BIBLIOGRAPHY

ANAS, JOSEPH C. APRIL 2013. Follow-up Study on the Efficiency of Honeybees (*Apis mellifera* Linnaeus) in Disseminating *Trichoderma* KA to Manage Gray Molds (*Botrytis cinerea* Pers) and Pollination in Strawberries (*Fragaria x ananassa* Duch). Benguet State University, La Trinidad, Benguet.

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ABSTRACT

The study aimed to: a) determine how far the *Trichoderma* spores will be disseminated by *Apis mellifera*; b) determine if the *Trichoderma* spores "dropped" by *Trichoderma*-laden honeybees are enough to prevent the rotting of the berries; and c) determine the pollination efficiency of the *Trichoderma*-laden honeybees. A modified *Trichoderma* Dissemination Box was used to contain the *Trichoderma*.

The honeybees successfully dessiminated the *Trichoderma* spores to the strawberry flowers from 20m to 70m away from the hive.

The fruit rot incidence in strawberry fruits was reduced by 58% from fruit setting to maturity and increased by 45% marketable yield.

There was no significant increase in the number of fruit set between the pollinated and unpollinated flowers. The *Trichoderma* spores that were disseminated were effective in significantly reducing fruit rot incidence by about 60%. However, an average of 20% disease reduction on marketable fruits was recorded. As a result, a significant increased in marketable yields by 45% was realized at harvest.

RESULTS AND DISCUSSION

Distances by which Honeybees can Disseminate
Trichoderma KA Spores

The treatment was found positive with *Trichoderma* KA growth. Mycelium and spore germination was observed from the different isolation done in each treatment. Honeybees are efficient in disseminating *Trichoderma* KA at a given distance. In T_1 which has a distance of 20m, colonies of *Trichoderma* KA were observed. The spores germinated at surface were surrounded by its mycelium (Fig. 18) while in T_2 which has a 40m distance away from the hive several colonies of *Trichoderma* KA growth were observed at the sides of the petriplates. The mycelium bears its spores as it continous to spread on the plated PDAs (Fig. 19). Moreover, in farther treatments the growth of the *Trichoderma* KA was noticed to have a small number of spores than the previous treatments which was closer to the hive. Spore germination was moderately compact at T_3 which has a 60m distance from the hive (Fig. 20) while in T_4 – 70m the mycelium growth of *Trichoderma* KA was dominant after incubation (Fig. 21). Indeed, the concentration of *Tichoderma* KA spores was observed from 20m to 40m distances away from the hive (Fig. 18 & 19). Based on these results, honeybees as they flew out to forage in farther distances it is possible that their was a loss of inocula from the bees resulting to lesser inocula to be deposited to the strawberry flowers. However, it is still evident that honeybees are efficient in disseminating the *Trichoderma* KA at a given distances.

According to Bilu *et al*. (2004) the amount that a bee deposits on a flower per visit is not known, but must be considerably less than that found on the bee's body upon leaving

the hive. Inoculum may be lost as the bee flies towards the flowers, and the amount delivered per flower is diluted by the many flowers that a bee visits per trip.

On the other hand, Saclangan claimed that honeybees can disseminate *Trichoderma* up to 30m away from the hive but, the result of this study showed that bees can disseminate *Trichoderma* spores from 20m distance and even as much farther as 40m, 60m and 70m away from the hive (Fig. 18-21).

The findings of Kovach (1999) provides further support to the result of this study where he proved that bees exiting from the hive on their way to the field, can pick up as many as 100,000 *Trichoderma* spores each. As they forage for nectar and pollen, the bees leave spores of *Tichoderma* 22 behind and on the flowers. *Trichoderma* 22 fights the familiar gray mold known as *Botrytis* fruit rot by outcompeting the rot, spore for spore.

In addition, the designed Biocontrol Agent Box which was based on Biocontrol Introduction box (BCAB) enabled the honeybees to disseminate *Trichoderma* KA spores five hours after their release at different distances from the hive reaching up to 70m.

The effectiveness of using bees as a biocontrol agent depends on several factors. Honeybees can disseminate *Trichoderma* inoculum to strawberry and effectively control gray mold (Maccagnani *et al*., 1999; Kovach *et al*., 2000). The success of the technique, however, depends on the type of inoculum dispenser (Bilu *et al*., 2004; Maccagnani *et al*., 2005), the *Trichoderma* strains, the carrier, and the attractiveness of the strawberry cultivar to the bees (Kovach *et al*., 2000).

