

Full Length Research Paper

Managed bees as pollinators and vectors of bio control agent against grey mold disease in strawberry plantations

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Pollination by bees and other animals significantly increase both crop yields and quality. Bees also support the transfer of bio-control agents for suppression of crop pests and diseases through bio-vectoring technology that has not been applied in Africa. Two farms were set up to test the ability of managed bees to disseminate *Trichoderma harzianum* to control *Botrytis cinerea*, on strawberries. At on-station farm, three treatments (bee-vectoring inoculum, spraying and control) with 4 replicates each were set up; while on-farm, normal farmer practices were employed. A nuclear beehive fitted with a two-way dispenser was loaded with two grams of *T. harzianum* inoculum. Fifteen bees and flowers from each treatment were picked and cultured in the laboratory. Fruits and flowers infected with *B. cinerea* were recorded, while healthy fruits were counted, weighed and equatorial and polar diameter determined. Each bee carried $22.4 \pm 4.9 \times 10^2$ colony-forming units of *T. harzianum*. Flowers from the sprayed treatment had significantly higher Colony-Forming Unit's F (3,140), ($P < 0.05$) than the bee-vectoring treatment. Grey mold disease levels on fruits were significantly lower ($P > 0.05$) in sprayed, bee-vectoring and control treatment than in farmer's practice treatment. Fruits from spray treatment weighed significantly higher than those from control treatment F (3,2122), ($P < 0.05$). The number of seeds, equatorial and polar diameter per berry were significantly higher ($F = 3, 2122, P < 0.05$) in farmer's practice treatment. Managed bees proved effective in vectoring *T. harzianum* but, sufficient Colony-Forming units had to be delivered for effective control of the disease.

Key words: *Trichoderma harzianum*, grey mold, strawberry, biocontrol agent, managed bees, bio-vectoring technology.

INTRODUCTION

Garden strawberry, *Fragaria x ananassa* Duch, is a perennial, herb that belongs to the phylum Spermatophyte and family Rosaceae. The strawberry does well in tropical

and subtropical regions of the world (Mir et al., 2019). Strawberry fruits are rich in well-balanced sugars and organic acids Guimarães et al. (2016), hence very

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nutritious for human consumption (USDA, 2015). Strawberry farming has picked over time and the annual world production greatly increased in quantity in the last twenty years to over 2.5 million tones (FAO, 2014). The demand and consumption have increased due to their antioxidants effect in human (Törrönen and Määttä, 2002).

In Kenya strawberry crop does well in acid soils ranging between 6-6.2 pH, and with good drainage capacity, but, irrigation is necessary during the growing period. However, due to poor crop management practices, strawberry pests and grey mold diseases caused by the fungi *Botrytis cinerea* are the major drawbacks in strawberry farming. According to IPM (2011), the convectional growers lose between 25-35% of their yield due to grey mold diseases as most of the fungicides used have become resistant to the pathogen. The fungicides further lead to the reduction of the market value of the strawberry due to their toxic residues in the fruit (Shen et al., 2008). According to Kovach et al. (2000) the viability of the strawberry reduces following the application of fungicides during the flowering stage as well as the harvestable yields. Further studies have also revealed that fungicides application is a great challenge to biodiversity conservation and a major threat to plant-pollinator interactions whose contribution to crop productivity is enormous (Ricketts, 2004).

Increasing production challenges have resulted in advancement of technology and innovation that ensured improved crop production in many countries of the world (Plan, 2016). However, it is necessary to validate such technologies in different geographic regions for the ease of adoption. Some of the notable new technologies that are transforming agriculture from a labor-intensive industry to capital intensive include bio-vectoring-technology (Mommaerts and Smagghe, 2011). This technology involves the use of insects as vectors of bio-control agents. The technology aims at minimizing the use of synthetic insecticides and development of pest resistance while maximizing on quality and yields of crops.

A successful outcome of entomovectoring technology has been realized mostly in some developed countries. The technology is especially useful in large variety of pollination-dependent crops. Managed bees, honey bees and bumble bees have been used to vector inoculum of fungi, bacteria and viruses from the hive to flowers (Kevan et al., 2003). The technology have been evaluated for the dissemination of *Trichoderma harzianum* T39 and *T. harzianum* 1295-22 against *B. cinerea* under field conditions using honey bees and bumble bees (Shafir et al., 2006; Kovach et al., 2000), *Metarhizium anisopliae* against pollen beetle, *Meligethes aeneus* and cabbage seed weevils, *Ceutorhynchus assimilis* the major pests of oilseed rape using honey bees (Carreck et al., 2007), *Trichoderma* spp against sunflower head rot, *Sclerotinia sclerotiorum*, the

bacterium *Bacillus thuringiensis* against moth, *Cochylis hospes* on sunflowers (Escande et al., 2002; Jyoti and Brewer, 1999), and viruses to control *Heliothis* in clovers (Gross et al., 1994). However, despite this innovative approach, many African countries including Kenya have not embraced it as part of integrated pollinator pest management (IPPM).

