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Swedish University of Agricultural Sciences

SLU Partnerskap Alnarp

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Projekttitel

SLU Partnerskap Alnarps projekt nr: PA1459

Kan jordgubbsplantans motstånd mot angrepp av gråmögel öka med Mold Guard biologisk bekämpning

Can the strawberry plant´s resistance to gray mold attack be increased with Mold Guard biological control

Projektledare: Lotta Nordmark

Författare till rapporten: Lotta Nordmark och Samar Khalil

Fakultet: LTV

Institution: Biosystem och teknologi

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Projektpartners: Mold Guard

Sammanfattning

Jordgubbar till färskvarumarknaden är den största produktionen inom svensk bärodling och har under de senaste åren odlats på i genomsnitt ca 2 400 hektar, vilket motsvarade 77 procent av den totala bärarealen 2020. Odlingsarealen har varit förhållandevis stabil över åren, medan skördeutbytet varierar beroende på väderlek och odlingstekniska insatser

Bland svamppatogener globalt och i Sverige anses gråmögel, Botrytis cinerea, vara en av de primära patogenerna på skördade jordgubbar som leder till betydande ekonomiska förluster av jordgubbsskörden. Svampen B. cinerea orsakar gråmögel i frukt och åldrande organ men kan också påverka vegetativa vävnader. Under förhållanden med regn och hög luftfuktighet kan mer än 80 % av jordgubbsblommorna och frukterna gå förlorade om växtmaterialet inte skyddas med avsedd kemisk eller biologisk behandling. Med jordgubbsblommornas fenologiska utveckling ökar behovet av upprepade behandlingar för att motverka patogenens utveckling. Upprepade behandlingar medför samtidigt en ökad risk att patogenen över tid utvecklar en resistens (Edin, 2011). Det medför behov av flera olika angreppsmekanismer mot patogenen.

Det finns dokumenterad effekt av naturligt förkommande essentiella oljor (Abd-Elkader et al. 2021) medverkar till att förhindra tillväxt av B.cinerea.

Mold Guard (NOMO Professional, MoldGuard Inc.) är en organiskt och 100% nedbrytbart mögelmedel, som förhindrar tillväxt av mögel, alger och svamp under lång tid. Produkten är registrerad på Kemikalie inspektionens samt ECHA (European chemicals angency). Den naturliga sammansättningen av bland annat Eucalyptusolja och Tea Tree Oil anses ha en låg negativ inverkan på människa, djur och miljö.

Syfte med projektet är att genomföra en undersökande förstudie om Mold Guards har en effekt mot gråmögel på jordgubbar och utarbeta behandlingsrekommendationer för att minimera angrepp av gråmögel på jordgubbar. Målet är att analysera bär vid skörd utifrån kvalitetskriterier enligt EU:s kvalitetsnormer för färskvarumarknaden och bedöma hållbarheten vid korttidslagring. Frågeställningar som projektet kommer att behandla är effekten av Mold Gurad effekt på patogenen samt i vilken koncentration. Vidare kommer också projektet besvara om formulan har någon påverkan på plantans tillväxt och utveckling samt skördeutbyte. Analys kommer att göras om formulans påverkan på mikroliv i substrat samt bärens kvalité.

Odlingen av jordgubbar som inokuleras med B. cinerea kommer att göras i substrat i tunnelodling och applicering av formula Mold Guard kommer att göras med upprepningar och koncentrationer. Analys av patogenen kvantifieras med hjälp av qPCR teknik och mikrobiell aktivitet. Efter två års projekttid kan följande slutsatser ge: Mold Guards formel (MF) är mest effektiv för att minska Botrytis cinerea vid en koncentration på 10 % i både in vitro- och in vivo-bedömningar. Formeln med 100 % koncentration visade en minskning av antalet frukter i tunnelförsöket. Ökad avkastning kunde uppnås med 10 % koncentration. Patogeninfektionen påverkades av koncentrationen av Mold Guard-formeln, valet av substrat och kultivar i tunnelförsöket samt vid undersökningar efter skörd.

Formelns koncentration på 10 % var mest effektiv vid utvärderingen av patogeninfektion två veckor efter skörd.

