BIBLIOGRAPHY

SACLANGAN, DAN A. APRIL 2008. Preliminary Study on the Field Application of *Trichoderma* spp. on Strawberry Flowers Using Honeybees (*Apis mellifera* Linnaeus). Benguet State University, La Trinidad, Benguet.

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ABSTRACT

The study was conducted to establish the efficiency of delivering *Trichoderma* spp. to strawberry flowers using honey bees, *Apis millefera*, and to observe any detrimental effect of the biological control agent (BCA) on the bee colony.

A BCA introduction box was designed and attached to a bee colony containing eight frames. The colony was situated on a strawberry farm of about 450 m^2 area. Two plots were covered with a white net to prevent the bees to forage on the flowers. Trial and observation were done for the first few days. The commercial *Trichoderma* spp. "Biocon" in powder form was placed in the BCA introduction box. After three days, strawberry flowers from different distances away from the bee colony and in the netted area were collected and isolated.

The isolated samples collected from the farm were positive of *Trichoderma* growth while samples from the netted area had other fungal and bacterial growths. The bees from the colony were able to disseminate *Trichoderma* to the strawberry farm as far as 30 meters away. Although the amount of food on the frames was reduced, bee population increase was noted during hive inspections on subsequent weeks after the experiment. There were no adverse effects of the BCA on the bee colony.

TABLE OF CONTENTS

INTRODUCTION

Biological control is a promising approach for the management of plant diseases but its use in commercial agriculture remains limited. Among the factors that limit the adoption of biological control into mainstream agriculture is the effective means of delivery to the crop. According to Dr. Gary E. Harman of the Cornell University (1992), delivery systems must ensure that biocontrol agents will grow well and achieve their purpose. Delivery and application processes must be developed on a crop by crop and application by application basis. No general solutions exist such that biocontrol systems must be developed for each crop.

Thorough knowledge and complete analysis of all the factors involved is necessary for developing the most effective biocontrol system. According to Dr. Miriam Zilberstain, an expert of Integrated Pest Management in Israel, the most effective way to manage a disease is to obtain all the needed information about the crop, the pathogen, the control measures implemented by the farmer and the conditions in the area. A comprehensive assessment of all the factors that affect the disease development should be done by the researcher.

The fungus that causes *Botrytis* fruit rot or gray mold of strawberry is widespread locally. It can infect strawberry flowers when spores landing on them are exposed to free water and cool temperatures. The disease is damaging because it extensively sporulates in strawberry flowers and can spread quickly, particularly under warm, wet conditions common at bloom time (McCandless, 1999). Spores that lay dormant in the flower

resume activity on the berry later in the season anytime before or after harvest when sugars increase and conditions become favorable to disease development.

Trichoderma spp. are free-living fungi that are common in soil and root ecosystems. Recent discoveries show that these are opportunistic, avirulent plant symbionts, as well as being parasites of other fungi (Harman, 2004). Most *Trichoderma* strains produce volatile and non-volatile toxic metabolites that impede colonization by antagonized microorganisms. *Botrytis cinerea* spore germination is impeded (fungistasis) by hydrolytic enzymes produced by *Trichoderma*. The mechanism of action involved in the suppression of *B. cinerea* by *Trichoderma* are prevention of spore germination and deactivation of harmful enzymes (Elad, 2000). Therefore, *Trichoderma* should be applied during bloom time so it would colonize the flower and prevent any activity of the pathogen on its surface. As the flower develops into a fruit, the volatile compounds produced by *Trichoderma* would inhibit *Botrytis* infection.

Honeybees are the most effective pollinators of the majority of agricultural crops. One reason for this is due to their physical characteristics. Their bodies are covered with fine hairs where pollen grains are attached to as they visit flowers (Fig. 9). The pollen grains that are attached to their bodies are easily dislodged as they visit other flowers. Another reason why bees are effective pollinators is their so called flower constancy or the flower fidelity behavior. Once the field bees decide to forage on one type of plant, they would visit that same kind of plant for the rest of the day. Therefore, pollen grains from one species are transferred to the same species. There would be no exchange of pollen grains with different plants because the foraging bee would only visit one type of plant per day.

It is in this context why the *A. mellifera* was thought of to be used in applying *Trichoderma* in the field. The *Trichoderma* combined in a powdery material was placed in a specially designed box connected to the entrance of the hive. As the forager bees exit the hive, they pass through the BCA. The bees, being naturally hairy, will pick up the dust material containing the *Trichoderma* and as they forage on strawberry flowers, the material would be deposited on each of the flowers visited (Fig. 13). Secondly, *A. mellifera* colonies for pollination contain thousands of foragers which would facilitate in the application of *Trichoderma*. Third, the honeybees are in movable hive boxes. These can be easily transferred to different farms and situated on the desired place. Lastly, honeybees improve strawberry pollination. Berries pollinated by bees were noted to be larger, perfect in shape and true to color. Thus, the use of *A. mellifera* to apply *Trichoderma* as BCA against *Botrytis* will not only minimize, if not prevent fruit rot but it will increase yield.

