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THE POTENCY OF LYSINIBACILLUS SP. IN CARBON FIBER AND ZINC OXIDE NANOPARTICLES MIXTURE TO SUPPRESS RALSTONIA SOLANACEARUM IN VITRO

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Abstract:
Ralstonia solanacearum is the cause of bacterial wilt on many Solanaceous crops. The antagonistic bacterium can be used to control the pathogen biologically. Lysinibacillus sp. was proven to be able to control R. solanacearum. Carrier materials are needed to formulate the biopesticide besides the active ingredients. The carrier material used in this study was 5% of 80 mesh carbon fiber as the site for the bacteria to attach and enriched with ZnO nanoparticles (ZnO Nps) as one of the plant micronutrients. The study's objective was to determine the concentration of ZnO Nps that is viable for Lysinibacillus sp. and able to suppress the in vitro growth of R. Solanacearum. The antagonism test was carried out using a completely randomized design with 11 treatments and 3 repetitions. The results showed that the largest inhibition zone, 8,30 mm, was caused by the treatment ZLd (ZnO NPs 1000 ppm + Lysinibacillus) without carbon, and 2,12 mm in the treatment of ZLKa ((ZnO NPs 250 ppm + Lysinibacillus + carbon fiber) with carbon.

Keywords: Carbon Fiber, Lysinibacillus Sp., R. Solanacearum, Zno Nanoparticles
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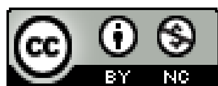

INTRODUCTION

Ralstonia solanacearum, which causes bacterial wilt disease, is widespread in the tropic and sub-tropic regions (James et al., 2003). R. solanacearum has a broad host range and includes nearly 200 plant species in 33 different families. The hosts of R. solanacearum include tomato, chili, eggplant, tobacco, peanut, and many solanaceous weeds (Semangun, 2004). One eco-friendly control that can be applied is to utilize an antagonistic bacterium such as Lysinibacillus. In this study, Lysinibacillus CKU_3 isolate was used. It was isolated from potato that was cultivated in Cikajang, Garut. Lysinibacillus CKU_3 was proven to suppress the development of some diseases, such as soft rot (Istifadah et al., 2016), bacterial wilt (Hersanti, 2016) and damping off (Istifadah et al., 2014).

Ant-antagonistic bacteria can be applied directly, but it has a short shelf life. Therefore, efforts that can be used to maintain the condition of the biocontrol agent remain effective by formulating antagonistic bacteria in a carrier that can support the survival of the biocontrol agent over a more extended time (Nawangsih et al., 2015). The formulation is a mixture of active ingredients, carrier materials and additives. Kloepper & Schroth (1981) stated that the application of live bacteria in plants requires a carrier that can maintain bacterial cell viability and is easy to apply.

The carrier material can increase bacterial stability and shelf-life, protect bacteria against storage environments and provide an initial source of nutrients after application (El-Hassan & Gowen, 2006). In addition, the carrier material can also facilitate the application of biological agents onto plants (Kloepper & Schroth, 1981). In general, some carriers in formulations have many disadvantages, such as biological agents being easily decomposed and only surviving within a short period. Carbon fiber is one of the ingredients that acts as a carrier material. Joni et al. (2009) stated

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that carbon fiber has the potency to be developed as a carrier material because carbon fiber can be attached by nano-sized minerals, which are added to the mixture of biopesticide as fertilizer.

One of the nano-sized minerals that can be added to a biopesticide mixture as fertilizer is ZnO nanoparticles (ZnO Nps). Zinc is one of the nutrients needed by plants. Zinc has a role in the formation of indole acetic acid (IAA), a hormone (auxin) that stimulates plant growth and development, and it is vital for plant physiological balance. ZnO has toxic properties when exposed to light because ZnO produces reactive oxygen species (ROS) and releases Zn2+ ions. Zn2+ ions in low concentrations can function as a nutrient for bacteria, while at a higher concentration as an anti-bacterial (Zhang & Xiong, 2015). Based on the description above, the authors chose the study's title, "The Potency of Lysinibacillus Sp. in Carbon Fiber and Zinc Oxide Nanoparticles Mixture to Suppress Ralstonia Solanacearum In Vitro".