The success of the technology, therefore, depends on the ability of the target vector to efficiently disseminate the biological control agent to the target crop. In the present study, we evaluated the effectiveness of African honey bees (managed bees) as vector of *T. harzianum* to strawberry crop under field conditions in central Kenya, evaluated the effectiveness of the *T. harzianum* against grey mold disease and determined the effect of simultaneous pollination and disease control using managed bees on strawberry quality and yields.

MATERIALS AND METHODS

Field sites and experimental design

The study was conducted on-station at the University of Nairobi (UoN) College of Agriculture and Veterinary Sciences (CAVS), and on-farm at Loresho in Kiambu county. The on-station farm is located off Kapenguria road, 15 km to the northwest of Nairobi at a geographical location E-1.25, S 36.742554 and elevation of 1840 m above sea level (ASL). The on station-farm is situated along Kaimoni road approximately five kilometres away from CAVS at a geographical location E-1.253, S 36. 751 and at an elevation of 1822 m ASL.

The on-station farm was divided into random experimental plots to form three treatments which included: Treatment 1- Open plot with one bee colony to enhance visits by managed bees and dispense the biological control agent, Treatment 2- Caged plot to enhance controlled manual spraying of crops with biological control agent (spray). The plot was caged using a stiff net that prevented entry of managed bees and other visitors into the plot, Treatment 3- Caged plot without managed bees and chemical spraying (control). The plot did not receive any biological control agent and there was no visitation by managed bees or other flower visitors. Each of the three treatments had 4 replicates. Loresho farm was classified as farmer normal practice. The plot was open to feral bees' visitation among other flower visitors.

Land preparation and strawberry planting

A piece of land measuring 50 m by 20 m at the on-station farm was cleared off the weeds. The plot was then divided into four blocks of 10 m by 20 m at two meters apart. Seven strawberry beds of one meter-wide and 10 m long at an interval of one meter- apart were made in each block. In each bed, twenty certified strawberry seedlings obtained from Kenya Agricultural and Livestock Research Organization (KALRO) were planted in a row. Spacing between plant to plant was 50 centimeters apart.

Weeding was done regularly to remove all the weeds that absorbed considerable nutrients and competed for space. Aerial irrigation was done for two hours daily during the first month of planting. Additionally, induced flower abortion was done on daily basis for the first two months after transplanting. This was done to enhance a healthy vegetative growth of the strawberry plants. To promote flowering and fruit set, Calcium Nitrate (CN) fertilizer was used (Republic of South Africa, 2008).

At on-farm site, two experimental plots each measuring 7 m by 10 m were established at an interval of 10 meters apart. The plots were then subdivided into two subplots each measuring 7 m by 2.5 m resulting to four replicates at 2 m apart. Three strawberry beds that measured one meter in width and 2.5 m in length were prepared on each subplot. The spacing between the beds and plant to plant was similar to that of on-station farm. In each strawberry bed, five certified strawberry seedlings obtained from KALRO were planted in each row resulting to fifteen strawberry seedling per subplot. Spacing was similar to that of on-station farm. Weeding was done weekly. Watering was done to supplement the little amount of rainfall received during the project duration. No chemical pesticides were applied to the strawberry plants on the on-farm site.

Installation of inoculated modified managed bee hive

A healthy colony of honey bees in a modified five- framed nuclear honeybee hive was installed at the on- station farm two weeks before flowering started. A modified two-way dispenser that consisted of an outlet (2 cm by 5 cm) and inlet (1 cm by 7 cm) pipes cylindrical in shape was fitted in the modified openings of the hive (Mommaerts et al., 2010). The outlet was made of transparent plastic material that transmitted light into the hive hence attracting bees to the exit, while the inlet had a landing platform from the outside that enabled bees to land on when coming back to the hive. The dispenser was designed to separate the outgoing bees from incoming bees for optimal dispensing of the biological control agent. At the onset of strawberry peak flowering, the dispenser was loaded with 2 g of *T. harzianum* inoculum prepared at 10^9 Colony-Forming Unit (CFU) g^{-1} with sterilized corn flour as a carrier- inert substance. The inoculum in the dispenser lasted for 6 days ensuring outgoing bees were well dusted with the inoculum on their hairy bodies (Freeman et al., 2004).