The information acquired from this research favors reduced reliance on chemicals for the control of fungal pathogens by farmers, reduced labor, enhanced environmental conservation and prolonged duration of disease management. Furthermore, most of our farmers do not put much attention on the significance of the pollination of crops. This study advocates the integration of using honeybee pollination on local agriculture and promotes heightened awareness of the benefits that beekeeping brings.

The objectives of this study are to establish the efficiency of delivering *Trichoderma* spp. to strawberry flowers using honey bees, *Apis millefera*, and to observe any detrimental effects of the biological control agent on the bee colony.

The study was conducted from September 2007 to March 2008. Field experiments were done on the strawberry field of Mr. Locloc Pa-at located behind the College of Arts and Sciences building at Benguet State University, La Trinidad Benguet, with an estimated area of more than 450m^2. Laboratory experiments were done at the Biocon Laboratory of the Horticulture Research and Training Institute (HORTI) and at Plant Pathology Laboratory of the College of Agriculture, Benguet State University, La Trinidad, Benguet.

REVIEW OF LITERATURE

Biological Control

Biocontrol is often viewed as a progressive and environmentally friendly way to control pest organisms because it leaves behind no chemical residues that might have harmful impacts on humans or other organisms, and when successful, it can provide essentially permanent, widespread control with a very favorable cost-benefit ratio (Newman *et al*., 1998).

One of the requirements for successful biocontrol is delivery and application methods should permit the full expression of the biocontrol agent. Delivery systems must ensure that biocontrol agents will grow well and achieve their purpose. In our experience, delivery and application processes must be developed on a crop by crop and application by application basis. No general solutions exist, and so biocontrol systems must be developed for each crop (Harman, 1992).

Trichoderma

Trichoderma strains exert biocontrol against fungal phytopathogens either indirectly, by competing for nutrients and space, modifying the environmental conditions, or promoting plant growth and plant defensive mechanisms and antibiosis, or directly, by mechanisms such as mycoparasitism. These indirect and direct mechanisms may act coordinately and their importance in the biocontrol process depends on the *Trichoderma* strain, the antagonized fungus, the crop plant, and the environmental conditions, including nutrient availability, pH, temperature, and iron concentration (Benítez, 2004).

Bees for Delivery

Over the past few years, several studies have shown that honeybees can successfully disseminate beneficial fungi, bacteria, and viruses to strawberries, pome fruits, and clover respectively. One of which is the study by Kovach and his colleagues at Cornell University from 1994-1997. They installed a 'footbath' in the box that fits across the bee hive entrance. As bees exit the hive on their way to the field, they walk across the 'footbath', picking up spores of the biocontrol agent.

Honey bee delivery of *Trichoderma harzianum* can be considered a useful technique for the biological control of *Botrytis* fruit rot. Bee delivered *Trichoderma* provides equivalent control as the present chemical fungicides available to growers and also maximizes strawberry yield through better pollination (Kovach).

There were no significant differences in the bee hive health parameters measured (bee longevity, brood size, and hive weight) between untreated hives and hives exposed to over 150 g of *Trichoderma harzianum* strain T.39 (Trichodex) over a 30 day period. Given the several years of observational data and the results of the experiment, the researchers believe that *T. harzianum* does not significantly impact honey bee health and that the risk associated with bees visiting crops sprayed with *T. harzianum* formulations or exposed to the concentrated product of this biocontrol agent are minimal (Kovach).

Honeybee Pollination

Self pollination of strawberry fruits amounts to 53%. Wind furthermore adds 14% of pollination and an additional 24% with the help of the honeybees (Delaplane and Mayer, 2000). Imagine that additional 24% to all the fruits that would be produced. The yield of the crop would increase significantly.

Strawberries plucked from plants that had been visited by bees weighed 26 to 40 percent more than those from plants that had not been treated. Strawberry growers do not generally keep hives because strawberry pollination occurs primarily via wind and gravity. Study from earlier research indicates that sending in the bees results in better pollination (Wong, 2000).

The Disease

Botrytis fruit rot (gray mold) is caused by the fungus *Botrytis cinerea* and is the most important disease of strawberry worldwide. This disease causes severe pre-harvest losses primarily due to infections of fruit and flowers, especially under humid conditions when daytime temperatures are moderate to warm (60º to 75ºF). Botrytis fruit rot is also an important post harvest disease, since the fungus grows at refrigeration temperatures. This pathogen infects a wide range of plants including many fruit, vegetable, and weed species (Legard *et al*., 2005).

In wet seasons, 80-90% losses of flowers and fruit can occur on unsprayed plants. The disease thrives during prolonged rainy and cloudy periods just before or during harvest, and on dense, lush, foliar growth (Ries, 1995).

Survival

B. cinerea is both very common and well adapted for survival. It occurs on a wide range of hosts and overwinters in dead leaves and decaying plant tissue. As the temperatures warm in the spring, infective spores are produced and disseminated to susceptible strawberry tissue by air currents, splashing rain or insects. When these spores contact water, they germinate and infect plants within hours. *B. cinerea* has an advantage

over many pathogens in that it has the ability to colonize either living or dead tissue. Many times, the fungus first becomes established in dead or dying tissue and then moves into healthy tissue (Hartman and Hershman, 1996).