Abstract

Strawberries for the fresh market are the largest production in Swedish berry cultivation and have been grown on an average of about 2,400 hectares in recent years, which corresponds to 77 percent of the total berry area in 2020. The cultivation area has remained relatively stable over the years, while harvest yields vary with weather conditions and cultivation techniques.

Among fungal pathogens globally and in Sweden, gray mold (Botrytis cinerea) is considered one of the primary pathogens of harvested strawberries, leading to significant economic losses in the strawberry harvest. The fungus B. cinerea causes gray mold on fruit and senescent organs, but it can also affect vegetative tissues. Under conditions of rain and high humidity, more than 80% of strawberry flowers and fruits can be lost if the plant material is not protected with the intended chemical or biological treatment. As strawberry flowers develop, the need for repeated treatments increases to counter the pathogen's development. Repeated treatments also increase the risk that the pathogen will develop resistance over time (Edin, 2011). This requires several different attack mechanisms against the pathogen.

There is documented evidence of the effect of naturally occurring essential oils (Abd-Elkader et al., 2021) in preventing the growth of B. cinerea.

Mold Guard (NOMO Professional, MoldGuard Inc.) (MF) is an organic, 100% biodegradable mold inhibitor that prevents the growth of mold, algae, and fungi for a long time. The product is registered with the Swedish Chemicals Inspectorate and the European Chemicals Agency (ECHA). The natural composition of Eucalyptus oil and Tea Tree Oil, among other things, is considered to have a low negative impact on humans, animals, and the environment.

The purpose of the project is to conduct an investigative preliminary study to determine whether Mold Guards has an effect on gray mold on strawberries and to develop treatment recommendations to minimize gray mold attacks on strawberries. The goal is to analyze berries at harvest according to EU quality standards for the fresh produce market and to assess shelf life during short-term storage. The project will address the effect of Mold Guard on the pathogen and the concentration at which it occurs. Furthermore, the project will also determine whether the formula affects plant growth and development and harvest yield. An analysis will be made of the formula's impact on microlife in the substrate and the quality of the berries. Strawberry cultivation inoculated with B. cinerea will be conducted in tunnel substrates, and Mold Guard formula (MF) application will be repeated at varying concentrations. Analysis of the pathogen is quantified using qPCR and microbial activity assays. After 2 years of project time, the following conclusions can be drawn: MF is most effective at reducing Botrytis cinerea at a 10% concentration in both in vitro and in vivo assessments. The formula with 100% concentration showed a reduction in the number of fruits in the tunnel experiment. Increased yield was achieved with a 10% concentration. Pathogen infection was affected by the concentration of the Mold Guard formula, the choice of substrate and cultivar in the tunnel trial, and in post-harvest studies.

The 10% concentration of the formula was most effective in evaluating pathogen infection two weeks after harvest.

BACKGROUND

Strawberries for the fresh market are the largest product in Swedish berry cultivation and have been grown on an average of about 2,400 hectares in recent years, which corresponds to 77 percent of the total berry area in 2020 (Swedish Board of Agriculture, 2020). The area under cultivation has been relatively stable over the years, while harvest yields vary with weather and cultivation techniques. On average, approximately 16,000 tons of strawberries are produced outdoors in Sweden each year (Swedish Board of Agriculture, 2020). As strawberries for the fresh market are primarily consumed fresh, unprocessed by consumers, there is great interest in berries from sustainable production systems with low inputs of non-biological products.

Among fungal pathogens worldwide, gray mold (Botrytis cinerea) is a primary pathogen of harvested strawberries, causing significant economic losses in the strawberry industry. The fungus B. cinerea causes gray mold on fruit and senescent organs, but it can also affect vegetative tissues. Under conditions of rain and high humidity, more than 80% of strawberry flowers and fruits can be lost if the plant material is not protected with appropriate chemical or biological treatment (Ries, 1995).

Methods to reduce or prevent attacks by B. cinerea include cultivation practices that are unfavorable to the pathogen's growth and development. Furthermore, using today's integrated pest management strategy, if necessary, treat before and during flowering with appropriate fungicides/biological preparations to counter pathogen attacks. As strawberry flowers develop, the need for repeated treatments increases to counter the pathogen's development. Repeated treatments increase the risk that the pathogen will develop resistance over time (Edin, 2011).