Bio-monitoring of *T. harzianum* transportation by managed bees

A sample of fifteen honey bees was picked when exiting the hive. The dispenser was refilled after every six days. The bees were captured using a sterile sweep net and picked using sterile forceps into sterile vials. Samples were taken to University of Nairobi (UoN) microbiology laboratory where they were cultured in potato dextrose agar (PDA) and incubated for four to seven days after which the total number of *T. harzianum* CFU was calculated.

Bio-vectoring of *T. harzianum* to strawberry flowers

A sample of fifteen strawberry flowers per treatments was excised using a sterilized pair of scissors and placed in manila paper bags. The samples were then taken for further processing at UoN, microbiology laboratory where the samples were cultured individually in PDA and after 4-7 days the number of *T. harzianum* CFU were calculated.

Determining grey mold disease incidence on flowers and fruits of strawberry

Healthy and grey mold diseased open flowers and ripe fruits from each plant per treatment were calculated and data recorded in the field data sheets. Strawberry fruits ripened on a weekly basis and hence data collection could only take place after every seven days for three months. The percent disease incidence was then calculated using the formula below as described by Waller et al.

(2002):

$$\text{Disease incidence\%} = \frac{\text{Number of Structures (flowers or fruits) with } B. \text{ cinerea}}{\text{Total number of Structures (flowers or fruits)}} \times 100$$

Determining the effect of simultaneous pollination and disease control using managed bees on strawberry quality and yield

The quality and quantity of strawberry fruits was determined using the following fruit parameters: total number of seeds per berry, the equatorial diameter, polar diameter and the weight of the berry (Colak et al., 2017). Harvesting of the fruits was done after every seven days for a duration of three months. Ripe berries characterized by red color were picked and packed for analysis at the National Museums of Kenya (NMK) in the Centre for Bee Biology and Pollination Ecology (CBBP). The diameter of the berries was measured using a vernier caliper in centimeters (cm) units, the weight was determined using analytical sensitive weighing balance in grams (g) units and the number of seeds per berry was counted from each fruit.

Statistical analysis

Data were analyzed using STATISTICA program (Stat Soft. Inc., 2007) version 8.0. The data were first tested for normality using Shapiro Wilk; s W- test and histogram. Where data did not show a normal distribution it was subjected to a normal distribution curve to check for outliers and also transformed at $\text{Log}_{10}(x+1)$. Statistical tests were set at a significant level of 0.05. The data on effectiveness of managed bees as a vector of the bio control agent, effectiveness of *T. harzianum* against grey mold diseases and the simultaneous effect of inoculated managed bees as pollinators were analyzed using a one-way analysis of variance (ANOVA) to show whether there was any significant difference among means. Where data showed a significant difference in means, the results were further analyzed using Turkey's test to show the level of significance difference.

RESULTS

Effectiveness of managed bees as vectors of *T. harzianum*

From the study, it emerged that an individual honey bee carried $22.4 \pm 4.85 \times 10^2$ Colony-Forming units of *T. harzianum* (Mean \pm SE) when exiting from the hive. Field observations showed that honey bees got dusted with the inoculum as they exited from the hives from the first day of refilling the dispenser with the inoculum. The highest mean of Colony-Forming Units ($25.8 \times 10^2 \pm 3.8 \times 10^2$) $P < 0.05$ were observed during the first day compared to honey bees sampled on the second day and third day respectively ($23.2 \times 10^2 \pm 2.4 \times 10^2$), $18.2 \times 10^2 \pm 4.4 \times 10^2$) after refilling the dispenser with inoculum ($F = 2, 12$, ($P < 0.05$) (Figure 1).