Cultural Control

Removal and destruction of dead or infected plant material is necessary to help reduce the amount of inoculum capable of producing new infections. It is also recommended to remove all ripe fruits during harvest as well as any fruit with signs of decay or rain damage. Use of plastic mulches prevents berry-soil contact, thus reducing disease. In addition, some cultivars have flowers and fruits that develop with an upright stature. This allows fruit to be exposed to better air movement and sunlight and reduces the risk of infection (Browne *et al*., 2005).

METHODOLOGY

Each of the factors that are involved in the study (the plant, the farm, the BCA, the bee colony and the BCA introduction box) was thoroughly considered and planned.

Internationally, it is established that *Trichoderma harzianum* T65 strain is the most effective *Trichoderma* species for *Botrytis cinerea.* But locally there has been no established *Trichoderma* species recommended for the management of *B. cinerea.* In search for different *Trichoderma* species, the researcher came across two locally available species: one from the Plant Pathology Laboratory of BSU, *Trichoderma koniingi* from Dr. Asuncion Nagpala and *Trichoderma* isolated from the commercial BCA named "Biocon", which was given by Dr. Virginia Cuevas of the University of the Philippines, Los Banos.

Prior to the field set up, bioassay tests were conducted and dusting of the BCA on strawberry flowers was done.

Bioassay Test

On Petri Plates

Bioassay tests were conducted to compare the efficacy of the two *Trichoderma* species which shall be used in the field application.

T0 no *Trichoderma*

T1 *Trichoderma koniingi*

T2 *Trichoderma* isolated from "Biocon"

A suspension of *Botrytis* spores (1.7×10^7 spore/ml) were placed on previously plated PDA. Then four paper discs dipped in two Trichoderma suspensions (*T. koniingi*

and “Biocon” isolate) were placed on the PDA. The plates were properly labelled and incubated at 33°. After two days, the plates were observed for fungal growths.

Botrytis is a slow growing fungus on artificial media. It took a week for the fungus to cover about 70% of the surface of PDA on petri plates. Another bioassay test was performed using 6 day old *B. cinerea*. Two suspensions were prepared, one containing *T. koniingi* and another containing *Trichoderma* isolated from commercial product Biocon. Three paper discs were dipped in each of the suspension then placed above the Botrytis growths. Observations were done after 2 and 4 days of incubation.

On Fruits

To evaluate further the efficacy of *Trichoderma* species in suppressing the disease, additional bioassay test was done using strawberry fruits. There were three treatments, with three replications, each containing three half-ripe strawberry fruits:

For each of the treatments sterile cotton was placed on 3 sets of plastic containers. A suspension of *Botrytis* spores were sprayed on the cotton of all the treatments. For the control, three half ripe fruits were placed above the damp cotton in each of the three plastic containers. For T_1, the fruits were first dipped in a suspension containing *T. koniingi* (1.7×10^7 spore/ml) and placed on properly labeled containers. While in T_2, the fruits were dipped in a suspension of *Trichoderma* isolated from Biocon then placed on separate containers. The plastic containers were then covered and incubated at room temperature. Observation was done after 3 and 5 days of incubation (Fig. 1).

Fig. 1. Dipping of fruits on *Trichoderma* suspension

BCA Introduction Box

The researcher believes that the success of the study revolves around the design of the box. He proposed that the box should contain a pollen trap to reduce the entry of the biocon agent in dust form into the colony. The researcher conferred the purpose of the study with local beekeepers on how the box should be designed. Mr. Ricky Tayaotao who conducts beekeeping trainings in La Trinidad, Benguet volunteered to design and build a box for the experiment.

This box is called the BCA introduction box since it is where the biological control agent was introduced (Fig. 2). The box is a modified pollen trap. It contains a movable plastic screen where the bees' body fits through. Before the bees would leave the box, they would pass through an area where the BCA is introduced. When the bees enter the hive coming from the field, the pollen packed on their hind legs are scraped off by this screen. This would prevent the entry of the pollens exposed to the BCA in dust

form. The pollens that were scraped off drop through another screen and into a sliding box or a drawer (Fig. 2).

Fig. 2. The unmodified BCA Introduction Box at different angles

Experimental Set Up

Location of Strawberry Field

The strawberry field needed was one with a wide area having numerous strawberry flowers. No chemicals should have been applied on the farm prior to and

during the field setup. Fortunately, the researcher was able to find a strawberry field satisfying these requirements. The farm is owned by Mr. Locloc Pa-at who agreed to cancel some of the needed farm activities in favor of the study.

The study was conducted during the peak months of strawberry bloom (December 2007 and January and February 2008) which reconcile with the best time to observe the behavior of bees foraging on strawberry flowers. It was noted that bees visited the crop starting at 8:45am until 3:00pm depending on the weather conditions.

Fig. 3. Mr. Locloc Pa-at's strawberry farm with an area of about 450 m^2.