Meanwhile, the occurrence of the contaminants such as fungus and bacteria in the isolated suspensions were relatively high since the flowers and honeybees cannot be sterilized and were highly exposed to an open environment.

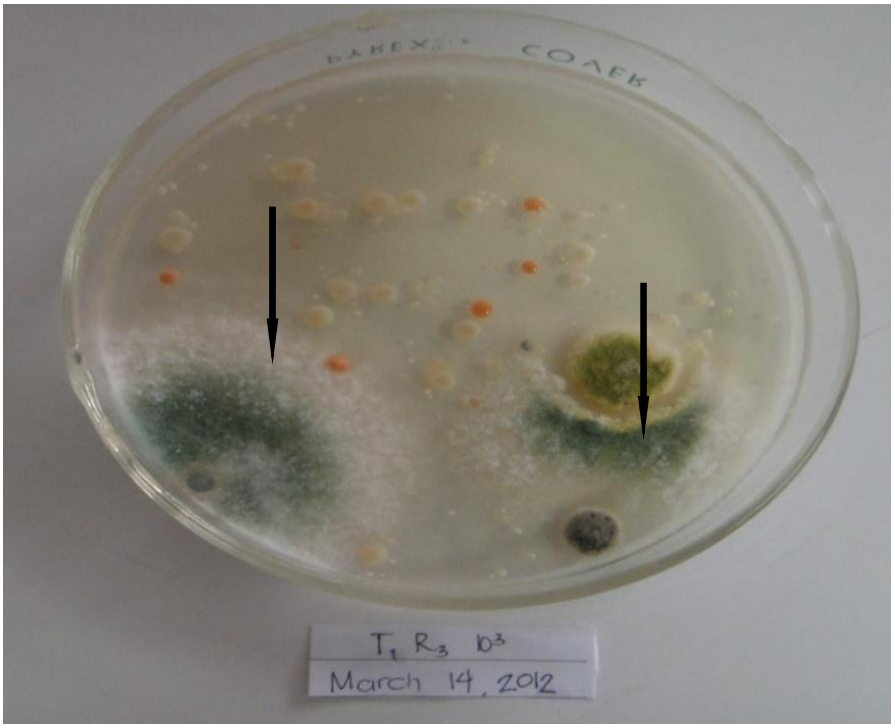

Figure 18. Mycelium and spore germination of *Trichoderma* KA isolated from strawberry flowers at 20m distance from the hive five hours after foraging

