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# Effect of different concentrations of *Beauveria bassiana* (Balsamo) Vuillemin against corn leaf aphid, *Rhopalosiphum maidis* (Fitch) (Hemiptera: Aphididae)

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## Abstract

An evaluation of the efficacy of different concentrations of the entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin against the corn leaf aphid, *Rhopalosiphum maidis* (Fitch) (Hemiptera: Aphididae) was carried out at Maejo University Biological Control Technology Learning Center (MJU-BCTLC) from August to October 2015. Ten concentrations of *B. bassiana* ranging from $1\times10^{1}$ to $1\times10^{10}$ spores/ml were used in the bioassay under the laboratory and greenhouse conditions. The laboratory bioassay revealed that all ten concentrations were found pathogenic to *R. maidis*. The mortality obtained ranged from 43.60% to 85.40%, highest at 85.40% of the concentration of $1\times10^{10}$ spores/ml and lowest at 43.60% of the concentration of $1\times10^{3}$ spores/ml, three days after treatment (DAT). However, the percent mortality values were not significantly different. They decreased with decreasing spore concentrations. Under the greenhouse condition the aphid infestation was at 65 days after sowing (DAS). At the concentrations of $1\times10^{10}$, $1\times10^{9}$, $1\times10^{8}$, $1\times10^{7}$, $1\times10^{6}$ spores/ml. The aphid population (5.6, 7.0, 9.8, 11.0 and 19.2 aphids per plant) at 65 DAS were drastically reduced at 72 DAS to 1.2, 3.0, 5.0, 9.4 and 11.2 respectively. In the control and at the concentration of $1\times10^{3}$, $1\times10^{2}$ and $1\times10^{1}$ spores/ml, the mean number of aphid population gradually increased at weekly interval starting from 58 DAS until the end of cropping period. The study showed that the concentration at $1\times10^{10}$ spores/ml was most effective to reduce the corn leaf aphid population.

**Keywords:** *Beauveria bassiana*, biological pest control, percent mortality, *Rhopalosiphum maidis*, spore concentrations

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## 1. Introduction

Corn (*Zea mays* L.) is one of the most important food crops worldwide, serving as staple food, livestock feed, and industrial raw material. It is vulnerable to the infestation of a number of insect pests. Among them, the corn leaf aphid, *Rhopalosiphum maidis* (Fitch) (Hemiptera: Aphididae), is a serious pest. *R. maidis* is of Asiatic origin. It is almost cosmopolitan in its distribution throughout the tropics, subtropics and the warmer temperate regions [1]. It is polyphagous and its alternative hosts include sorghum, millets, sugarcane, wheat, barley, rice, and other graminaceous crops [2]. *R. maidis* damage occurs when large populations attack the corn plant at a late whorl stage before anthesis. Both nymphs and adults suck plant sap resulting in yellowish mottling, malformed kernels, reduction in the number of kernels, and even barrenness [3] and severe infestation can cause yield reduction through feeding damage and the tassel covered with honeydew. In addition to direct plant damage, it also can transmit plant viruses such as maize dwarf mosaic virus and barley yellow dwarf virus [4, 5].

At present, the management of aphids is mainly by using insecticides. However, developments of resistance against pesticides, pest resurgence, and adverse effect on biocontrol agents, health problems and the environment have arisen due to this method [5]. Considering the negative impact of insecticides, a pest management strategy using its natural enemies such as predators, parasitoids and microbial control agents are acceptable alternative methods. The entomopathogenic *Beauveria bassiana* (Balsamo) Vuillemin is used worldwide, and it can provide an environmentally responsive substitute to chemical pesticides without damage to non-target organisms [6]. *B. bassiana* also has a wide host range [7], and its occurrence is worldwide.

The entomopathogenic fungi are naturally occurring and have potential as biocontrol agents against a

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number of important insect pests of agricultural crops [8]. They are–relatively cheap for mass production, easy for storage and effective over a wide range of temperature and humidity resulting in a rapid kill at economical doses. Previous investigations related to the efficacy of different entomopathogenic fungi such as *B. bassiana*, *Metarhizium anisopliae* and *Lecanicillium* spp. to control aphids have been conducted and shown a great success [9, 10]. Due to little information on the efficacy of *B. bassiana* against *R. maidis*, the objectives of the present study were to evaluate the efficacy of different concentrations of *B. bassiana* against the corn leaf aphid under laboratory and greenhouse conditions.