Field Set Up

Before the sun was up at 5:30am in the morning, the ten framer box containing eight frames of bees was positioned on the western part of the strawberry field (Fig. 10). The purpose for situating the hive there is to make the flowers of the crop the first flowers that the bees would be exposed to. When the bees would commence their orientational flights, the strawberry crop would be in between their hive and the sun which is their main basis for direction. The hive is two meters away from the first row of strawberries. One of the factors affecting the visit of bees to flowers is the proximity to their hive (Fig. 4).

Two plots in the center of the strawberry field were covered with a white net to prevent the bees to forage on the flowers. This netted area served as control (Fig. 5).

The BCA introduction box was connected into the entrance of the hive box before the bees came out and oriented on the new location (Fig. 6). The setup was maintained in that condition for two days. On the third day, trial was done to test the box. A small amount of the Biocon was placed onto the box and observation was made. From the result of the trial, the researcher made modifications on the box to improve its effectiveness (Fig. 7). On the fourth day at 8:40 am, the BCA was placed on the box. At 10:00am, Biocon that dropped on the drawer was again used. At 11:00am, Biocon was refilled. On the fifth day at 8:40 am, the BCA introduction box was filled with Biocon and refilled throughout the day when necessary (Fig. 8). During this two days of BCA introduction, the amount placed in the BCA introduction box was 45 grams. There were still Biocon left on the drawer of the box after the field application.

Fig. 4. The bee colony placed two meters near the strawberry plants

Fig. 5. Netted area that served as the control

Fig. 6. The bee colony with the attached BCA introduction box

Fig. 7. Modification of the BCA introduction box

Fig. 8. The BCA introduction box containing the commercial *Trichoderma* (Biocon)

Fig. 9. Bees that had contact with the BCA in powder form; note the fine hair of the bees' body

Collection of Strawberry Flowers

On the sixth day, strawberry flowers were collected in different distances away from the hive. To accomplish this, a tagged string was used (Fig. 10). Two and one-day old flowers were collected. Each of the samples was placed on sterile plastic cellophane to prevent contact among the flowers. The different distances where strawberry samples were collected from are: 2-6m, 7-12m, 13-18m, 19-24m, 24-30m and the netted area.