There are documented fungicides with different mechanisms of action that inhibit fungal growth and prevent them from breaking down plant cells. There are biological preparations based on beneficial fungi, bacteria, and yeasts that break down B. cinerea. There is also evidence that naturally occurring essential oils (Abd-Elkader et al., 2021) help reduce B. cinerea growth (Javanmardi, 2022).

Mold Guard (NOMO Professional, MoldGuard Inc) is a completely organic, 100% biodegradable mold agent that prevents the growth of mold, algae, and fungi for a long time. The product is registered with the Chemicals Inspectorate and the European Chemicals Agency (ECHA). The natural composition of, among other things, Eucalyptus oil (Taghavi et al. 2021) and Tea Tree Oil is considered to have a low negative impact on humans, animals, and the environment.

Dr. Podlipskiy, who developed the product, has been working on solutions for mold problems since the late 90s. He researched naturally occurring compounds with antifungal properties. He found a solution inspired by nature's balance. This led to the creation of various formulas for food, agriculture, and industrial applications.

The formula currently on the market is used for mold remediation in properties. The focus has primarily been on treatment inside and outside properties. Still, tests on infestations in banana and coffee plantations in Honduras and Christmas tree farms in New Zealand have also been carried out, with a visually positive response. Therefore, in this project, the company wants to explore the product's broad range of applications in a perennial crop such as strawberries, which have documented

problems with pathogen infestations, including B. cinerea. Several of the essential oils included in this formula have been tested individually on strawberries (Taghavi et al., 2021).

Strawberries are a crop for which we know the plant's growth and development, the damage caused by the fungus B. Cinerea, and its impact on reduced productivity and increased food waste (Benediktsson, 2022; Persson, Hovmalm, & Nordmark, 2023). However, effective strategies for controlling gray mold remain a concern.

OBJECTIVES

The purpose of the project is to conduct an exploratory, preliminary study to determine whether Mold Guard formula (MF) affects gray mold on strawberries and to develop treatment recommendations to minimize gray mold attacks. The goal is to analyze berries at harvest against quality criteria in line with the EU's quality standards for the fresh produce market and to assess shelf life during short-term storage.

RESEARCH QUESTIONS

1. How effective is Mold Guard's formula against Botrytis cinerea (BC), the causal pathogen of grey mold on strawberries?

2. What concentration is suitable to achieve the intended effect against the attack by BC?

3. Is there an inhibitory effect on the strawberry plant's growth, development, and yield potential?

4. How does the old Guard's formula affect the microbiota on leaves and in the substrate?

5. How does the choice of growing substrate affect the efficiency of Mold Guard's formula?

6. How does Mold Guard's formula affect strawberry quality?

METHOD

The project was carried out in three steps, as described in Figure 1.

