Boffin Access
FOSTERING KNOWLEDGE
Open Access Journal | Volume: 1.1

Agronomy and Agricultural Techniques

Entomovector Technology Research of *Botris Cinerea,* Using Honey Bees and Bumblbees, Which Cause Grey Mold in Strawberries

Ismagulova A[1*]
Tulegenova Zh[1]
Spanbayev A[1]
Eshzhanov T[1]
L.N. Gumilyev[1]

[1]Eurasian National University, 112657, Nur-Sultan, Akmola, Kazakhstan

Keywords

Grey mold; Entomovector; Bee,bumblebee; Trichoderma

Introduction

Phytopathogens, which cause various diseases in berry plants, reduce their productivity. Accordingly, this may lead to economic losses. Modern agrochemicals used in phytopathogenic bacteria control do not solve the problem completely.

Today from the perspective of Environment Protection Organization, biological method for plant disease treatment is very efficient. Biologicals are made using living micro-organisms, antigens or based on their biomass. Essentially, they can be a good alternative to chemicals and protect the environment from pesticide pollution [1].

Consequently, studies related to the efficient methods of pathogen control in the conditions of country cottage and field is an actual issue.

Entomovectorial technology is a relatively new term. The term "entomovectorial technology" was introduced by Fin researchers as Hokkanen and Menzler-Hokkanen in 2007 [2]. Entomovectorial technology consists in the transference of agent, which is under the vectorial bio-control. For instance, biological is stuck on a wasp like a pollen-grain, and wasps will contact with a spore of bio-control agent material, when they leave and enter the cap. As a result, they transfer a great deal of biologicals. Despite of short period of time, this technology demonstrates good results. Though, the efficiency of entomovectorial technology, some specific features, local climate and other factors require additional extended studies.

In the above mentioned example, bees are demonstrated as bio-control agents. It was pointed out that the efficiency of plant diseases control in the fields depends on the frequency of the flights of bees.

The followings can serve as good examples of that: when the disease in blueberries, produced by *Monilinia vacciniicorymbosi,* was moved to *Bacillus subtilis* biological the damage was decreased from 21-67% to 7-44%, raspberry infestation with *Botritis cinerea* fungus was reduced from 90 to 68%, *Sclerotinia sclerotiorum* infestation in sunflower was stopped in 31 days being replaced by *Trichoderma* [3]. Therefore, we can get good results, if the bio-control agents of flowers reach optimum capacity.

Kapongo and others [4] report about prevalence of bee (*Bombus impatiens*) bio-agents in different plants. For instance, bees spread over about 1,000-5,000 CFU to flowers, in the result of this *Botritis cinerea* in tomatoes and sweet pepper was reduced to 57-59% [4].

Climate change and wholescale distribution of fungal agent diseases in Kazakhstan demand studies applying new methods of biological phytopathogene control.

Botrytis cinerea belongs to *Ascomycota* division, *Pezizomycotina* type, *Leotiomycetidae* class, *Helotiales* order, *Sclerotiniaceae* family, *Botrytis* genus. This pathogene affects nearly 200 plants in the world, which leads to serious economic losses [5].

As far as the *Botrytis cinerea is* one of the important necrotrophic fungal species with a high causative pathogen, today, it is frequently applied for molecular research.

The strawberry and raspberry are widespread and important cultivated berry plants. The productivity of strawberry depends on fungi diseases, particularly grey mold, caused by *Botrytis cinerea. Botrytis cinerea* is an economically essential phytopathogene. Therefore, studying the infection and damage in strawberries, caused by this pathogen and searching for new methods of controlling the disease in order to minimize the increasing use of

Article Information

DOI: 10.31021/AAT.20201101
Article Type: Review
Journal Type: Open Access
Volume: 1 **Issue:** 1
Manuscript ID: AAT-1-101
Publisher: Boffin Access Limited
Received Date: 26 September 2020
Accepted Date: 07 October 2020
Published Date: 09 October 2020

***Corresponding author:**
Altynai Ismagulova
Eurasian National University
112657, Nur-Sultan
Akmola, Kazakhstan
E-mail: altynai14.02@mail.ru

Citation: Ismagulova A, Tulegenova Zh, Spanbayev A, Eshzhanov T, L.N. Gumilyev. Entomovector Technology Research of *Botris Cinerea*, Using Honey Bees and Bumblbees, Which Cause Grey Mold in Strawberries. Agronomy Agric Technol. 2020 Oct;1(1).101