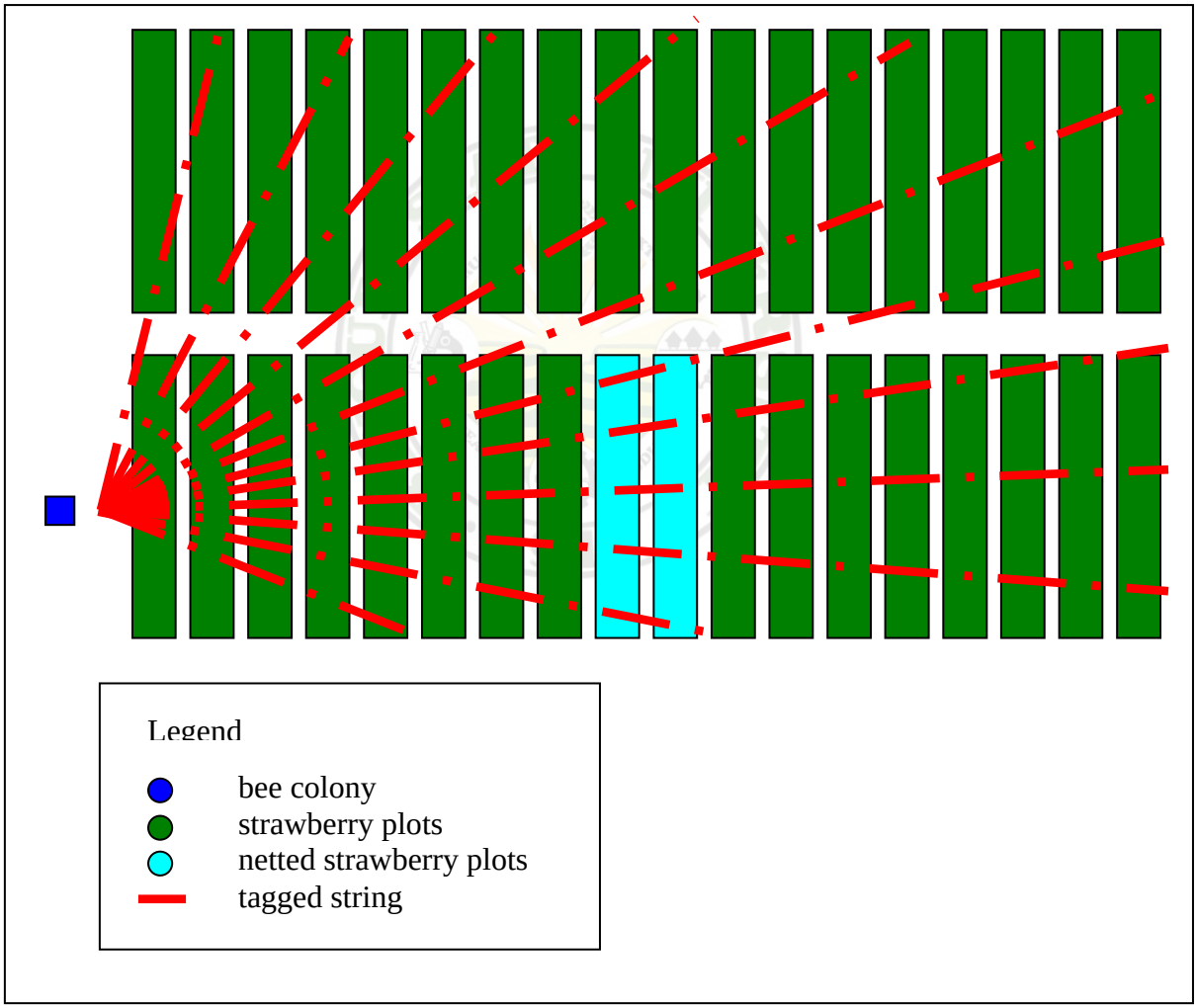

Fig. 10. Field set up and collection from different distances away from the hive

Isolation of *Trichoderma* spp.

The flower samples were handled aseptically. These were placed on sterilized paper and sliced into four pieces using sterilized scalpel and forceps. The four pieces were then planted on previously plated potato dextrose agar (PDA). After each of the tissue planting method was done, the scalpel and the forceps were dipped in denatured alcohol and heated on alcohol lamps to prevent the transfer of spores from one flower to another. The sterile paper used was replaced after each flower isolated (Fig. 11). The plates were then incubated in an incubation cabinet with a temperature of 33° to promote fungal growth.

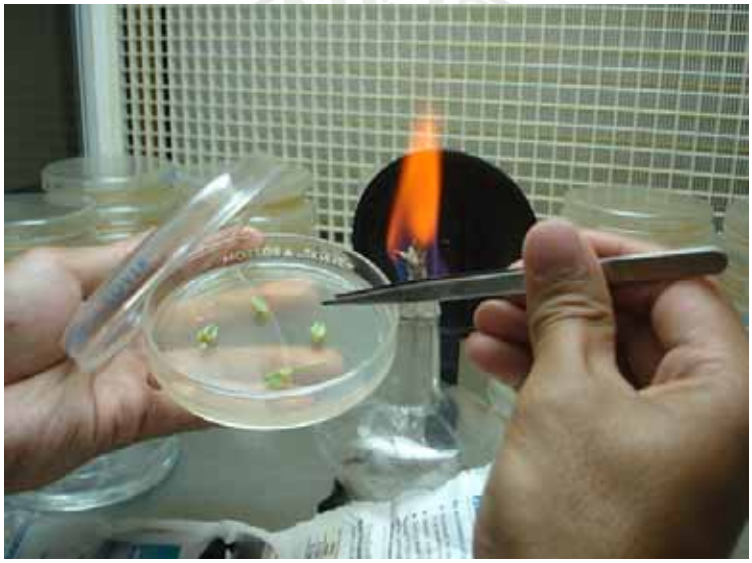

Fig. 11. Isolation of *Trichoderma* spp. from strawberry flowers

Bee Colony Examination

The hive used was examined to determine any detrimental effect of exposing it to the BCA. Inspections were performed a week after the BCA introduction and the following weeks thereafter. The colony components inspected were: egg laying of the queen, brood cycle, increase or decrease of adult bees and stored food.

Fig. 12. The researcher on the field set up of the study

Fig. 13. Bees foraging on strawberry flowers

RESULTS AND DISCUSSION

Bioassay Test

Using the Same Day Old *B. cinerea*

and *Trichoderma* Isolates

After three days of incubation, the *Trichoderma* isolates grew rapidly covering about 80% of the media. There was no *Botrytis* growth seen on both treatments. (Fig. 14a & 14b)

