KnE Life Sciences

PGPR 2021
First Asian PGPR Indonesian Chapter International e-Conference 2021
Volume 2022

Research article

Natamycin Treatment for Control of Rhizopus Mold on Strawberries (Fragaria Virginiana)

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Abstract.
Fragaria virginiana (strawberries) were discovered in Garut, West Java, Indonesia, and they have a high economic value in the food industry. However, due to the lack of effective natamycin treatment methods, the problem of postharvest disease caused by *Rhizopus sp*. has yet to be solved. The goal of this study was to examine how different natamycin concentrations affect *Rhizopus* sp. mold control. The dip coating method was used to apply the natamycin to *F. virginiana*. The concentrations of natamycin used were 250 and 500 ppm. During the seven days of storage at 25°C, the total incidence of disease caused by *Rhizopus* sp. and the average weight of *F. virginiana* were observed. The natamycin treatment by dip coating was found to be effective at preserving *F. virginiana* at lower concentrations.

Keywords: Natamycin treatment, Post harvest disease, mold, Strawberies

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Published 07 June 2022

Publishing services provided by Knowledge E

Selection and Peer-review under the responsibility of the PGPR 2021 Conference Committee.

1. Introduction

Fragaria virginiana (Strawberries) originated from Garut, West Java, Indonesia. *F.virginiana* are one of the popular fruits in Indonesia, which have the highest climatic adaptation. *F.virginiana* is commonly known as a fruits with small size shape, thin and easy peeling rind with bright red skin color when ripe [1]. However, *F.virginiana* have a problem about postharvest disease. Postharvest disease cause considerable losses to harvested fruits and vegetables during transportation and storage [2]. One of the problem postharvest disease in citrus is green mould caused by *Rhizopus* sp. wherever it is grown and causes serious losses annually [3].

Rhizopus sp.is identified by the mass of olive-green spores produced on infected fruits and their prolific production ensures that this fungus is found wherever fruit is present, including field, packing house, equipment, degreening and storage rooms, transit containers and in the marketplace [4]. These pathogens occur in almost all

citrus growing regions of the world [5]. These *Rhizopus* sp. species are strict wound pathogens, they are ubiquitous and produce profuse amount of asexual conidia that are readily disseminated by air [6,7]. One of solution to decreasing postharvest disease caused by fungal is antifungal such as natamycin.

The antifungal natamycin is a commonly used organic food additive [8]. Natamycin has board antifungal active against mould and yeast but not bacteria, because polyenes macrolids have ring structures that interact with membrane sterols with high affinity, thus causing the alteration of membrane structure and leading to the leakage of cellular material [9,10]. Natamycin shows activity over a wide pH range (2-7) and effective at low concentrations (as low as 20 mg/l) and it shows no toxic effects even when ingested as high as 500 mg/l [11,12]. The effectiveness of natamycin to control *Rhizopus* sp.with spray treatment was incidence about 40%. Comparable with wrapped treatment on lemons with approximately 5mg/ml was about 40-50%.

However antifungal effect of natamycin to control green mould on artificially inoculated *Rhizopus* sp.has not been evaluated with liquid treatment and storage at 25°C.In this study, antifungal effect of natamycin to control green mould on artificially inoculated *Rhizopus* sp. was evaluated and its impact on decay control and storability were assessed in room storage condition.

2. Methodology

2.1. Preculture plant Pathogen

2.1.1. Pre-incubation for Spore Production

Select a frozen vial (-70 °C glycerol stocks) or freeze dried tube from the fungal strain of concern (the culture must be a monoculture and have the status of a public deposited strain). Prepare suitable mould-agar plates (PDA) depending on preference of the strain. Transfer 100 µl of the culture stock under sterile conditions to the agar plate and disperse the liquid with a sterile spreader. Incubate for 5-14 days at 25°C until sufficient spores (conidia) have formed, continue incubation if more sporulation is needed.

2.2. Harvesting Spores

When spore-formation is confirmed, flood the agar plate with 5 ml sterile Peptone Physological Salt (PPS) + 0,05 % Tween 20. Subsequently, scrape off the fungal spores carefully with a sterile spreader. Collect the spore containing liquid in a sterile jar. Prepare, under sterile conditions, multiple screw capped vials containing 0,5 ml of 20%

glycerol. Disperse 0,5 ml spore containing liquid under continuously shaking to each glycerol vial. Store the vials under frozen conditions (-20°C, preferably -70°C). When frozen, thaw one vial to determine the viable cell count (spore density) of the frozen capped vials.