## 2. Materials and methods

### 2.1 Experimental site and duration

The experiment was carried out from August to October 2015 at Maejo University Biological Control Technology Learning Center (MJU-BCTLC), Faculty of Agricultural Production, Maejo University, Chiang Mai, Thailand.

### 2.2 Rearing of aphids

The corn leaf aphids were collected from the corn fields in the Maejo University Experimental Farms by collecting aphid-infested corn plant parts such as leaves and tassels. They were kept in plastic bags, placed in the cooler and brought back to the laboratory. They were then reared on potted corn plants in the greenhouse. All agronomic practices (e.g. application of fertilizers) were given except insecticide application. The aphid culture was thus maintained for both laboratory and greenhouse assays until all experiments were complete.

### 2.3 Fungus culture

The entomopathogenic fungus, *B. bassiana,* was obtained from Maejo University Biological Control Technology Learning Center (MJU-BCTLC), Maejo University Biological Control Research Center (MJU-BCRC). This fungal isolate was subcultured on Potato Dextrose Agar (PDA) and incubated at room temperature for 14 days. The conidia from 14 days-old culture surface were harvested by scraping them off the medium gently with the help of a sterilized glass rod, and were suspended in distilled water for further uses. The hyphal debris was removed by filtering the mixture through a double-layered muslin cloth. The filtrate were mixed with a known quantity of 0.02% Tween 80. The conidial concentration of the final suspension was adjusted with haemacytometer to obtain $1\times10^{10}$ spores per ml from which the lower concentrations were prepared by serial dilution technique for bioassay study.

For greenhouse experiment 1 cm diameter of 14 days old culture of *B. bassiana* was mass produced on autoclaved boiled broken rice and incubated for 14 days. After 14 days the suspension was filtered through a double-layered muslin cloth and the conidial concentrations were accordingly adjusted as in the laboratory experiment.

### 2.4 Bioassay

The efficacy of *B. bassiana* against corn leaf aphid in the laboratory was determined by using detached leaf method. Ten concentrations of spore suspensions, namely, $1\times10^{1}$, $1\times10^{2}$, $1\times10^{3}$, $1\times10^{4}$, $1\times10^{5}$, $1\times10^{6}$, $1\times10^{7}$, $1\times10^{8}$, $1\times10^{9}$ and $1\times10^{10}$ spores per ml were used. For each treatment, aphid infested corn leaves were cut and the leaf petioles were kept immediately wet with water-soaked sterilized cotton wool wrapped in aluminum foil to keep them moist and for protecting them from dehydration. They were placed on moistened filter paper inside a clear plastic container ($10\times10\times6$ cm), covered with muslin cloth. The number of aphids on infested leaves was counted and sprayed with 3 ml of respective conidial concentrations. In the control, aphids were treated with 3 ml of sterilized distilled water containing 0.02% Tween 80. The plastic boxes were covered with lids.

For the greenhouse bioassay, 30 days old corn plants were used, and in each treatment one corn plant per pot was used. The natural infestation of the corn leaf aphid was recorded and 100 ml of each concentration of *B. bassiana* spores was applied at 30 days after sowing and at weekly intervals afterward until the end of cropping period.

### 2.5 Data analysis

The laboratory experiment was conducted using a completely randomized design (CRD) with five replications for each treatment. The mortality data were recorded separately at 24 h intervals in comparison to control. The dead corn leaf aphids from the treatment and control were collected and isolated on PDA plates for the observation of mycosis effect. The data and the means were analyzed by using SPSS21 software for ANOVA. The corrected percent mortality was obtained by using Abbott's formula [11] and the time-dose mortality determination for $LC_{50}$ and $LT_{50}$ were analyzed by using Probit analysis of the Finney's test [12].