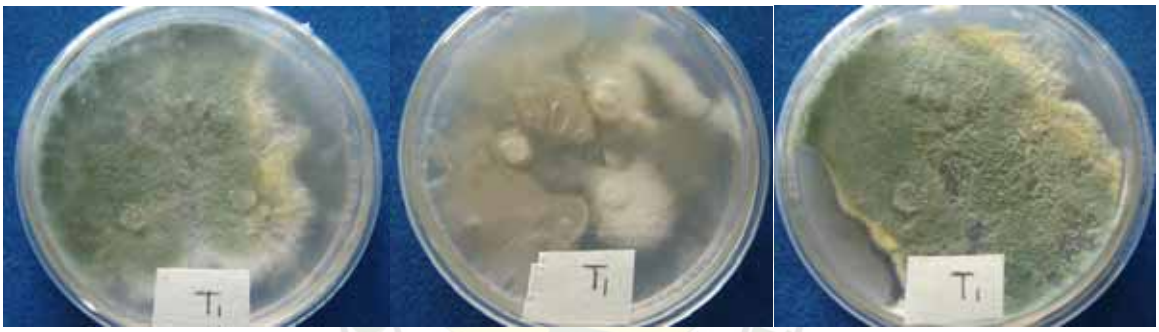

Fig. 14a. Treatment using *T. koniingi*

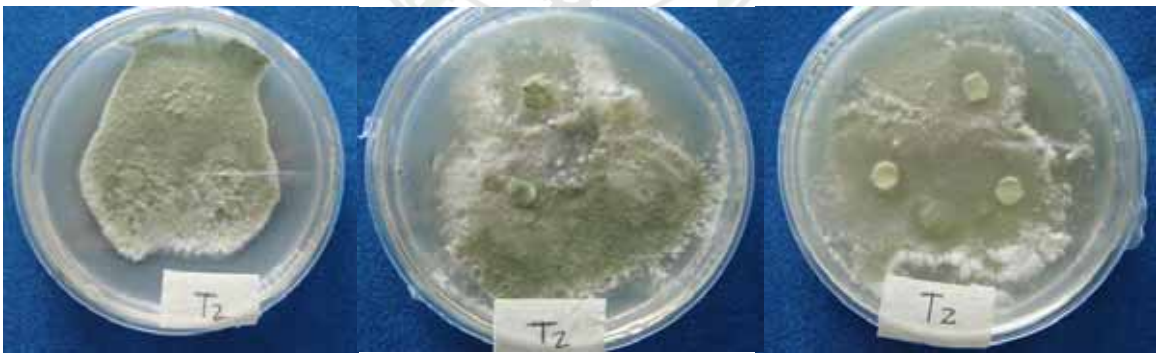

Fig. 14b. Treatment using the commercial preparation of *Trichoderma* spp. ("Biocon")

Using Six Day Old *B. cinerea*

After two days of incubation, mycelial growth was observed on the paper discs containing *T. koniingi*. The advance of the *T. koniingi* was hindered when it was placed above *Botrytis* growth (Fig. 15a). While in T_2 fruiting structures were already evident on the fungal growth from the *Trichoderma* isolated from Biocon (Fig. 15b). Therefore the Biocon isolate grows more rapid compared to the *T. knoniingi* when placed above *Botrytis* growth.

Fig. 15a. Bioassay using *T. koniingi* against *B. cinerea* after two days of incubation

Fig. 15b. Bioassay using Biocon isolate against *B. cinerea* after two days of incubation

After four days of incubation, it is evident that the growth of the *Trichoderma* on the T_2 treatment is more rapid compared to the *Trichoderma* on the T_1. In T_2, the *Trichoderma* isolate has already covered the total surface of the media. (Fig. 16b)

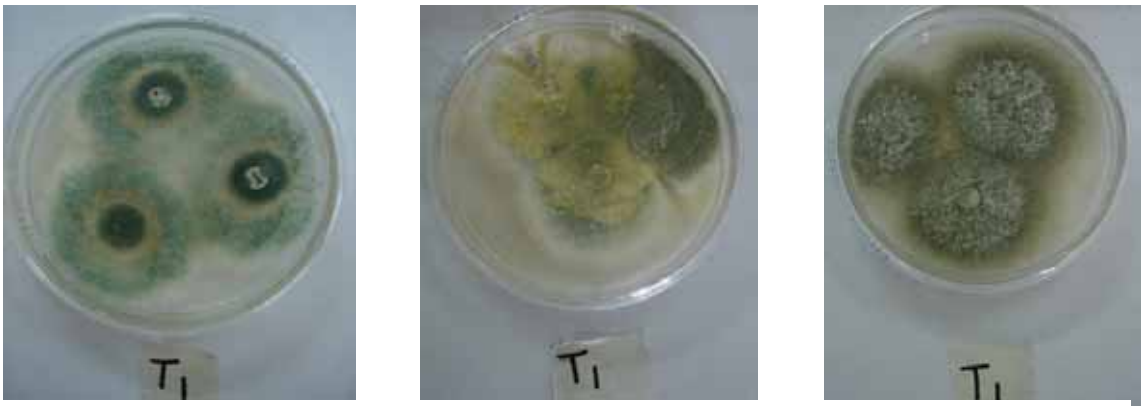

Fig. 16a. Bioassay using *T. koniingi* after four days of incubation

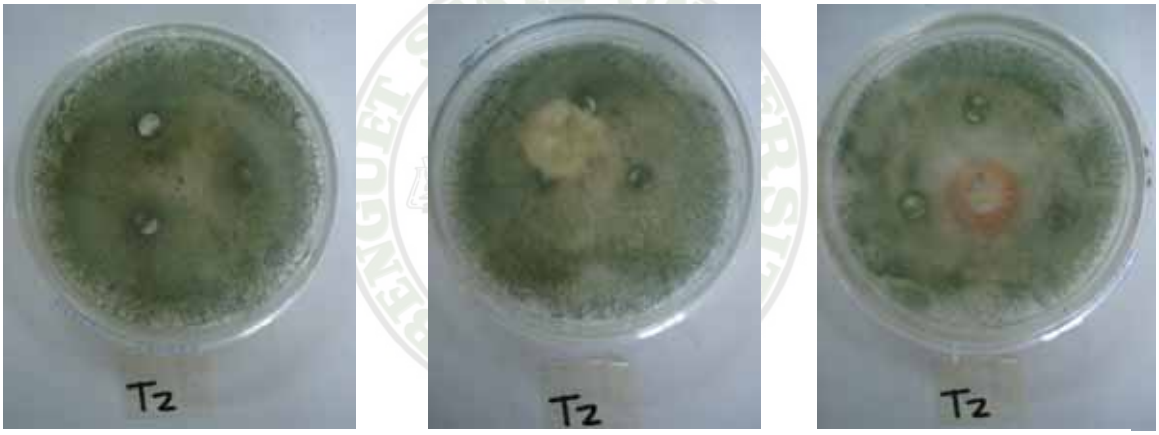

Fig. 16b. Bioassay using Biocon isolate after four days of incubation

Bioassay Test Using Half Ripe Fruits

After three days of incubation, there were severe infections of Botrytis on the treatment without *Trichoderma*. T_1 had three fruits infected with the disease while T_2 had two fruits infected. (Fig 17a, 17b and 17c)