2.3. Pre-selection Fruits

Fruit selection is done based on the following conditions:

1. fruits are free from visible damage, plagues or diseases before the start of the trial;
2. fruits were not subjected to any postharvest treatment before the start of the trial;
3. preferable organic grown crops are selected to minimize the interference of fungicides added prior to the start of the trial;
4. the selected fruits originate from one single batch. This comprises: same variety, grown on the same plantation, grown at the same time and grown under similar conditions; and
5. to minimize the variation in visual judgment, fruits have a standard size, uniform color and are similar from shape.

2.4. Decontamination of Fruits

Surface-sterilize the fruits in a decontamination bath containing 100-mg/liter sodium hypochlorite solution. Subsequently, rinse the fruits with fresh water. Repeat the rinsing step, if necessary. Finally air dry the samples.

2.5. Treatment Post Harvest Fruit

2.5.1. Wound Inoculation

A cork borer which can be regulated in depth is used to wound the fruits in a uniform manner (standard depth and size). Decontaminate the cork borer before use and with regular time intervals. Wound each fruit once. Perform handling and method in a uniform manner during the complete process of wound infection (practice if necessary). Thaw the spore suspension of the pathogenic fungus under room temperature conditions. Dilute the spore suspension in PPS to obtain a final dilution of 106 CFU/ml. Inoculate each wound directly with 10 µl of spore-suspension using a micropipette. Incubate the fruit samples for 3 hours at room temperature (wound placed upside). Plate the (diluted)

spore suspension on agar medium for confirmation of the total microbial load added to the fruit wounds.

2.6. Fungicide Treatment

Use plastic disposable boxes with a transparent lid for fruit storage. The use of polystyrene foam (preformed) to keep the fruit samples in place is advised. Divide the fruit samples in batches of 40 fruit samples per treatment. Each treatment consists of 5 replicates, considering each treatment a box of 8 fruit samples. The treatment process is done by placing the fruits in an onion bag (or equivalent) and add the bag fruit to a dipping bath (10 ~25 liter bin) containing either one of the treatments mentioned in table 1. Dip the fruits for one minute by gently shaking. Finally, remove the fruits from the container and let it drain for 15 seconds. The complete treatment process is done under ambient conditions. Transfer the fruits to the storage boxes with the wound placed upside and subsequently close all boxes.

2.7. Storage

Storage temperature and moisture content is conform the commercial practice. Store sample boxes in random order to avoid deviations from storage conditions. Keep sample boxes away from direct sunlight. Cover with black foil, if necessary. Monitor temperature during the complete trial (additionally monitor moisture content, if possible). The sample boxes are labelled/coded unrecognizable to the person executing the assessment.

2.8. Assessment post-harvest Fruit

Assessment of the fruits is done by measuring pathogen incidence and severity at regular time interval (depends on the tested fruit and/or fruit contaminant).

3. Result and Discussion

3.1. Effectiveness of Natamycin to control postharvest green mould on F.virginiana

3.1.1. Incidence of Rhizopus sp. on F.virginiana

In this study, the ability of natamycin treatments to protect *F.virginiana* from green mould *Rhizopus* sp. was investigated. Postharvest natamycin application effectively

controlled decay on *F.virginiana*, but the effectiveness of natamycin was dependent on the concentration used. For instance, after days of incubation, the incidence of green mould on *F.virginiana* untreated was 43% at days 4th (Fig. 1).

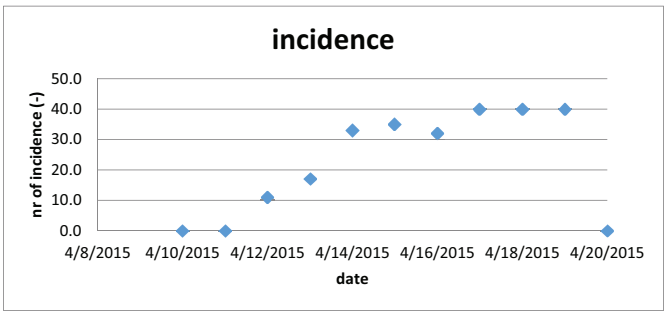
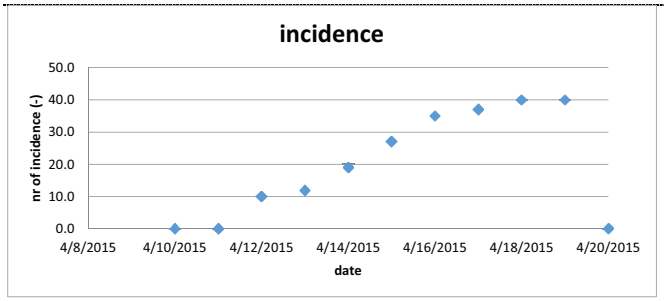
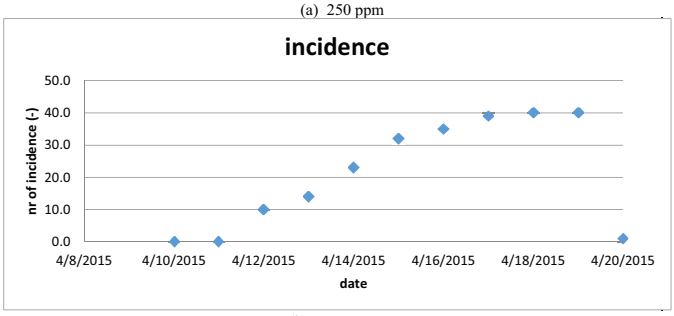


Figure 1: The incidence of *F.virginiana* by *Rhizopus* sp. controlled treatment.