Figure 1: Culture and spore *Botrytis cinerea*

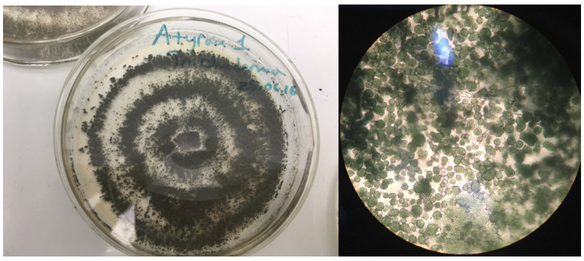

Figure 2: Culture and spore of *Trichoderma viride* taken from the local soil

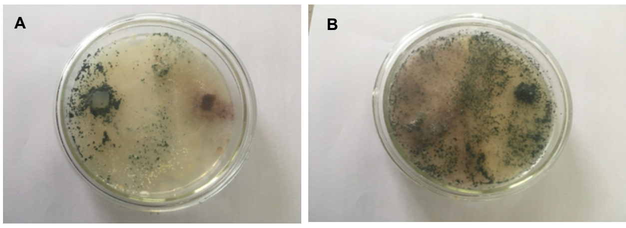

Figure 3: The result of parallel seeding of *Trichoderma viride* fungi and *Botrytis cinerea* culture in the nutrients medium.

synthetic fungicides are topical issues. Studies show the efficacy of Trichoderma as bio-control agent against the *Botrytis* [6,7]. But frequent application of BCA costs for farmers is high. This study suggests the efficacy of transferring pesticide on Carpathian wasps in the field conditions. Two year studies indicate significant decrease of Botrytis infection.

Materials and Methods.

Inoculum, Vectors and Dispensers

Botrytis cinerea is omnicoius. They grow in sugar and they can also treat cellulose. They also can grow in apple, vine, formylic acid, glycerin, flax-seed and cotton-seed oil, tannin, 2 % nicotine, they use nicotine as a source of nitrogen. And except for those above mentioned, they grow in peptin and asparagines. In the case of nitrogen deficiency, they have a specific characteristic of gulping it from the air.

A.I. Osparin and O.I. Kuplenskaya [8], researching Botrytis mycelium, which feed on nitrogen, conclude that dry weight of mycelium grown in glucose is 0,06 gr., while dry weight of mycelium grown in lactose and mannite is 0,04 (Osparin and Kuplenskaya, 1931). Potassium, phosphorus, nitrogen, magnum, sulphur and manganese are vitally important elements for normal growth of Botrytis (Figure 1).

As a result of research works, we come to conclusion that *Trichoderma viride* inhibits the growth of *Botrytis cinerea.* For this purpose, the antagonistic properties of fungi *Trichoderma viride* were considered in the laboratory (Figure 2), taken from the local soil, describing the fungal culture.

The effect of *Trichoderm* fungi on the radial growth of *Botrytis cinerea* culture was studied. In the control sample of gray mould pathogen mycelium within six (Figure 3A) and twelve days (Figure 3B) reaches 38.7 mm and respectively 41 mm to the edge of the Petri dish. Studies show while inoculating them in agar medium, due to struggle for nutrients and place 3*106 spores/ml implemented by the presence of titres (Figure 3).

Spray seeding of fungal spores and mycelium is not an easy way. Water-diluted preparation is effective only in re-production, since only in this way the bio-control agent lands on the infected flower organs. Economically this method is not beneficial, because it splashes over the whole area of planting field, soil and leaves.

In the process of realization of bio-control measures with the help of *Trichoderma viride* fungi, multi hives of Carpathian bees are used. The large multi hive is resistant to various climate changes, polystyrol hives consist of three separate parts, each of which consists of colonies with a Queen and 350 working bees. All hives are equipped with two-way dispenser (with or without preparats). With the help of two-way dispenser Carpathian wasps get out of a hive through dispensing tray with biopreparats, internal movements take place through an empty tunnel, so that not to pollute the inside of the hive.

The studies were conducted for two years during the growing season in the strawberry valley in Staraya Krepost' village, in Semey (2016-2017) (Figure 4). Perennial plantings of strawberry were sown in August, 2016 (8,0 ha). Planting area is built in single-type places, 6 pieces per meters. Each line is 0,6 × 125 м. Strawberry fields are surrounded by fruit trees, berry trees. The Carpathian wasps are located within a radius of 0.05 km to open planting fields. All strawberries are made in accordance with the recommendations for growing in organic conditions; no chemical agents were used to protect plants during the studies.

GPS coordinates: width: 50º 29^{1}29ı 52º C

Length: 80º 5^1 52 17º B,

Studied strawberry cultivar is "Simphonia", and this is the most common variety of strawberry. As noted for two years, the flowering period begins on May 25-28 and ends on June 10-14 (2016, 2017) (Figure 5).

Eight experimental fields located at a distance of 5-25 m from hives (Figure 6). Each area consists of 12 strawberry plants. Four fields are separated with different colored neutral anti-insect nets (size 0.44 × 0.77 mm, 9-10% shadowiness). This allowed us to

Figure 4: The map of the strawberry planting. Staraya Krepost', Semey, Eastern Kazakhstan Oblast. The strawberry plantings are surrounded by rectangles; Points in the field indicate experimental planting fields and position of hives.