For the greenhouse experiment a randomized completely block design (RCBD) was used with five replications for each treatment. Data on the number of

**Table 1** Percent mortality and time mortality response of *R. maidis* at various concentrations of *B.bassiana* at three days after treatment

| *B. bassiana* (spores per ml) | Percent Mortality (mean±SD) | $LT_{50}$ (Days) |
|---|---|---|
| $1\times10^{10}$ | $85.40\pm20.02^{ns}$ | 1.43 |
| $1\times10^{9}$ | $74.20\pm41.00^{ns}$ | 1.47 |
| $1\times10^{8}$ | $70.20\pm25.42^{ns}$ | 1.49 |
| $1\times10^{7}$ | $68.20\pm35.45^{ns}$ | 1.71 |
| $1\times10^{6}$ | $67.20\pm46.68^{ns}$ | 1.94 |
| $1\times10^{5}$ | $62.80\pm37.57^{ns}$ | 1.94 |
| $1\times10^{4}$ | $55.20\pm39.68^{ns}$ | 2.00 |
| $1\times10^{3}$ | $43.60\pm43.87^{ns}$ | 2.02 |
| $1\times10^{2}$ | $53.20\pm48.69^{ns}$ | 1.49 |
| $1\times10^{1}$ | $80.00\pm44.72^{ns}$ | 1.36 |
| Control | $70.00\pm21.26^{ns}$ | - |

corn leaf aphids on corn plants were recorded weekly before fungal application and dead aphids were taken to the laboratory and isolated on PDA plates for mycosis confirmation. Data were evaluated by using XLSTAT and the differences were presented by Duncan's Multiple Range Test (DMRT) [13].

## 3. Results and discussion

### 3.1 Laboratory bioassay

The data of corrected percent mortality due to *B. bassiana* with different concentrations at three days after treatment are presented in Table 1. It indicates that the mortality increased with an increase of the higher concentrations. The recorded mortality of corn leaf aphid at different concentrations ranged from 43.60% to 85.40%. The highest percent mortality was 85.40% of the highest concentration of $1\times10^{10}$ spores per ml. The lowest percent mortality of 43.60% was recorded at the concentration of $1\times10^{3}$ spores per ml at three days after treatment. However, they were not significantly different among all concentrations (Table 1). Loureiro and Moino [14] reported percent mortality of three aphid species, namely, *Myzus persicae*, *Aphis gossypii* and *Aphis craccivora* at $1\times10^{6}$ to $1\times10^{7}$ spores per ml after four days and *B. bassiana* and *M. anisopliae* caused 100% mortality of *M. persicae* at $1\times10^{6}$ and $1\times10^{7}$ spores per ml, respectively. *B. bassiana* used in the present study caused 67.20% to 68.20% mortality response to corn leaf aphid at $1\times10^{6}$ to $1\times10^{7}$ spores per ml at three days after treatment. A progressive reduction in the mortality of aphids was observed with decreasing concentrations. Nirmala *et al.* [15] showed that two isolates of *B. bassiana* did not cause any mortality on *R. maidis*. However, in the present study, all the concentrations of *B. bassiana* were found to be pathogenic to *R. maidis*.

### 3.2 Median lethal concentration ($LC_{50}$)

The $LC_{50}$ value obtained in the present study was $1.2\times10^{3}$ spores per ml. The mortality of aphid began 24 h after treatment. The $LC_{50}$ value obtained was lower than that reported by Ujjan and Shahzad [16] for *B. bassiana* PDRL 1187 ($2.7\times10^{5}$ spores per ml) against the mustard aphid, *Lipaphis erysimi.*

### 3.3 Median lethal time ($LT_{50}$)

Variation in $LT_{50}$ values at different concentrations of *B. bassiana* are presented in Table 1. A low $LT_{50}$ value (1.43 days) was recorded at $1\times10^{10}$ spores per ml and the highest $LT_{50}$ value (2.02 days) was at $1\times10^{3}$ spores per ml. Nirmala *et al*. [15] obtained the $LT_{50}$ value (3.17 days) at $1\times10^{8}$ spores per ml against *Aphis craccivora*. In the present study, $LT_{50}$ value (1.49 days) was recorded at $1\times10^{2}$ and $1\times10^{8}$ spores per ml. According to Loureiro and Moino [14] the genetic variation in the fungal strains, difference in bioassay method, and aphid species involved could affect the difference of the virulence.