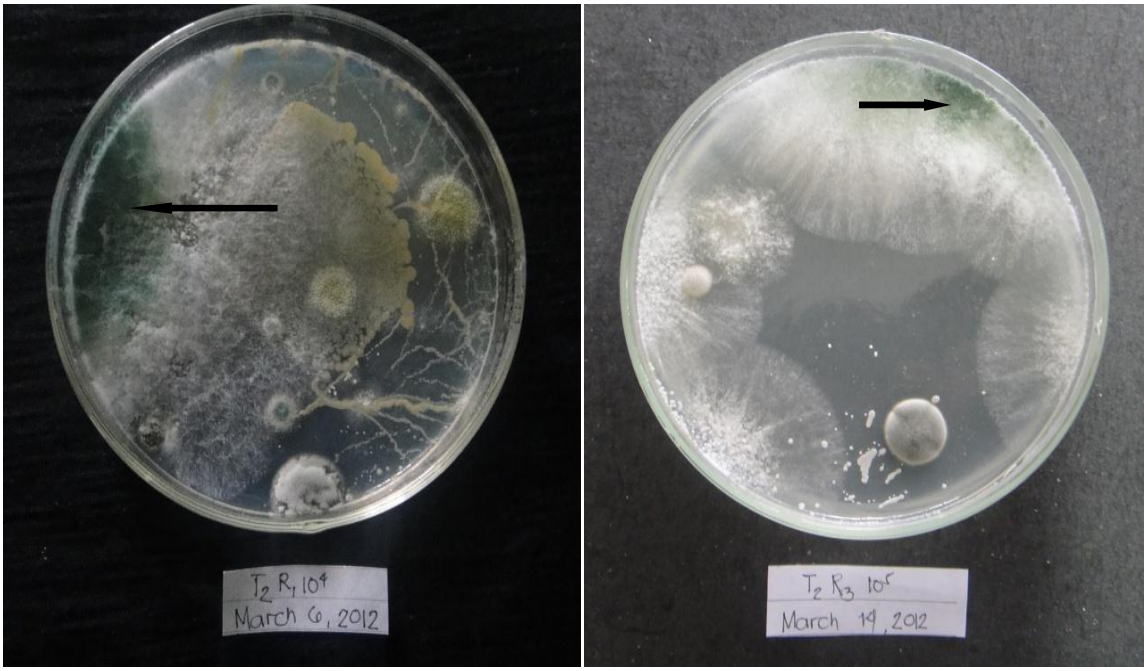

Figure 19. Mycelium and spore germination of *Trichoderma* KA isolated from strawberry flowers at 40m distance from the hive five hours after foraging

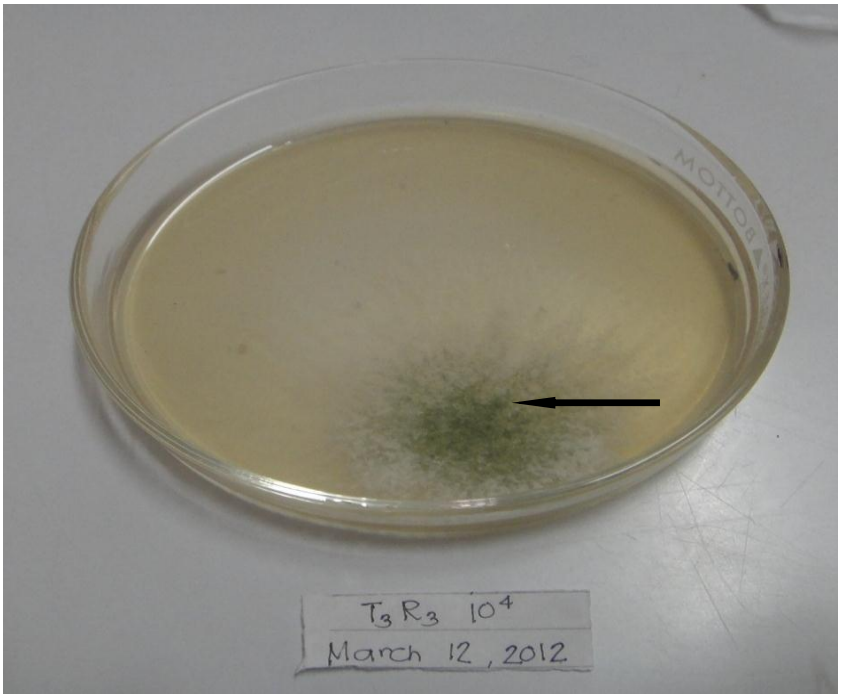

Figure 20. Colony growth of *Trichoderma* KA isolated from strawberry flowers at 60m distance from the hive five hours after foraging