Colony-forming units of *T. harzianum* on strawberry flowers

There was a significant difference in number of CFU of

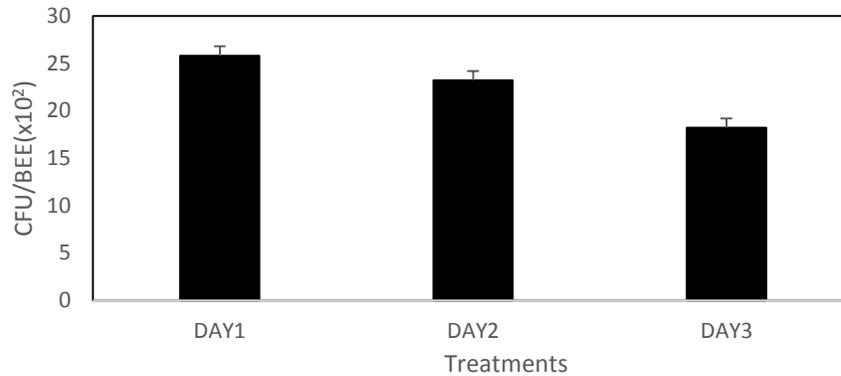


Figure 1. Number of *T. harzianum* spores per sampling day.

T. harzianum per strawberry flower among treatments ($F=3,140$, $P<0.05$). Spray treatment recorded highest mean of CFU of *T. harzianum* per flower (20.56 ± 0.48 (10^2) while bio-vectoring technology (BVT) treatment recorded 11.86 ± 0.44 (10^2) CFU of *T. harzianum* per flower ($P<0.05$). Control treatment and farmers practice treatment recorded the lowest mean of CFU of *T. harzianum* per flower (0.36 ± 0.11 and 0.33 ± 0.08) 10^2) respectively ($P>0.05$).

Effectiveness of *T. harzianum* on Grey mold diseases control

It was evident that *T. harzianum* had significant effects on disease control on ripe fruits of strawberry. Fruits harvested from the four treatments showed a significant difference in their diseases incidence ($F=3.12$, $P<0.05$). Spray treatment showed significantly low fruit diseases incidence (16%) as compared to farmers practice plot (38%) $P<0.05$. There was no significant difference in fruit disease incidence among BVT (26%), control (26%) and spray plot. $P>0.05$. However, there was a significant difference in percentage disease incidence on strawberry flowers among treatments ($F=3.12$, $P<0.05$). The farmers practice plot recorded significantly lower mean of flower disease incidence (18%) as compared to BVT (30%) and control treatment (29%). There was no significant difference among spray plot (23%), BVT and control plot ($P>0.05$).

The effect of pollination by inoculated managed bees on strawberry quality and yield

Data on total harvest, weight, polar diameter, equatorial diameter and the number of seeds in a strawberry fruit were used in this study to assess the simultaneous effect of inoculated managed bees on strawberry quality and yield. There was no significant difference among the

means of the total berry harvested in all the plots ($P>0.05$). However, the spray plot showed significantly the highest weight of 4.89 ± 0.20 kg as compared to caged plot (4.13 ± 0.22 kg) ($F=3,2122$, $P<0.05$). There was no significant difference among weights of berries in BVT (4.84 ± 0.18 kg), farmers practice (4.48 ± 0.42 kg) and spray plot ($P>0.05$). Polar diameter of fruits obtained from spray, caged and farmers practice showed a significant difference ($F=3,2122$, $P<0.05$). Fruits obtained from farmers practice plot recorded significantly the higher polar diameter (2.48 ± 0.18 cm) as compared to caged plot (1.64 ± 0.09 cm) $P<0.05$. Similarly, there was a significant difference in equatorial diameter of fruits obtained from all the treatments ($F=3,2122$, $P<0.05$). Fruits with the highest equatorial diameter were recorded from farmers practice (1.93 ± 0.20 cm), while the lowest mean of equatorial diameter was recorded from caged plot (1.31 ± 0.05 cm). Lastly, the mean number of seed per berry had a significant difference among all the treatments $P<0.05$. Farmers practice recorded the highest mean (242 ± 11) of the number of seeds while caged recorded the lowest mean number of seed per berry (125 ± 6). ($F=3, 2122$, $P<0.05$) as shown in (Table 1). Wild bees and other flower visiting insects recorded in the study sites before and after installation of managed bee's hives included managed bees, solitary bees, dipteran, lepidopteran, coleopteran, orthopteran, Aranea and wasps. Dipterans were proportionally the most numerous of the flower visitors in both the on- station and on-farm sites while solitary bees were many in the on-farm site. However, close observations confirmed that only bee species were in contact with both anthers and stigma while visiting the strawberry flowers and would thus be able to transfer pollen and hence enhancing cross-pollination.

DISCUSSION

From the results, it was evident that honey bees were

Table 1. Effect of pollination by managed bees and *T. harzianum* application on strawberry quality and yields.