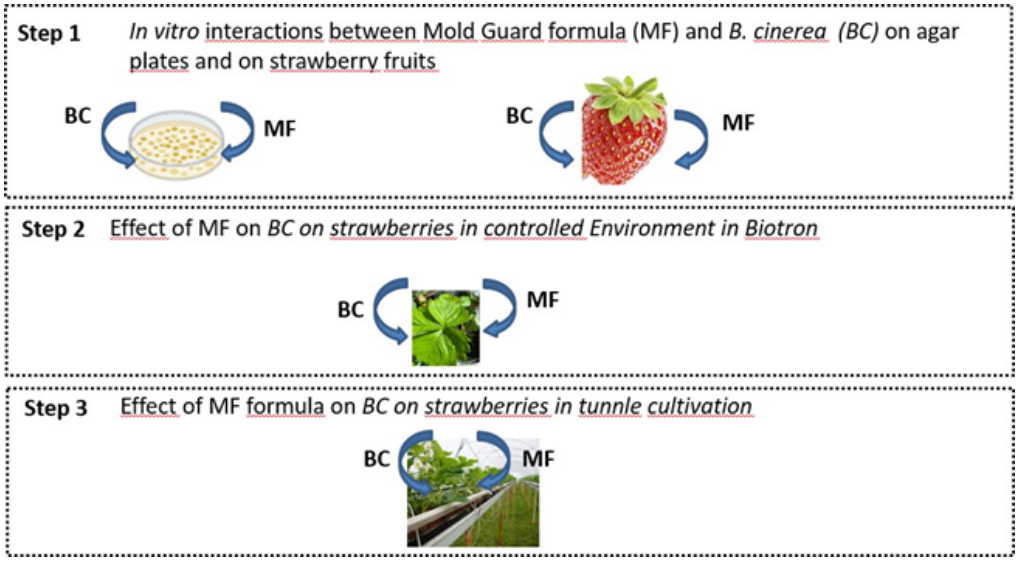

Figure 1. The steps followed in the project performance

RESULT AND DISCUSSION

Step 1

In vitro interactions between MF and BC on agar plates and on strawberry fruits

The first step was performed in the laboratory using a dual-culture test on agar plates and spraying MF onto strawberries, as illustrated in Figure 2. The MF was investigated at the concentration of

1% ,2,5% ,5% ,10% or 25%. In the control treatment, water was used. The MF concentrations were mixed with agar in the dual-culture test, and a piece of BC mycelium was placed in the center of the plate. The mycelial growth was then evaluated as an indicator of pathogen growth.

In the fruit assay, the MF was sprayed on the fruits using the same concentrations as in the dual test. The pathogen suspension was sprayed on the fruits one week after MF application. Infection score following the scale: 0 = 0% infection 1 = <10% infection; 2 = 10-20% infection; 3 = 20-30%

infection; 4 = 30-40% infection; 5 = 40-50% infection; 6 = 50-65% infection; 7 = 65-80% infection; 8

= >80% infection was used. The assessment lasted for two weeks.

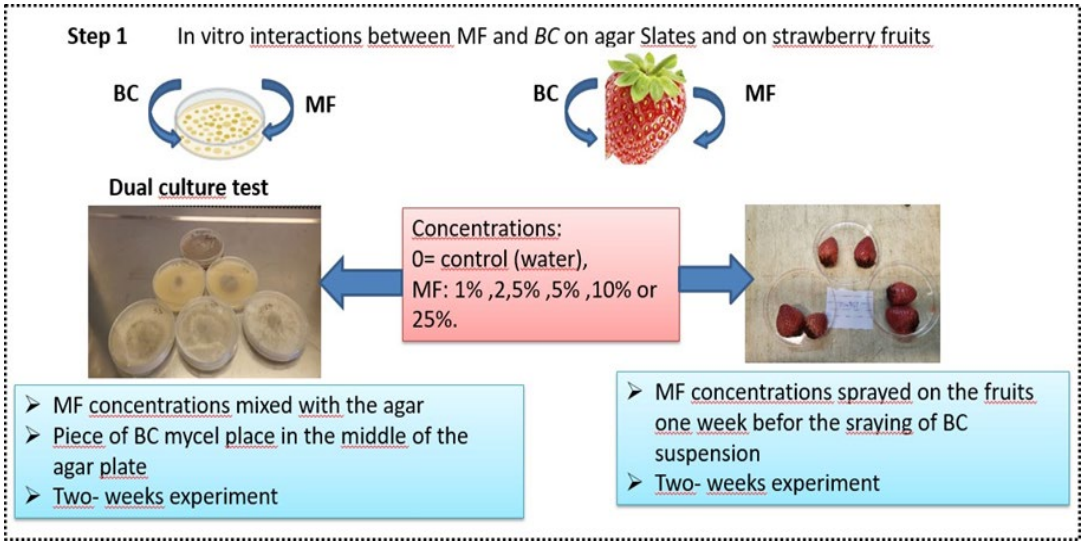

Figure 2. Illustration of in vitro studies of the impact of Mold Guard formula (MF) on the growth of Botrytis cinerea (BC)

In vitro interactions between MF and BC on agar plates and on strawberry fruits. Reduction of mycel growth of BC in the MF concentrations of 10% and 25% (Figure 5). In the fruit assay, the growth of BC was reduced in all the treatments except for the 25% and 5%, which indicated growth on the scale of 1-2.