METHODS

The research method through In vitro antagonism test of Lysinibacillus CKU3 in the formulation of Carbon fiber and ZnO Nps against R. Solanacearum and Chitinase Test. The experiment was carried out 7 days after the formulation was mixed. The experiment was arranged in a completely randomized design consisting of 12 treatments and 3 replications. The treatments were (control, carbon fiber, ZnO nanoparticles, ZnO nanoparticles + carbon fiber, ZLa, ZLb, ZLc, ZLd, ZLKa, ZLKb, ZLKc, ZLKd). The antagonism test was begun with spread inoculation of R. solanacearum suspension (107 cfu/ml) on the surface of the N.A. medium. Filter paper discs (0.8 cm in diameter) were saturated with the treatment mixtures (1010 cfu/ml) and allowed to stand overnight. Then they were placed on the N.A. plates that had been inoculated with R. solanacearum, and incubated at 37 °C for 3 x 24 hours. The width of the inhibition zones was measured.

The experiment was arranged in a completely randomized design consisting of 9 treatments and 3 replications. The treatments were (control, ZLa, ZLb, ZLc, ZLd, ZLKa, ZLKb, ZLKc, ZLKd). The Chitinase test uses a disk method in the colloidal chitin agar medium and filter paper. The Chitinase test was carried out after the formulation was mixed. Filter paper discs (0.8 cm in diameter) that were dropped 25 µl/disc of formulation were placed on the surface of the colloidal chitin agar and incubated at room temperature for 5-7 x 24 hours. The width of the inhibition zones was measured.

RESULT AND DISCUSSION

The results of the study showed the ability of the Lysinibacilus CKU3 in the carbon fiber and ZnO N.P.s mixture to suppress the growth of R. Solanacearum was indicated by the inhibition zones around the filter paper discs on the medium (Figure 1, Table 1).

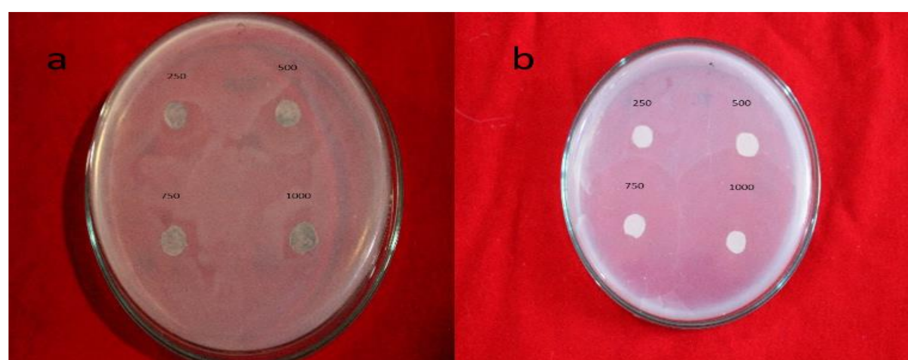

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Figure 1. The inhibition zones. (a) Mixture of *Lysinibacillus* CKU_3, carbon fiber and ZnO N.P.s (250 ppm, 500 ppm, 750 ppm) 24 hours after application, (b) Mixture of *Lysinibacillus* CKU_3 and ZnO N.P.s (250 ppm, 500 ppm, 750 ppm, 1000 ppm) 96 hours after application.

The data in Table 1. shows that all treatments inhibited the development of the pathogen R. solanacearum except the treatment of ZnO NPs 250 ppm, ZnO NPS 250 ppm + carbon fiber, ZLa (ZnO NPs 250 ppm + Lysinibacillus CKU3), and ZLb (ZnO NPs 500 ppm + Lysinibacillus CKU3). Single treatment of ZnO NPs 250 ppm did not cause any inhibition zone. It indicates that ZnO N.P.s can not inhibit the in vitro growth of R. solanacearum. At the same time, a single treatment of carbon fiber caused the inhibition of R. solanacearum with an inhibition zone of 3.35 mm. Carbon fiber is suspected of having anti-bacterial properties both against pathogenic bacteria as well as antagonistic bacteria. The results also showed that the treatment of a mixture of ZnO N.P.s + carbon fiber + Lysinibacillus CKU3 formed the inhibition zones 24 hours after application. However, the width of the inhibition zone did not increase over time. The width of inhibition zones caused by treatment of Ly bacillus CKU3+ ZnO N.P.s increased daily. The mixture of ZnO N.P.s and carbon fiber did not show any inhibition, likely with the addition of ZnO nanoparticles on carbon fiber causing carbon fiber to be inactive and inhibiting the growth of R. solanacearum.