Treatment	Mean ± SE					
	No. of flowers	No. of fruits	Weight (g)	PD (cm)	ED (cm)	No. of seeds
BVT	186.25 ± 21.07 ^a	69.75 ± 2.78 ^a	4.85 ± 0.18 ^{ab}	1.96 ± 0.08 ^a	1.55 ± 0.04 ^a	160 ± 4.8 ^a
Spray	204.5 ± 28.6 ^a	85.5 ± 2.50 ^a	4.89 ± 0.20 ^a	2.02 ± 0.08 ^{ac}	1.44 ± 0.05 ^{ab}	135 ± 5.1 ^b
Caged	157.25 ± 11.50 ^{ab}	57.5 ± 3.38 ^a	4.13 ± 0.20 ^b	1.64 ± 0.09 ^b	1.31 ± 0.05 ^b	125 ± 5.8 ^b
FP	88.75 ± 21.99 ^b	54.25 ± 20.14 ^a	4.48 ± 0.42 ^{ab}	2.48 ± 0.18 ^c	1.93 ± 0.10 ^c	241 ± 11.1 ^c

Means with same letters within a column are not significantly different ($P > 0.05$).

loaded with *T. harzianum* spores as they exited the hive. The inoculum was heavily loaded on the upper part of their entire bodies and was dispensed consistently from the dispenser throughout the day. The findings agreed with the work done by Bilu et al. (2004) on use of Triwaks; a dispenser type that dispensed a high level of *Trichoderma* spp consistently. Flowers collected from all the treatment showed the presence of *T. harzianum* although at different concentrations. The results were an indication that honey bees were able to carry and disseminate the biological control agent to the target crop. The study results agreed with those of Kovach et al. (2000) who observed that honey bees could successfully deliver *T. harzianum* to strawberry plant for biological control of *Botrytis* fruit rot.

The study also revealed that honey bees carried more of the BCA on day one than on day two and three after loading the dispenser with the inoculum. It was evident that the inoculum was reducing every time the honey bees were exiting the hive hence the loading of the dispenser had to be done after every six days. The results concurred with findings of Freeman et al. (2004) where application of biological control agent after every two days resulted in better control of *B. cinerea* than less frequent application of every seven to ten days.

The findings of the study further revealed that spores of *T. harzianum* were recorded more on flower samples derived from sprayed and BVT plots as compared to those derived from control and farmers practice plots. BVT and spray treatments received biological control agent through managed bees and spraying respectively while control and farmers practice plot could have received the biological control agent as drift or as naturally occurring spores in the soil. That observation agreed with findings by Shafir et al. (2006) where on average 22000 CFU of *T. harzianum* per flower were delivered by honey bees to strawberry plants through a Triwaks dispenser. Even though the spray treatment showed the highest number of spores landing on the flowers, the practice is not economically viable for large scale or commercial farming. This is because the practice is labor-intensive and much amount of water is consumed leading to higher expenses. Shafir et al. (2006) found out a different challenge of application of biological control agent whereby frequent use of a sprayer may cause

mechanical damage to the fruit and foliage. The use of managed bees to deliver the biological control agent requires little manpower and managed bees double up as pollinators of the target crop.

The flower samples obtained from BVT plot were assumed to have lost some *T. harzianum* spores. This was attributed to the movement of honeybees from one flower to another (Shafir et al., 2006). Additionally, biological control agent could have been lost during the process of capturing bees using a sweep net, drifting by the air or being swept away by sprinkled water during the irrigation process. However, traces of *T. harzianum* spores were found on flowers in the control and farmers practice plots. This observation provided evidence that the fungi (*T. harzianum*) could have naturally occurred in the soil at a very small concentration, or likely that the *T. harzianum* spores were drifted through the stiff net from the spray and BVT plots to the non *T. harzianum* loaded plots. Those findings were supported by work done by Shafir et al. (2006). Research done by Peng et al. (1992) and Kovach et al. (2000) indicated that drift effect of biological control agent occurred whereby non-target areas were found to have the inoculum. Similar observations were made by Elad and Yunis (1993) who also found a significant population of *T. harzianum* in untreated control plots when the biological control agent was sprayed in the treated plots of cucumber plants.

The presence of *B. cinerea* spores obtained from the laboratory after inoculating a gram of soil samples collected from the study sites indicated that the grey mold disease was present in the soil before the introduction of the biological control agent. Results from this study revealed that *T. harzianum* used in the strawberry plots either through usage of managed bees or spraying had *B. cinerea* disease suppression effect on strawberry fruits. When the biological control agent (*T. harzianum*) was applied on strawberry plants, it was evident that the results of the disease suppression were expressed more on fruits than on flowers. Those results could be justified by the fact that the transition from a flower to a fruit took place within one to three days while fruit ripening took between five to seven days. Additionally, high disease incidence on BVT flowers could be as a result of lower number of CFU of *T. harzianum* delivered by managed bees as compared to those delivered via spraying.