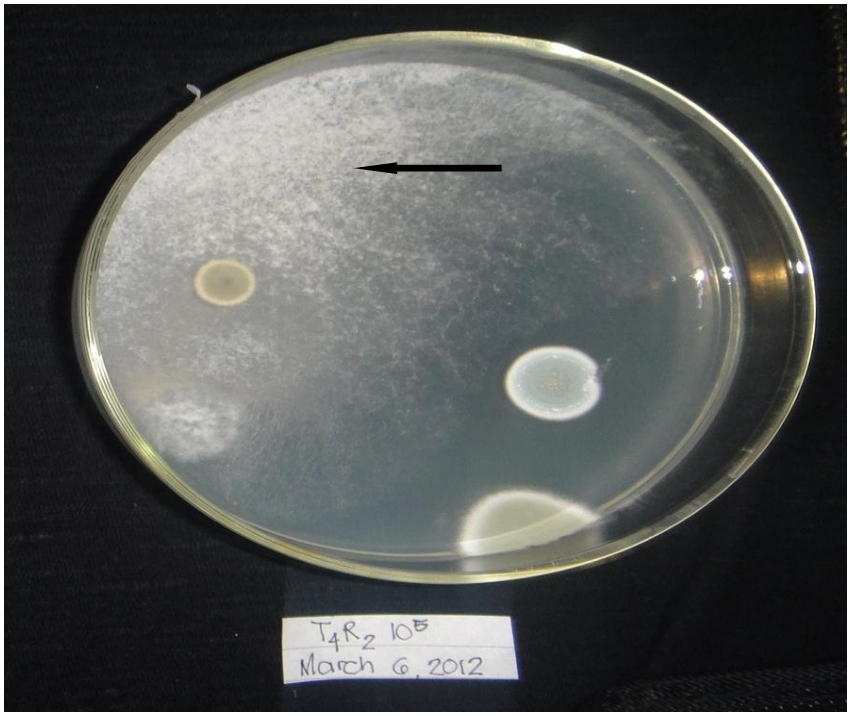

Figure 21. Mycelia of *Trichoderma* KA isolated from strawberry flowers at 70m distance from the hive five hours of foraging

Evaluation of *Trichoderma* KA for *Botrytis* Management

The result in Table 1 shows the *Botrytis cinerea* rate of infection in both treatments. There was no *Botrytis* infection observed on the tagged sample flowers. However, after the fruit set, formation of *Botrytis cinerea* was observed. Symptoms of rotting started on the developing fruits on both treatments. This corroborates with the findings of Legard *et al*. (2005) as cited by Saclangan (2008) showed that *Botrytis cinerea* Pers causes severe preharvest losses primarily due to infections of fruits, especially under humid conditions when daytime temperatures are moderate to warm (60^{o}F to 75^{o}F).

The use of honeybees as an applicator of the *Trichoderma* KA for the *Botrytis* management was evaluated on fruits. Out of the samples, the treatment using honeybees had a 42% disease incidence from fruit set to maturity while 68% of infected fruit incidence was recorded on the treatment without using bees. Significantly, the study found a 58% reduction of disease infection when using bees as disseminators of *Trichoderma* KA.

It corroborates with the early study of Saclangan (2008) wherein he conducted a bioassay test on fruits to evaluate the efficacy of *Trichoderma Koniingi* in suppressing the disease. A suspension of *Botrytis cinerea* was sprayed in the sterilized cotton contained in plastic containers in which the sample fruits, which were dipped on *Trichoderma* suspension, were then placed and got incubated for three days. About 56% of the untreated fruits were infected with the fruit rot disease as compared to the treated fruits with *Trichoderma Koniingi*, a total of 33% of the fruits were infected while an average of 67% disease management reduction on fruit samples.

The disease assessment using honeybees in disseminating *Trichoderma* KA was nearly similar to the result of the bioassay test. The established BAB which was modified

from the former BCA box was one of the factors that made the study successful in attaining the percentage reduction of *Botrytis cinerea* in strawberries.

Previous studies showed that the effectiveness of using bees as a biocontrol agent depends on several factors. Honeybees can disseminate *Trichoderma* inoculum to strawberry and effectively control grey mold (Maccagnani *et al*., 1999; Kovach *et al*., 2000). The success of the technique, however, depends on the type of inoculum dispenser (Bilu *et al*., 2004; Maccagnani *et al*., 2005), the *Trichoderma* strains, the carrier, and the attractiveness of the strawberry cultivar to the bees (Kovach *et al*., 2000).

Table 1. Percentage *Botrytis* infection on tagged samples from fruit setting to maturity

TREATMENTS	FLOWERS	FRUITS
Without honeybees	0%	68%
With Honeybees loaded with *Trichoderma* KA	0%	42%

Pollination Efficiency of Honeybees

The flowers that were exposed to *Apis mellifera* produce a true-to-type shape of strawberries. Sweet Charlie Strawberry Variety has two types of shape – wedge and conical shape (Fig. 23a & 23b). Pollination and fertilization of the ovules resulted to normal development of fruits.

Number of Fruit Set

The number of fruits that set after the flowers were secluded or exposed to the bees is shown in Table 2. The number of fruit set in plants secluded from the bees was 50 out of 50 which has a computed percentage fruit set of 100%. On the other hand, the fruit set in sample flowers exposed to the bees was 50 which is also equivalent to 100%. The data showed that there was no difference between the fruit set percentages of both exposed and covered strawberry flowers. However, in order to achieve complete pollination, self fertile strawberries require pollinators such as bees to reduce deformities and to attain bountiful quality of fruits for strawberry production.

