksa® (Yellow sophora)</td>
<td>18.1±0.6 A</td>
<td>17.3±0.4 A</td>
</tr>
<tr>
<td>Jinsamiplus® (Yellow sophora + derris)</td>
<td>16.8±0.5 A</td>
<td>17.3±1.0 A</td>
</tr>
<tr>
<td>Allcatch® (Yellow sophora)</td>
<td>18.8±0.6 A</td>
<td>18.7±1.0 A</td>
</tr>
<tr>
<td>Bogumeeco® (Bead tree + yellow sophora)</td>
<td>19.7±0.9 A</td>
<td>20.8±0.5 A</td>
</tr>
</tbody>
</table>

* Means with the same letters in each EFAM are not significantly different (P < 0.05, LSD
Figure 1. Application of eco-friendly agricultural materials and *B. bassiana* GHA on greenhouse cucumbers for sweet potato whitefly control: (A) EFAM treatment, (B) plants kept for 7, 4, or 0 days in greenhouse after spraying, (C) *B. bassiana* GHA treatment at 7, 4, and 0 days after EFAM spray.

Figure 2. Influence of eco-friendly agricultural materials on germination rate (A) and mycelial growth (B) of *B. bassiana* GHA. Three different concentrations of EFAM (0.5X, 1X and 2X the recommended concentrations) were mixed with GHA suspensions for 0.5 h and inoculated on media. Different letters of each concentration (0.5X, 1X and 2X) above the graph indicate significant differences among EFAMs of the same concentration (P < 0.05, LSD Test). “*” above the graph indicate significant difference among different concentrations of the same EFAM (P < 0.05, LSD Test).

Figure 3. Effect of treatment interval on *B. bassiana* GHA and fungicidal eco-friendly agricultural material for the control of sweet potato whitefly (*Bemisia tabaci*). Means from the same day with different letters are significantly different (P < 0.05, LSD Test). (DBT: days before GHA treatment).

Figure 4. Effect of treatment interval between *B. bassiana* GHA and insecticidal eco-friendly agricultural material to control sweet potato whitefly (*Bemisia tabaci*). Means of the same
day with different letters are significantly different (P < 0.05, LSD Test). (DBT: days before GHA treatment).
Fig. 1.
Fig. 2.

A. Germination rate

B. Mycelial growth
Fig. 3.

A. Chameleon

B. Whalshasan

C. Eunhasu
Fig. 4.

A. Eungbaksaf

B. Jinsamiplus

C. Allcatch

D. Bogumeco