Figure 1 result difference significantly with treated sample with natamycin at 250 ppm was about 30% and 500 ppm was about 35% (Fig. 2). The incidence of *F.virginiana* by *Rhizopus* sp. controlled treatment wider than with natamycin treatment (250 ppm and 500 ppm).



(a) 250 ppm



(b) 500 ppm

Figure 2: The incidence of *F.virginiana* by *Rhizopus* sp. 250 ppm and 500 ppm natamycin treatment.

Figure 2. showed the incidence of *F.virginiana* by *Rhizopus* sp. 250 ppm and 500 ppm natamycin treatment. The result showed that the lower concentration of natamycin has been effective than high concentration. However, in another case concentration of natamycin more effective at 8-10 ppm in vanilla-flavoured yoghurt to reduced yeast growth than lower concentration of 5-7 ppm [13]. Beside that, the times of storage and temperatures used to incubation sample needed about 4 days to reduced *Rhizopus* sp.

was 43% (controlled treatment), 30 and 35% (250 and 500 ppm) at 25C with dip coating method. Compare with [8] experiments result that after 4 weeks of storage at 4C, the incidence of *Rhizopus* sp. mould on lemons with 5 mg/l natamycin spray treatment was reduced by 15% after 14 days of incubation.

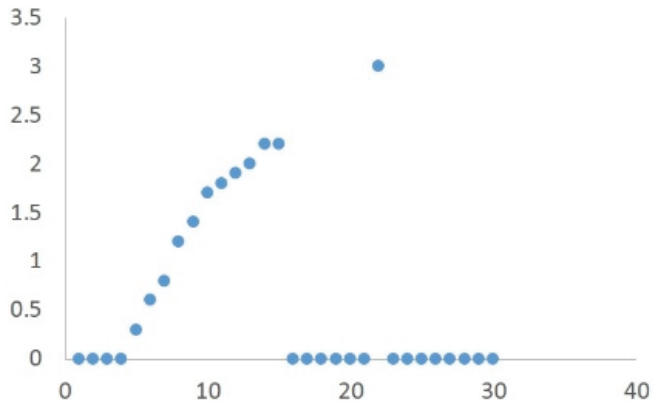


Figure 3: The weight average of *F.virginiana* controlled treatment.

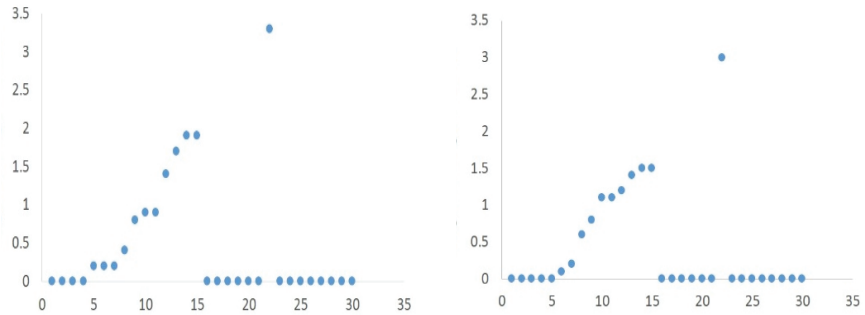


Figure 4: The weighted average of *F.virginiana* by *Rhizopus* sp. 250 ppm and 500 ppm natamycin treatment.

The effectiveness of natamycin to control *Rhizopus* sp. also can seen by weight average on *F.virginiana*. For instance, weighted average of *F.virginiana* at 17 days was 3.0 (controlled treatment) (Fig. 3), 3.0, and 3.1 (250 and 500 ppm of natamycin treatment) (Fig. 4). This results show that controlled treatment and natamycin treatment were similar impact to weighted average of *F.virginiana*.

4. Conclusion

In conclusion, our result highlights show that the natamycin treatment with 250 ppm reduce incidence about 32% *Rhizopus* sp while non treated reduce incidence of green mould on artificially inoculated about 7%. Future research will be aimed a investigating

about effectiveness of natamycin on another fruits such as citrus and another fungal such as *Penicillium digitatum*., *Alternaria alternate* ect.

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