The flowers of all the current commercial strawberry cultivars (*Fragaria x ananassa* Duch) are hermaphrodite and self-fertile. However, these flowers may not be completely self-fertilized; thus, the role of bees as pollinators is important in order to reduce deformities and increase yield for commercial strawberry production (McGregor, 1976).

Table 2. Number of fruit sets and its total percentage

TREATMENTS	FRUIT SET	PERCENTAGE
Without honeybees	50	100%
With Honeybees loaded with *Trichoderma* KA	50	100%

Number of Marketable and Deformed Harvest

Table 3 shows the percentage of harvests noted on marketable (Fig. 23a & 23b) and not marketable fruits (Fig. 23c) on both treatments. Based on the tagged samples, 26% were deformed (not marketable) and only 6% were normal fruits among all the fruits which were treated without using the bees. Whereas, a total of 8% were deformed and 50% were normal fruits among all the fruits treated using honeybees. Basing from these data percentages on both treatments, there was a 69% calculated reduction of deformities using honeybees and an average of 88% increase in true-to-type fruits was calculated on the exposed strawberry samples for pollination.

Honeybees pollinated 11% of achenes on their first visit, so to produce a normal berry (fertilization rate of 87%) about 11 visits by honeybees/flower are required to achieve a normal-sized berry (Anonymous, 2013).

This result determines the pollination rate of honeybees in reducing deformities and promoting a good quality for strawberry production. However this was attained by using bees as pollinator and with the aid of other pollinators since the field was open for insect visitation.

Strawberry flowers are hermaphroditic (having both male and female reproductive organs) and self-fertile and 80% of fruit production is due to abiotic factors such as gravity and wind; however, pollinating insects play as essential role in obtaining maximum fruit as well as reducing deformities (Chagnon *et al*., 1989).

The use of pollinator such as bees is important to improve pollination by increasing mobility of pollen grains and consequently, ensuring ovule fertilization and achene development (Leech *et al*., 2000). Pollination by insects often enhances yield and fruit

quality compared with non-pollinated crops. In addition, Chagnon *et al*. (1989) stated that honey bees (*Apis mellifera* L.) are recognized as the main pollinator of the strawberry crop.

Table 3. Percentage of marketable fruits and non-marketable fruits at harvest

TREATMENTS	NOT MARKETABLE (Deformed Fruits)	MARKETABLE
Without honeybees	26%	6%
With Honeybees loaded with *Trichoderma* KA	8%	50%

Pollination and Dissemination Evaluation on overall Harvests

The result in Table 4 shows the total harvest that includes the normal, deformed and the infected fruits in both treatments. Of all the berries exposed to bees in the treatment process, 58% of the harvests were normal while 24% were infected. On the side of the berries secluded from the bees, a total of 32% were normal while a total of 30% was infected with *Botrytis cinerea*.

Out of these results, the idea of combined pollination and dissemination of beneficial fungi using bees was evaluated in this study. The calculated total average increase in marketable fruits out of the treatments was 45% while the use of *Trichoderma* KA was proven effective in managing the *Botrytis cinerea* by a 20% disease reduction in the harvest. This means that out of 45% marketable harvests, the combined pollination and dissemination comprises 20% reduction of the disease and increase in percentage harvests. In relation to the previous study by Lab-oyan in 2001, he noted that pollination alone by honeybees increased yield by 25%; therefore, the efficiency of honeybees as pollinator and

disseminator of the *Trichoderma* KA for *Botrytis* control increased marketable fruits up to 45%.