Table 1. Width of the inhibition zones on 7 days after incubation.

No.	Treatments	Inhibition Zone Width (mm)
1.	Control (*Lysinibacillus* CKU_3)	7,95 b
2.	Carbon fiber	3,35 ab
3.	ZnO Nanoparticles	0 a
4.	ZnO Nanoparticles + carbon fiber	0 a
5.	ZLa (ZnO NPs 250 ppm + *Lysinibacillus*)	0 a
6.	ZLb (ZnO NPs 500 ppm + *Lysinibacillus*)	0 a
7.	ZLc (ZnO NPs 750 ppm + *Lysinibacillus*)	4,71 ab
8.	ZLd (ZnO NPs 1000 ppm + *Lysinibacillus*)	8,30 b
9.	ZLKa (ZnO NPs 250 ppm + *Lysinibacillus* + carbon fiber)	2,12 ab
10.	ZLKb (ZnO NPs 500 ppm + *Lysinibacillus* + carbon fiber)	1,40 ab
11.	ZLKc (ZnO NPs 750 ppm + *Lysinibacillus* + carbon fiber)	0,69 a
12.	ZLKd (ZnO NPs 1000 ppm + *Lysinibacillus* + carbon fiber)	0,52 a

* The numbers followed by the same letters in the column are not significantly different according to the Duncan Multiple Range Test at 5%

Source: Author, 2022

The results showed that the treatment that produces the highest inhibition zone was ZLd (ZnO NPs 1000 ppm + Lysinibacillus), which was 8.30 mm in the treatment without carbon, and ZLKa (ZnO NPs 250 ppm + Lysinibacillus + carbon fiber) which was 2,12 mm in the treatment with carbon. The inhibition zone in the treatment with carbon fiber produced a smaller inhibition zone. However, it was formed faster, i.e., 24 hours after application, while the inhibition zone in the treatment without carbon was formed longer, i.e., at 96 hours (4 days) after treatment. The inhibition zone can be seen in Figure 1. The time difference in the formation of inhibition zones between the treatment of the mixture of ZnO N.P.s + Lysinibacillus and the mixture of ZnO N.P.s + Lysinibacillus + carbon fiber may be caused by the utilization of carbon by the bacteria for their growth. Therefore, the presence of carbon fibers might increase the ability of Lysinibacillus to produce an inhibition zone faster than the treatment without the carbon fiber.

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Lysinibacillus CKU3 is an endophytic bacterium that can degrade chitin (Figure 2). The chitinolytic activity was shown by the formation of clear zones around the Lysinibacillus CKU3 colonies that could be seen at 96-120 hours after the bacteria were cultured and incubated on a medium containing colloidal chitin as a carbon source. Tsujibo et al. (1999) stated that the chitinase enzyme produced by bacteria degrades chitin into chitobiose which will be transformed into a source of carbon (energy) and nitrogen.

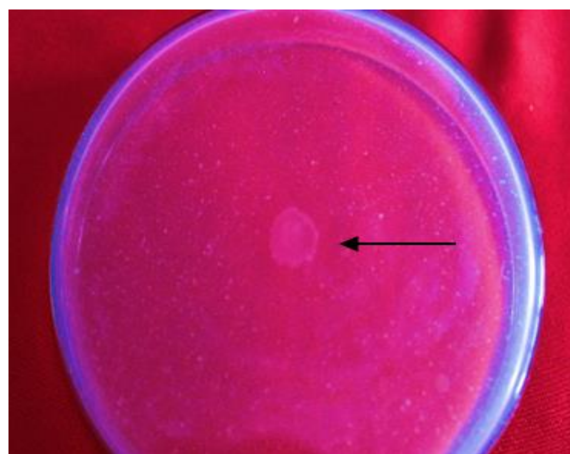

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Figure 2. Clear zone caused by Lysinibacillus in the colloidal chitin medium.