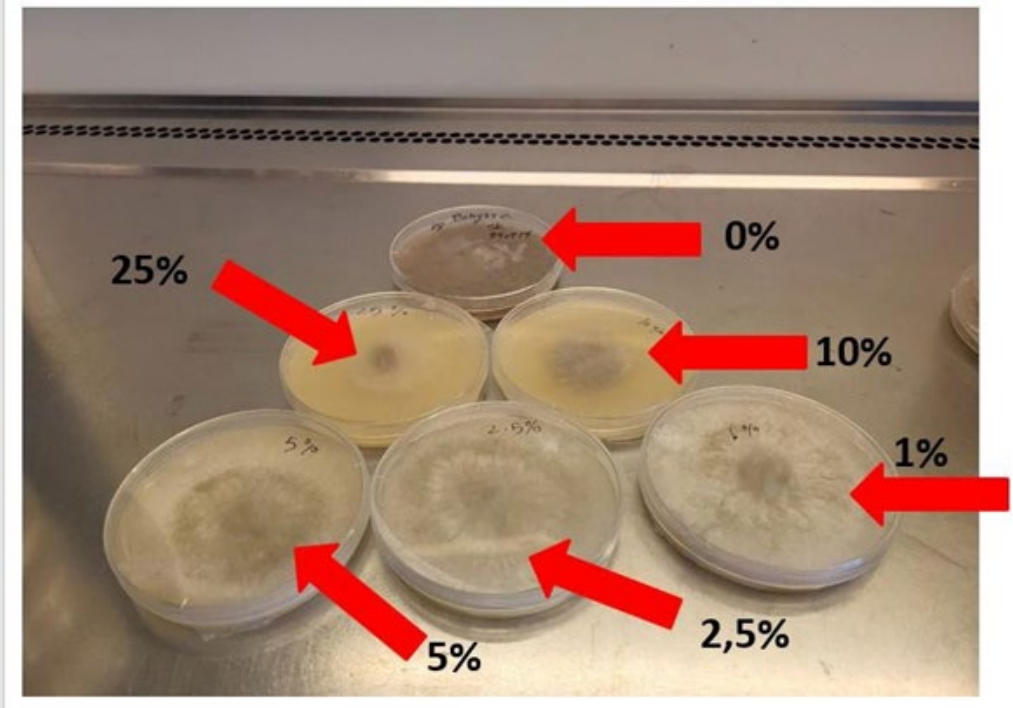

Figure 5. In vitro growth of BC mycel on agar plates mixed with different MF concentrations

Step 2. Effect of MF formula on BC on strawberries in a controlled environment in Biotron

The investigations were carried out in the Biotron using two strawberry cultivars, Favori and Sonata, and peat as the growing medium. We applied two concentrations of the MF, 10% and 25%, to the strawberry plants and flowers twice during the eight-week cultivation period (Figure 3). The pathogen suspension was applied one week after MF application.

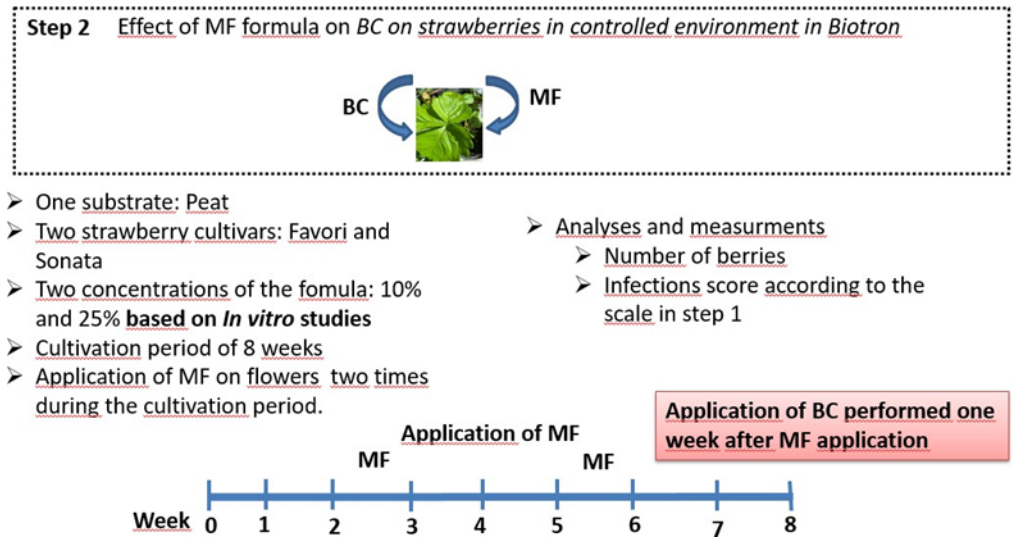

Figure 3. Illustration of the investigations regarding the effect of the MF formula on BC on strawberries in a controlled environment in Biotron