Figure 5: The growing season of strawberry

Figure 6: The bee hive and dispenser of Carpathian bees

Figure 7: The level of gray mold infection tilled by Trichoderma. Fertilization of observing patch with the help of Carpathian bee.

pollinate with the help of the wind during the flowering period. In other areas, except the wind, strawberry flowers are pollinated with the help of the Carpathian wasps through transferring *Trichoderma*. Registration devices for data collection on specific humidity and air temperature (HAXO-8 for temperature and Logger for humidity, MicroDAQ.com NH 03229 USA) are installed above the covered with net and open plots. There are no differences in the microclimatic state on the area covered with net and not covered (RH:T = 0.05, DF = 23, P = 0.95; air temperature: t = -0.38, DF = 23, P = 0.7).

Bio-control of the agent, spread out from the strawberry field, was evaluated by collecting fresh strawberry flowers at a distance of 0, 25, 50, 75 and 100 m from bee colonies. At each distance there were four repetitive selected sample plots. Planting plots are located in four segments formed between beehives. Planting plots will be open to prevent the entering of bees. Ten flowers from each repetition of plots are collected and put into plastic bags. Flowers are collected on different days of flowering season. All flowers are put into refrigerator and stored at a temperature of -20° C. before analyses. The collection of flowers was collected on effective days for the Carpathian bees.

The number of Carpathian wasps settled among the flowers were determined, in the days when they search for food, by the method of calculation in transect in the strawberry field of Staraya Krepost' in 2016. The number of insects was considered bidaily at a distance of 0, 25, 50, 75 and 100 m from beehives for every 10 m sections (N = 4) in the section of 100 m from each place.

To study the effectiveness of biopesticides transferred with the bees, 6 gr. of Trichoderma was put into dispenser. Every other day the powder was renewed. It was renewed both at high humidity of air, and at bad quality of a powder. To study the effectiveness of the pollination in 2016, the Carpathian bees, which do not have a bio-products, were used.

Gathering time of strawberries starts on 26-28 June and lasts until July. Strawberries are gathered every third day. The number of defected and non-defected fruits are counted and this information are used to define the level of gray mold infection. The weight of ten fruits take from different places are investigated to know the effectiveness of pollinating. The grow of non-defected fruits is counted.

Results and Discussion

Weather condition was different in two years. Average temperature in June was 15° C in 2016 and 20° C in 2017. Average temperature in June was 23°C.

Volume of average raining in June was high, in 2016 140 %, in 2017 136%.

Total monthly indication of raining in June 2016 was 128%, in June and July 2017 was 193%. Average indication of raining was 92% in 2016, 78% in 2017.

There was twice as much rain as in 2017.

According to nonparametric analysis, qualitative analysis of cycle (Cq) indication of flower (36,5 ± 0,12) in all bee hive distance(0, 25, 50, 75 and 100 м) and time interval (4 , 5, 6 and 7 days) are equal; (distance: F = 0,44 DF = 3 P = 0,43; day: F = 0,33 DF = 3 P = 0,78; distance X day: F = 0,83 DF = 12 P = 0,62).Therefore, on the average 123% of *Trichoderma* spores simultaneously widespread to the flowers, that is percentage feeder is went on with beehive. During the experiment spores simultaneously widespread as well.

The bees were counted 0,13 ± 0,02 10 m distance from transect patch, this number does not change during the period of flowering ($F_{2,251}$ = 2,15 p = 0,14).The number of bees sitting on the flowers is similar in different distance from the beehive (5, 25, 50, 75, 100 м) ($F_{4,251}$ = 0,81, P = 0,53). In spite of bees there are other insects in strawberry flowers: some groups of flies (in one patch 0,68 ± 0,08 units), wax making bee (0,46 ± 0,04), wild bees (0,21 ± 0,03) and flies (syrphid) (0,10 ± 0,01). The number of these insects does not depend on the distance (total P> 0,05).

The effectiveness of biological observing according to infection of gray mold

The level of gray mold infection distinguishes during the observing years in observed patch ($F_{1,152}$ = 1,284.6, p<0,001) (7-picture). When trichoderma drug was used, it immediately decreased gray mold infection ($F_{1,152}$ = 1,284.6, P <0,001). The effectiveness of treatment increased every year ($F_{1,152}$ = 39,44, p <0,001). In two years level of gray mold infection has decreased (2016: т = -9,30, DF = 152, p<0,001; 2017: т = -8,20, DF = 152, p<0,001) Figure 7.