Table 4. Percentage of infected and uninfected fruits at harvest

TREATMENT	UNINFECTED	INFECTED
Without honeybees	32%	30%
With Honeybees loaded with *Trichoderma* KA	58%	24%

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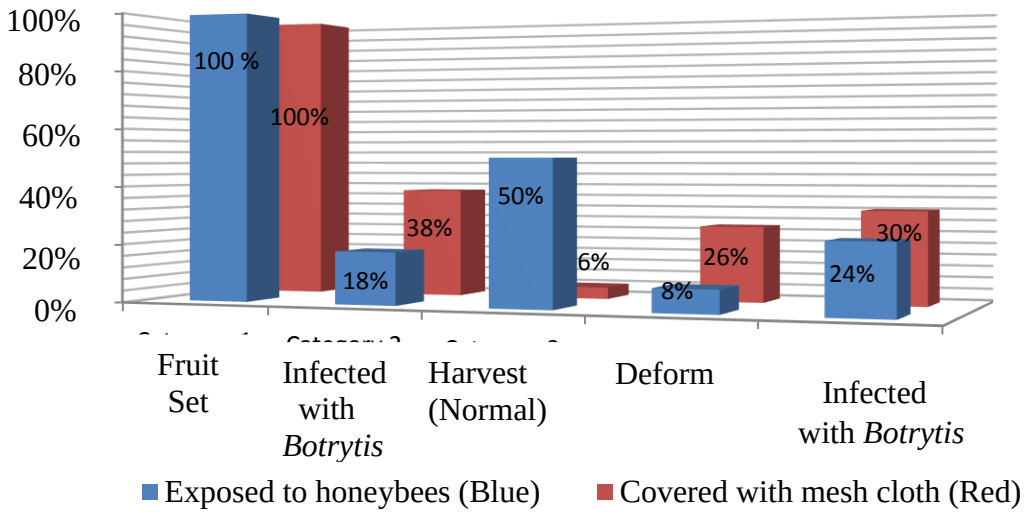

Figure 22. Total percentage of normal, deformed and infected strawberries from fruit set to harvest

