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**Root-Knot Nematodes (*Meloidogyne* spp.) Prevention on
Tomato Plants by Utilizing Rhizosphere Bacteria for
Sustainable Agriculture**

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Research Highlights

Root-Knot Nematodes are one of roots damaging nematodes. Their existence leads to decrease in tomatoes productivity in Indonesia as much as 46.2%. Currently, the pest and damage prevention is focused on biological control to attain the sustainability in agriculture. Utilizing of rhizosphere bacteria is an alternative for this purpose by both direct mechanism and indirect mechanism through induced resistance. This research is proposed to obtain the effective rhizosphere bacteria as an agent of tomato plants resistance induction on nematodes and observe its influence on the growth and productivity of the tomato plants. The methods conducted are exploration, isolation, selection, and laboratory scale experiments. Exploration in five tomato planting regions provided 189 isolates. The further laboratory scale selection resulted in 18 candidates of bacteria having the potency in growth and superiority in antagonist, and nine appropriate consortium isolates were obtained in the next compatibility test. On the final test in the field, it was concluded that P7 consortium isolate consisting of (LM24 and CK212), P52 (LM38+CN26+CK27+CK212) simply suppressed the presence of nematodes more over P40 (LM24 + CN26) solely provided the proper growth, whereas P31 (LM24+CN26+CK27) was not only able to inhibit nematodes but also provide the satisfying growth and productivity.

Graphical Abstract

Table 1. Consortium isolates and the corresponding codes

No	Consortium isolate combination	Code
1	(LM24+CN26+CK27+CK212)	P2
2	(LM24+CK212)	P7
3	(LM38)	P10
4	(LM38+CK27)	P12
5	(LM38+CN26+CK27)	P22
6	(LM24+LM38+CW222+CK212)	P28
7	(LM24+CN26+CK27)	P31
8	(LM24+CN26)	P40
9	(LM38+CN26+CK27+CK212)	P52
10	Control	K

Figure 1. The treatment effect of various rizhosphere bacteria consortiums induction on the plant height at 10th week after plantation, K = control.

Figure 2. The treatment effect of various rizhosphere bacteria consortiums induction on the number of leaves at 10th week after plantation, K = control.

Figure 3. The treatment effect of various rizhosphere bacteria consortiums induction on the weight of tomato harvest, K = control.

Figure. 4 The effect of various rizhosphere bacteria consortiums induction on the number of unit gall on roots, K = control.

Figure. 5 The effect of various rizhosphere bacteria consortiums induction on the number of unit II larvae *Meloidogyne* spp. on roots, K = control.

Table 2. Reduction percentage of larvae II in tomato roots due to rizhosphere bacteria induction

Treatments	Average number of II larvae units in 100 mL of soil	Larvae II repression (%)
P2	1019.44	1.56
P7	589.89	43.04
P10	960.67	7.23
P12	781.44	24.54
P22	648.44	37.38
P28	746.11	27.95
P31	566.22	45.32
P40	671.56	35.20
P52	557.56	46.16
Control	1035.56	-

Figure 6.Biplot analysis of the rizhosphere bacteria

Note: P1 - P10 = treatments; Ttan = the plant height; JD = number of leaves; JG = number of gall; JLII = number of larvae II; H = Harvest; I – VI = vector positions

Research Objectives

This research is proposed to obtain the effective consortium rizhosphere bacteria as an induction agent for improving the resistance to *Meloidogyne spp.* and provide information about its influence on growth and productivity of tomato plant

Methodology

This research was conducted in the field according to design of experiment by the applying of randomize design. There were 189 rizhosphere bacteria isolates from tomato plants at plantation center in West Java. Further assay provided 18 isolates having potency in inhibit *Meloidogyne spp* and increase the growth of the tomato plants. From those there were only six isolates having high compatibility in the subsequent test. There were eight consortium

variations from the previous result, one single isolate, and one control in the treatments. The experimental data were taken nine times.

Nematodes (*Meloidogyne spp*) inoculum preparation

The nematode was isolated from 6 – 12 days old *Meloidogyne spp*-infected tomato roots. The roots were cut in one centimeter and then treated with 0.5% (v/v) NaOCl solution. The mixture was ground by using mortar and pestle, and left for four minutes. NaOCl solution containing mashed roots was filtered by using stage filtration with the filter size 75µm, 50 µm, and 35 µm, respectively. Eggs and II-larvae in the filter were rinsed with aquadest, and collected in particular storage for further multiplication. The multiplication was conducted according to method proposed by Huettel (1985) by using carrots as media with the addition of nematode supplements. The carrots were peeled and cut in 3 cm width. It was sterilized by soaking in 1.5% NaOCl solution for 15 min, and then rinsed by sterile aquadest. The carrot pieces were placed at sterile petri disk, after that the eggs and larvae were added by using sterile pipette and incubated in 27°C for two months. It was then used as inoculum source.