In step 2, the effect of the MF formula on BC on strawberries in a controlled environment in the Biotron. The highest number of fruits was in 10% of MF in both cultivars, Favori and Sonata, at three occasions. Favori had a higher number of fruits at 25% of MF compared with Sonata. Impact of Strawberry cultivars and MF concentration on yield. 10 % concentration of MF showed a positive impact and a reduction of BC growth on strawberry fruits in both cultivars.

Step 3. Effect of MF on BC on strawberries in tunnel cultivation

The investigations were carried out in a tunnel using four strawberry cultivars as described in Figure 4 regarding the methodology and analyses.

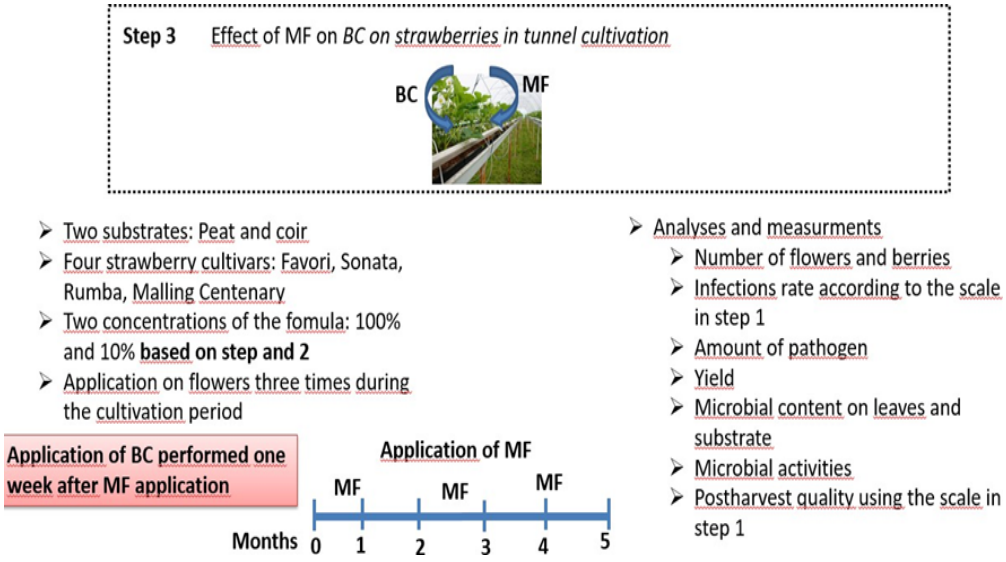

Figure 4. Illustrations of the investigations carried out to study the effect of MF on BC on strawberries in tunnel cultivation

In step 3, the effect of MF on BC on strawberries in tunnel cultivation

The highest yield is in the 10% MF treatments compared to 100% MF. No impact of either cultivar or substrate. Negative impact of pathogen infection on yield. The best reduction in the growth of BC is in the treatment with 10% MF + BC

In post-harvest studies, BC enhanced growth after 2 weeks of shelf life. Furthermore, had both cultivar and substrate impacts on the development of BC growth

CONCLUSIONS

The Mold Guard´s formula is most effective for the reduction of Botrytis cinerea at a concentration of 10% in both in vitro and in vivo assessments.

The formula of 100% concentration showed a reduction in fruit number in the tunnel experiment.

Enhanced Yield could be achieved using 10% concentration

The pathogen infection was affected by the concentration of Mold Guard formula, the choice of substrate, and of cultivar in the tunnel experiment and at postharvest investigations

The 10% concentration of the formula was the most effective in the evaluation of pathogen infection at postharvest of two weeks

All cultivars indicated pathogen growth at postharvest using 100% and also 10% concentration

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