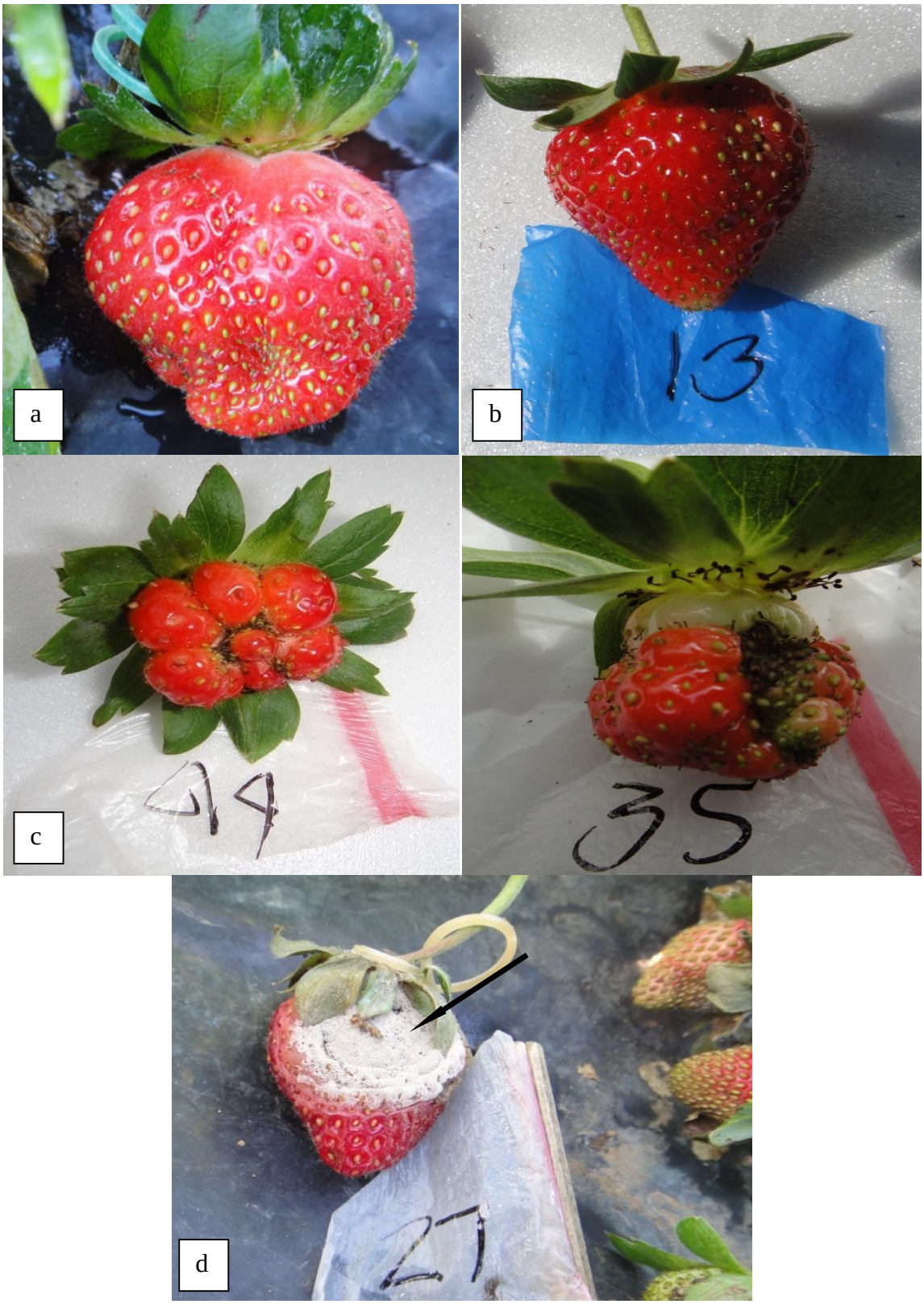

Figure 23. Sample of true-to-type shape of Sweet Charlie Strawberry Fruit a) wedge shape b) cone shape c) deformed fruit d) infected fruit with *Botrytis* rot

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

The study aimed to a) determine how far the *Trichoderma* spores will be disseminated by honeybees; b) determine if the *Trichoderma* spores "dropped" by *Trichoderma*-laden honeybees are enough to prevent the rotting of the berries; and c) determine the pollination efficiency of the *Trichoderma*-laden honeybees.

The efficiency of the honeybees as a disseminator of the *Trichoderma* spp. that was reported by Saclangan (2008) confirms the success of this study. As recommended, the researcher modified the box prior to the release and assessment of *Trichoderma* KA in managing *Botrytis cinerea* in strawberries.

The isolated dilutions from different distances were positive of *Trichoderma* KA growth. Honeybees can disseminate *Trichoderma* on different distances ranging from 20m, 40m, and 60m up to 70m distances away from the hive.

In relation to the dissemination of the *Trichoderma* KA for *Botrytis* management on the tagged samples, a total of 68% of the berries secluded from bees got infected while a total of 42% of the berries subjected to bee dissemination were found infected. However, the calculated percentage of disease reduction from fruit set to maturity was 58%. Therefore using bees as disseminators of *Trichoderma* KA reduced the incidence of *Botrytis* from fruit set to maturity; and this was attained by using the modified Biocontrol Agent Box (BAB). Indeed, the previous studies were correct on stating that the design of the dispenser affects the results of the treatment.

Additionally, on the number of fruit sets after the flowering of the strawberries, both the exposed and covered flowers attained 50 out of 50 or 100% fruit sets as recorded.

The combined pollination and dissemination of honeybees promoted true-to-type shape of fruits by 88% and reduced deformities by 69% on strawberry fruits. The data recorded on the overall harvest was 58% for the exposed samples while 32% was recorded on the samples secluded from the bees. Based on the disease assessment using *Trichoderma* KA to suppress *Botrytis cinerea* in strawberries, there was an average of 20% increase in marketable fruits at harvest. However, out of this percentage, the calculated average on the efficiency of honeybees as disseminator of *Trichoderma* KA for *Botrytis* management and as pollinators of strawberries was 45% increased in the number of true-to-type marketable fruits at harvest.

Conclusions

The Biocontrol Agent Box (BAB) successfully enables the honeybees to deliver *Trichoderma* KA resulting to the significant reduction of fruit rot incidence on the developing strawberries until harvests that were located as far as 70m away from the hive. Marketable fruits also significantly increased.

Recommendations

The Biocontrol Agent Box (BAB) can be used in similar studies. The amount of *Trichoderma* KA deposited by honeybees to the flower per visit is not known. Further studies are recommended to take a sample of a honeybee loaded with *Trichoderma* K a certain distance, likewise, to take a sample of the flower which received pollination first and dissemination of the biocontrol agent.

It is strongly recommended that bee colony density would be relatively high, in order to achieve saturation pollination and a higher percentage of large, well-formed fruits.

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