T_0

Fig. 17a. Control

T_1

Fig. 17b. *T. koniingi*

T_2

Fig. 17c. *Trichoderma* isolated from "Biocon"

Isolation of *Trichoderma* spp. on Strawberry Flowers

There were no *Trichoderma* growths on the flowers that were collected from the netted area: instead other fungal and bacterial growths were noted (Fig. 18a). All the flower sample's collected from 7-12m had *Trichoderma* growths (Fig. 18c). There was only 1 flower that had *Trichoderma* from 13-18m, while the other flowers that were collected from 2-6m, 19-24m and 25-30m had 2 to 3 flower samples with *Trichoderma* growth. The results show that the bees were able to disseminate the *Trichoderma* at different distances from the hive reaching as far as 30 meters away while no *Trichoderma* was observed from the flowers planted in the area secluded from the bees.

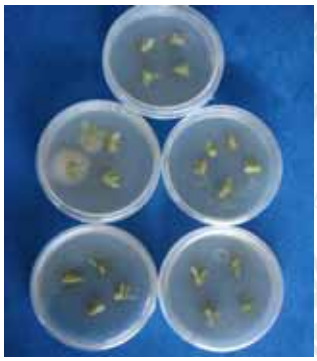

Fig. 18a. Netted

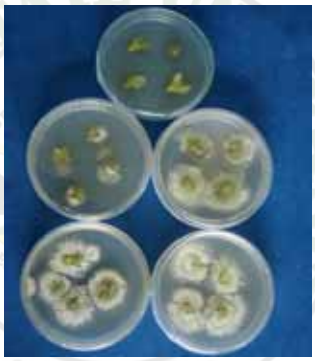

Fig. 18b. 2-6m

Fig. 18c. 7-12m

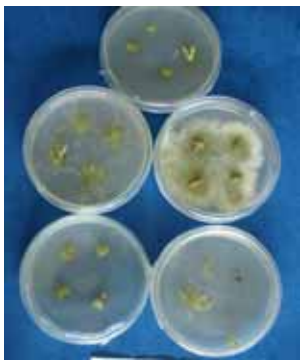

Fig. 18d. 13-18m

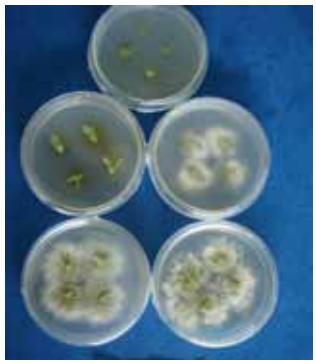

Fig. 18e. 19-24m

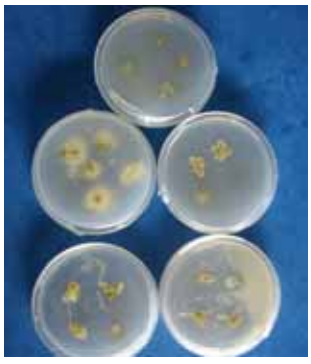

Fig. 18f. 25-30m

Fig. 18. Isolated *Trichoderma* from different areas in the strawberry field

Meteorological Data

Foraging behavior of the honey bees is greatly influenced by the weather. Thus, meteorological data were acquired from Philippine Atmospheric, Geophysical and Astronomical Services Administration (PAG-ASA) Balili, La Trinidad, Benguet (Table 1). The prevailing temperature favors bee foraging. However, there were frequent occurrences of rain shower in the afternoon. Hence, minimal bees were seen in the field during this time. The light rain may have limited the foraging time for the bees. Wind speed was also high. This may have affected the detachment of the powder material on the bees as they forage. Though it was cloudy, there was no fog occurrence.

Table 1. Meteorological data gathered during the introduction of the BCA

	02- 21- 2008	02- 22- 2008
Relative Humidity	76%am	68%am
Average Temperature	20.65°C	21.5°C
Rainfall	traize 2:00pm	rainshower 2:00pm
Wind Speed	04 knots/sec	06 knots/sec
Wind Direction	180°S	180°S
Daylength	753 min	752 min
Fog Occurrence	None	None

Colony Examination

Inspection of the hive shows that the egg laying of the queen was not hindered (Fig. 22). The presence of different brood stages proves that the brood cycle was continuous. The increase in the number of adult bees was also evident on the hive entrance. Prior to the field setup, there was high quantity of stored food. After the study, it was observed that there was a slight decrease. Nevertheless, the food left was still high (Fig. 21). The maintained food collected proves that there was no sudden loss of foragers that were in direct contact to the BCA. Two weeks after the study, a new frame was added to the colony (Fig. 23). The colony increase was not interrupted. Overall, this would mean that there was no adverse effect on the hive after its exposure to the BCA.