### 3.4 Greenhouse bioassay

The analysis of variance showed that the corn leaf aphid populations were affected significantly upon using different concentrations of *B. bassiana* on a weekly interval basis (Table 2). The mean aphid population was significantly lower at $1\times10^{10}$ spores/ml in all weeks. Similarly, the concentrations of $1\times10^{9}$, $1\times10^{8}$, $1\times10^{7}$, $1\times10^{6}$ and $1\times10^{5}$ spores/ml were found effective.

It was observed that the mortality of aphid population was detected at 58 days after sowing (DAS). Everly [17] stated that infestation of large

**Table 2** Mean number of corn leaf aphids per plant at different DAS and concentration of *B. bassiana*.

| Days after sowing | Treatment (spores per ml of *B. bassiana*) | | | | | | | | | | Control |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $10^{10}$ | $10^9$ | $10^8$ | $10^7$ | $10^6$ | $10^5$ | $10^4$ | $10^3$ | $10^2$ | $10^1$ | |
| 58 | 3.0 | 4.4 | 7.0 | 8.0 | 11.0 | 15.8 | 20.4 | 23.4 | 28.6 | 32.2 | 40.2 |
| 65 | 5.6 | 7.0 | 9.8 | 11.0 | 19.2 | 30.6 | 40.6 | 48.2 | 53.4 | 61.4 | 189.8 |
| 72 | 1.2 | 3.0 | 5.0 | 9.4 | 11.2 | 32.6 | 91.8 | 109.0 | 135.0 | 167.6 | 408.6 |
| 79 | 0.4 | 2.8 | 3.2 | 6.4 | 8.2 | 24.8 | 44.2 | 137.0 | 188.8 | 235.2 | 543.0 |
| 86 | 0 | 1.0 | 2.4 | 3.2 | 3.8 | 11.2 | 25.6 | 177.2 | 278.0 | 337.0 | 970.0 |
| Mean | **0.93** | **1.66** | **2.50** | **3.45** | **4.85** | **10.45** | **20.24** | **44.98** | **62.16** | **75.76** | **195.60** |

population of *R. maidis* build up in the corn whorl before anthesis. Douangboupha *et al.* [18] and concluded that higher infestation in the dry season was found during the tassel stage at 49 DAS. The present study shows that aphid population was found at tasseling in all treatments. However, in control and at lower concentrations of *B. bassiana* the aphid populations gradually increased until the end of cropping period. At the concentrations of $1\times10^{10}$, $1\times10^9$, $1\times10^8$, $1\times10^7$, $1\times10^6$ spores/ml, the maximum numbers of aphid (5.6, 7.0, 9.8, 11.0 and 19.2 aphids per plant) were recorded at 65 DAS. They were then drastically reduced at 72 DAS. Foott [19] reported that the corn leaf aphids were commonly found at the time of tasseling and after tassel emergence. Hunger *et al.* [20] found that the minimum infestation of corn leaf aphids was lower at the silking stage. It was also observed that the maximum aphid numbers (32.6 and 91.8 aphids per plant) were recorded at the concentrations of $1\times10^5$, and $1\times10^4$ spores/ml, respectively at 72 DAS. In control and at $1\times10^3$, $1\times10^2$ and $1\times10^1$ spores/ml the mean numbers of aphid gradually increased at a weekly interval starting from 58 DAS until the end of the cropping period. Applications of *B. bassiana* at the concentrations of $1\times10^{10}$, $1\times10^9$, $1\times10^8$, $1\times10^7$, $1\times10^6$, $1\times10^5$ spores/ml significantly reduced aphid numbers when compared with control and other concentrations (Table 2). The greenhouse bioassay thus indicated that the higher the concentrations of *B. bassiana*, the higher reduction of the aphid infestation.

## 4. Conclusions

The results obtained from the laboratory and greenhouse assays in the present study showed that all concentrations of *B. bassiana* were found pathogenic to *R. maidis*. Under the greenhouse conditions, the higher concentrations of *B. bassiana* had proven to be more effective than lower concentrations. The concentrations of *B. bassiana* from $1x10^6$ to $1x10^{10}$ spores per ml used in this study warrants further investigation under field conditions such that it can be further used as an entomopathogenic biocontrol agent for the management of the corn leaf aphid. It can also be incorporated in an integrated pest management program designed for the corn leaf aphid management together with other compatible insect pest management tactics.

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