Finally, the effect of high humidity as a result of irrigation and increased managed bees may have contributed to the growth and multiplication of *B. cinerea* on BVT flowers. Dedej et al. (2004) found that the incidence of mummy berry disease increased as a result of increased honey bee density, but the mummy berry disease incidence was reduced when the honey bees disseminated *Bacillus subtilis* to blueberry plant.

The study also proved the effectiveness of *T. harzianum* against grey mold as also recorded by Hokkanen et al. (2015) who recorded a 66% grey mold reduction under light disease pressure when honey bees were used to disseminate *Gliocladium catenulatum* to strawberry plants. Similar findings by Peng et al. (1992) and Yu and Sutton (1997) also reported sixteen percent reduction of *B. cinerea* in strawberry using *Clonostachys rosea* vectored by honey bees. The use of biological control agent therefore, proved to be effective in suppressing *B. cinerea* that causes grey mold disease to strawberry plants. It was evident that the spray treatment recorded the lowest disease incidence as compared to the other treatments. Although the control treatment did not receive any biological control agent, disease incidence showed a decrease from what was recorded from flowers as compared to the fruits. Those findings revealed the protective effect of the stiff net in preventing entry of strong winds that would have drifted the pathogen, direct sunlight and water droplet or contaminated insects coming from open environment.

It was therefore apparent that pollination by insects and wind on strawberry plant played a significant role in influencing the quality and yield of strawberry crop. The findings of this study showed a significant difference in the number of seeds in a fruit, weight, total harvest and the diameter of strawberry fruit across the treatments. The number of managed bees, solitary bees and wind pollination could have influenced the quality and quantity of the strawberry fruits. Studies by Albano et al. (2009) showed a relationship between the number of fertilized ovules and berry size of strawberry when pollinated by both honey bees and solitary bees. Therefore, it may be assumed that the high number of pollinating solitary bees could have contributed to quality and quantity of strawberry yield from on-farm site. Comparison of the strawberry yield between BVT plot and farmers practice plot revealed a strong contribution of solitary pollinators. Managed bees alone were found to be effective in strawberry pollination. Colak et al. (2017) found a significant difference in weight for honey bee pollinated strawberry. However, reliance on several pollinators would lead to better quality and quantity of strawberry fruits. The results of this study agreed with the results of Albano et al. (2009) on diversification of pollination sources, avoiding the dependence on a single specific group of pollinators.

The consistent higher fruit weight, polar diameter and number of seeds in spray plot compared to control plot

was likely to have resulted from the effects of *T. harzianum* as both plots were enclosed. Those findings agreed with those of Kovach et al. (2000) who showed that stiff net frequently used by fruits and vegetable growers, protected plants from cold and hence accelerated plant growth. However, despite the influence of pollinators on strawberry yield, it was confirmed from this study that the commercial strawberry cultivar had a degree of self-pollination and wind pollination. According to Albano et al. (2009) flowers of all the commercial strawberry are hermaphrodites and self-fertile. It was noted that flowers of both open (BVT and FP) and stiff-netted plots (control and spray) were similarly shaken under windy conditions, indicating that the stiff net was not a major barrier to wind pollination. That observation agreed with the findings of Kuvanci et al. (2010) that showed a significant increase in strawberry yields following honey bees, wind and native bee's pollination. It was further noted that on installation of honey bee's colony within or close to the BVT, solitary bees were displaced. Those findings agreed with Roubik (2009), who showed that increased managed bees in an area silently competed with solitary bees interfering with their foraging activity. However, higher quality of strawberry yield was recorded from on-farm site due to frequent pollination by solitary bees. That is in contrary to the findings revealed by Hansted et al. (2015) that showed a rise in fruit set and yield when bees were kept close or within an orchard.

CONCLUSION AND RECOMMENDATION

Conventionally, control of grey mold disease in strawberry plantation is achieved through application of chemical pesticides. The pesticides used are harmful to the environment, human and to beneficial insects such bees. In order to eliminate the negative effects of chemical pesticides in the environment, BVT where managed bees are used to disseminate biological control agent could be an alternative solution to pesticides application. The present study recommends: further research on the effect of increased managed bee to the environment, the long term effect of biological control agent (*T. harzianum*) to the bees and to the environment

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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