Brzezinska & Donderski (2001) stated that each bacterium could degrade chitin differently, depending on several factors such as pH, temperature, incubation time, and substrate. The speed of diffusion and the type of chitinolytic enzyme excreted by bacteria into the medium influence the speed of formation of clear zones (Apriani, 2008). The chitin degradation by the chitinase enzyme produces GlcNAc, which is at the beginning of fermentation used by bacteria for its life process. Brzezinska & Donderski (2000) stated that GlcNAc is a source of carbon and nitrogen for bacterial growth. The ability of Lysinibacillus CKU3 to degrade chitin in the different treatment mixtures can be seen in Table 2. Lysinibacillus CKU3 mixed in ZnO N.P.s in various concentrations did not reduce its ability to degrade chitin, except for 500 ppm ZnO which the width of the clear zone in colloidal chitin-containing media was slightly narrower compared to other ZnO concentration treatments.

However, the ability of Lysinibacillus CKU3 to degrade chitin was lost when the mixture was added with carbon fiber. No clear zone was detected on the colloidal chitin medium on the treatment of Lysinibacillus CKU3 in all the concentrations of ZnO N.P.s, and carbon fiber tested. The loss of the ability of Lysinibacillus CKU3 to degrade chitin is thought to be related to the presence of carbon fiber. The 5% carbon fiber added to the mixture of Lysinibacillus CKU3 and ZnO N.P.s became a carbon source and was utilized by Lysinibacillus CKU3 so that the bacteria did not degrade the chitin contained in the medium. Allegedly Lysinibacillus CKU3 preferred to use carbon fiber, which was available earlier on the filter paper disc, rather than chitin which was available later on the medium. Another possibility was that the mixture of ZnO N.P.s and 5% carbon fiber was not supportive to be used as a biopesticide formulation with active ingredient Lysinibacillus CKU3 because the chitinolytic activity of Lysinibacillus CKU3 was lost in the mixture.

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Table 2. The Clear Zone Width Formed in the Chitinase Test

No.	Treatments	Clear Zone Width (mm)
1.	Control (Lysinibacillus CKU3)	3,018 c
2.	ZLa (ZnO Nps 250 ppm + Lysinibacillus CKU3)	3,075 c
3.	ZLb (ZnO Nps 500 ppm + Lysinibacillus CKU3)	2,095 b
4.	ZLc (ZnO Nps 750 ppm + Lysinibacillus CKU3)	3,125 c
5.	ZLd (ZnO Nps 1000 ppm + Lysinibacillus CKU3)	2,929 c
6.	ZLKa (ZnO Nps 250 ppm + Lysinibacillus CKU3 + carbon fiber)	0 a
7.	ZLKb (ZnO Nps 500 ppm + Lysinibacillus CKU3 + carbon fiber)	0 a
8.	ZLKc (ZnO Nps 750 ppm + Lysinibacillus CKU3 + carbon fiber)	0 a
9.	ZLKd (ZnO Nps 1000 ppm + Lysinibacillus CKU3+ carbon fiber)	0 a

* The numbers followed by the same letters in the column are not significantly different according to the Duncan Multiple Range Test at 5%

Source: Author, 2022

Aside from the loss of the ability of Lysinibacillus CKU3 to degrade chitin, the mixture of ZnO N.P.s and carbon fiber also reduced the antagonistic capability of Lysinibacillus CKU3, as indicated by the smaller size of the inhibition zone in all mixed concentrations of ZnO N.P.s + carbon fiber (Table 2). The antagonistic activity was suspected between ZnO N.P.s and carbon fiber. In a previous study in which Lysinibacillus CKU3 mixed with silica N.P.s and carbon fiber, the inhibition was more significant when compared with Lysinibacillus CKU3 + nano silica observed at 24 hours after application (Pawestri, 2017).

CONCLUSION

Conclusions from the results of the experiment, it can be concluded that: 1) Lysinibacillus CKU3 in the 5% carbon fiber and ZnO N.P.s formulation showed different suppression periods. The inhibition zones in the treatment with carbon were formed 24 hours after application, while the inhibition zones in the treatments without carbon were formed 96 hours after treatment; 2) The largest inhibition zone (8.30 mm) in the treatment without carbon was caused by the treatment ZnO NPs 1000 ppm + Lysinibacillus CKU3, and ZnO NPs 250 ppm + Lysinibacillus sp. + Carbon fiber 5% (2.12 mm) in treatment with carbon.

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