Fig. 19. The colony one week after the experiment

Fig. 20. The noted increase in the number of adult bees a week after the experiment

Fig. 21. A frame showing that the stored food was still abundant

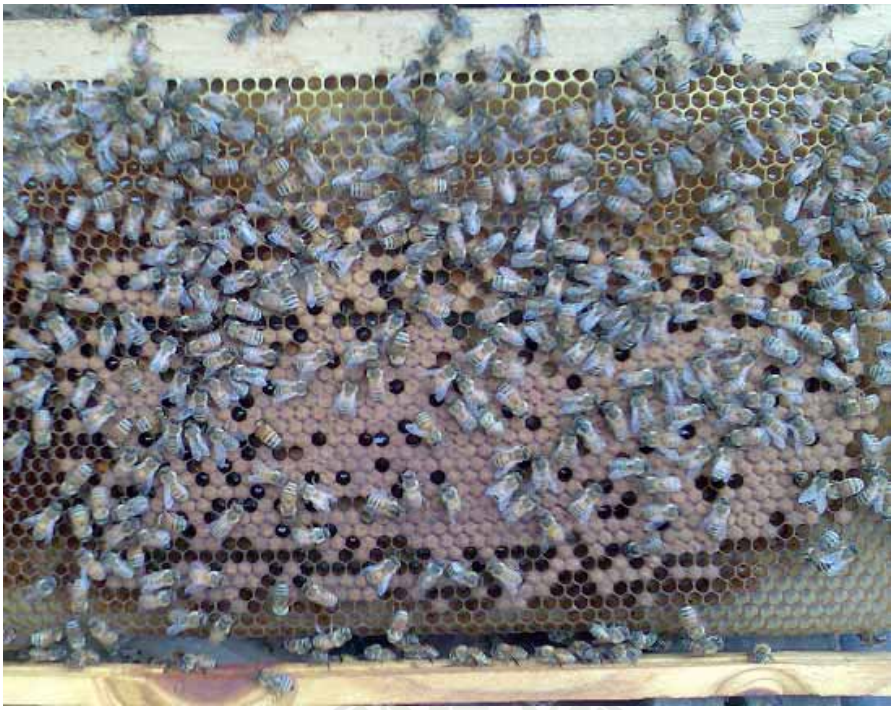

Fig. 22. Hive inspection show uniform laying of the queen after the experiment

Fig. 23. The bee colony two weeks from the experiment; note one frame was added

SUMMARY, CONCLUSION AND RECOMMENDATION

Summary

The study was conducted to establish the efficiency of delivering *Trichoderma* spp. to strawberry flowers using honey bees, *Apis mellifera*, and to observe any detrimental effect of the BCA on the bee colony. The study was conducted from September 2007 to March 2008. Field experiments were done at the strawberry field of Mr. Locloc Pa-at located within the Benguet University grounds. On the other hand, laboratory experiments were conducted at the Biocon Laboratory of HORTI and at Plant Pathology Laboratory of the College of Agriculture, Benguet State University, La Trinidad, Benguet.

The preparation of the colony to be used, BCA selection, designing and modification of the BCA introduction box and locating an experimental field were all accomplished prior to the main phase of the study.

Using a bee hive colony containing eight frames, the BCA introduction box was attached and placed in the strawberry farm. After three days of orientation for the bees, the commercial BCA in powder form was introduced for two days. For the untreated control, an area was covered with net to prevent bees from the hive to have contact with these flowers. On the sixth day of the field setup, samples were collected on different distances away from the bee hive and on the netted area. The samples were placed on previously plated PDA to observe the presence of the BCA on the flowers.

The isolated samples collected from the farm at different distances from the bee colony were positive of *Trichoderma* growth while samples from the netted area showed growth of other fungi and bacteria.

Hive inspections show that the food stored was slightly reduced but an increase of bee population was noted on subsequent weeks after the experiment.

Conclusion

Using the BCA introduction box, the commercial *Trichoderma* "Biocon" was successfully delivered to strawberry flowers using honeybees. Thus, it is feasible to use *A. mellifera* in the field application of *Trichoderma* to strawberry flowers. The BCA that the bees were exposed for four days had no adverse effect on the colony.

Recommendation

Since the study is only preliminary, further experiments should be conducted prior to the promotion of the use of this method of delivery. There is no established local BCA that has proven effective against *B. cinerea*. It is strongly recommended to first evaluate other potential antagonists against this disease of strawberry. There is also a need to modify and improve the BCA introduction box. The big challenge is to design it in such a way that the bees would contact the BCA as they exit the colony but will prevent the BCA's entry into the colony when bees return to the hive. Further studies should also be conducted to compare the effectiveness of this method and spraying as a technique of delivering the biocon as control against *B. cinerea* gray mold of strawberry with chemical fungicides.

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