The influence of effective fertilization on the fruit weight

The average weight of ten fruits is shown in wide diapason 63,2 ± 3,2 g (since 2016) and 182,8 ± 9,8 g (since 2017). There is no difference noticed from the effect of other pollinating (wind + bee, other insects) ($F_{1,250}$ = 0,05, P = 0,8). In the weight of ten fruits the average indication between wind pollinating and free pollinating shows 0,8 ± 3,8 g.

The influence of fertilization and biological observing on total weight

Using Trichoderma while pollinating through Carpathian bee effects on strawberry's total weight ($F_{1,18}$ = 9,7, p = 0,006), but pollinating by themselves did not give any results ($F_{1,12}$ = 0,14, P = 0,7). Characteristics of total expenses in years (pollinating with Trichoderma: $F_{1,18}$ = 33,31, p<0,001; pollinating : $F_{1,12}$ = 113,3 p<0,001). Pollinating through insects and Trichoderma in the first year 123% (2016: т = 2,32, DF = 18, P = 0,03), in second year 112% (2017: т = 1,58, DF = 18, P = 0,13).

Bees who fertilized flower serve as additional fertilizer for increasing the weight of fruits.

Vector system of bees has several services – controlling the invaders and relation between plants and vector. There are some more problems appeared during the transportation the drugs with the bees in open places than in the fields. Main obstacles are pollinating those plants, which are not so important and weather conditions [9]. Carpathian bees are considered to be pollinating bees; they are wide sectioned and can be fertilized for different plants at the same time (2-4). They can also transport to other not important plants .

Conclusion

The research results show that using bio fungicide with the help of Carpathian bees is one of the effective way to fight against gray mold infection in strawberry plant. However, controlling depends on the weather as well. In 2016 and 2017 the level of gray mold infection decreased 2,8 to 1,6. According to the results, Trichoderma drugs widespread 100 m distance far from the beehives. Carpathian bees used about 70% of Trichoderma drugs in first 60 seconds. When counting Carpathian bees sitting on the flowers pollinating materials showed low level. If total areal of wide spreading of Carpathian bees in beehive reaches about 253 м, 40 % of them fertilize plants in 100 m. It is noticed that in average 1 Carpathian bee flies 100 m vertically along the strawberry patch. Total number of what Carpathian bees gathered is fifth of other plant pollinating materials gathered. This result is not surprise because Carpathian bees are workers and carriers, they have abilities to fertilize other plants too. In our point of view in spite of Carpathian bees, other insects are using to fertilize strawberry's flowers. Other types of pollinating were successful in entomovector technology.

References

1. Peng G, Sutton JC. Kevan PG. Effectiveness of honey bees for applying the biocontrol agent Gliocladium roseum to strawberry flowers to suppress Botrytis cinerea. Can J Plant Pathol. 1992 Jan;14(2):117-129.
2. Hokkanen HMT, Menzler-Hokkanen I, Lahdenpera ML. Managing bees for delivering biological control agents and improved pollination in berry and fruit cultivation. Sustain. Agric Res. 2015;4(3):89-102.
3. Samuel S, Veloukas T, Papavasileiou A, Karaoglanidis GS. Differences in frequency of transposable elements presence in Botrytis cinerea populations from several hosts in Greece. Plant Dis. 2012 Sep;96(9):1286-1290.
4. Kapongo JP, Shipp L, Kevan P, Sutton JC. Co-vectoring of Beauveria bassiana and Clonostachys rosea by bumble bees (Bombus impatiens) for control of insect pests and suppression of grey mould in greenhouse tomato and sweet pepper. Biological Control. 2008 Sep;46(3):508-514.
5. Williamson B, Tudzynski B, Tudzynski P, Van Kan JA. Botrytis cinerea: the cause of grey mould disease. Mol. Plant Pathol. 2007 Sep;8(5):561-580.
6. Mcquilken MP., Gemmell J, Lahdenperä M. Gliocladium catenulatum as a potential biological control agent of damping-off in bedding plants. J Phytopathol. 2001 Oct;149(3-4):171-178.
7. Prokkola S, Kivijärvi P. Effect of biological sprays on the incidence of grey mould, fruit yield and fruit quality in organic strawberry production. Agric. Food Sci. 2007 Jan;16(1):25-33.
8. Oparin AI, Kuplenskaya OI. Uprashennaya metodika issledovaniya fiziologicheskih svoistv kagatnyh mikroorganizmov. – In: Hranenniye sahornoi svekly. Sbornik materialov I statei po voprosam hraneniye sahornoi svekly. 1931;45-50.
9. Maccagnani B, Bazzi C, Biondi E, Tesoriero D, Maini S. Potential of Osmia cornuta as a carrier of antagonist bacteria in biological control of fire blight: a comparison with Apis mellifera. Acta Hortic. (ISHS) 2006;704, 379–386.