Research Location in the field test

The field test were conducted in Dusun Pangkalan Desa Margajaya Kecamatan Tanjungsari, the location has altitude at 850 m above sea level, Andisol type of soil, and C type of rainfall (rather wet) according to Schmidt and Ferguson (1951). The application field in this experiment was never planted with tomato plants or other *solanaceae* class plants before. Therefore there is no root-knot nematodes found. Soil assessment was performed in order to determine the existence of the nematodes in the field. Composite soil was taken in 10 cm depth in each of five certain spot in the field as much as 100 g. The number of nematode in the soil samples were calculated according to modified Corong Bearmann method.

Treatments

The tomato seed comes from Ratna cultivars. In initial step, it had been immersed in each bacterial consortium of for 24h. This step was purposed to attach the bacteria to the seed surface, therefore it could penetrate into the seed tissue, and the systemic induced resistance would occur due to its presence. The next step, the seed was planted in polybag nursery containing sterile soil. After four leaves emerged the plant was then placed in targeted field. Tomato planting in the field was conducted when the seed was at 2 weeks old after nursery. Inoculation of *Meloidogyne spp* was performed at the same time by adding the II-larvae suspension into the holes surrounding the plant roots, and there were 3000 II-larvae in each hole. After that the roots would be covered by soil and watered by a little water.

Data analysis

The data would be analyzed by Analysis of Variance (ANOVA) followed by Duncan test at 5% level of significance. Biplot analysis was performed in order to determine the relation between plant growth and root-knot nematode attack by using Microsoft®Excel

2007/XLSTAT Version 2009.3.02. The maximum distance from center point in determination of general identifier character influenced by the treatment was estimated by Hotelling test (Johnson and Wichern, 2014) according the Eq. 1:

$$r_i \pm \sqrt{\frac{p(n-1)}{(n-p)}} F_{(\alpha, p, n-p)} \sqrt{\frac{S^2_{PC_1}}{n}} \quad [1]$$

Where:

- r_1 = ellipse radius for PC
- p = used PC component
- n = Number of the tested treatment
- $(\alpha, p, n-p)$ = F distribution at 5% probability with db p and $n-p$
- $S^2_{PC_1}$ = variance for PC_1 score.

Results

Every treatment influenced the growth and induced resistance of tomato plants to reduce root-knot disease caused by nematode. At the tenth week after plantation (WAP), all of induction treatments provided higher height rather than the control. The highest plant was 126.67 cm with P31 induction and the increase was about 32.56% compared to the control. At that moment, the same treatment provided the highest number of leaves at 21.11 and the increase was calculated at 34.72% more than the control.

The bacteria are able to increase the growth through synthesis of the growth hormone i.e. IAA, in addition to phosphate solubilization and nitrogen fixation. The treatments significantly ($p = 0.05$) increased the growth of tomato plants due to the synergistic mechanism attributed to the presence of bacteria. It was resulted that the treatments increased the mass of the harvest significantly ($p = 0.05$) at the end of plantation period. The highest weight was obtained from P31 treatment and measured to be 1036.44 g. The treatment provided 46.21% more mass, whereas others in the range of 766.78 – 919.22 g, or with the increase as much as 8.17 – 29.67% than the control.

Several studies reported that the biological agent in the form of bacterial consortiums provided better growth and productivity of the plants. There was an evident that bacterial consortiums led to more preferable productivity in rice and sunflower plants than individual bacterium treatment (Fitri & Gofar, 2009). It was reported that a number of microorganism isolated from rizhosphere were able to increase the wet and dry mass of cucumber plant (Estrada et al, 2004). The presence of rizhosphere bacteria led to increase the plant growth rate since the ability to provide nutrients and assist the plant in its absorption in addition to

synthesis and transform various phytohormones as growth enhancer (Kloepper, 2003; Timmusk & Wagner, 2004).

The utilization of rhizosphere bacteria as inducer and growth enhancer is an effective method because it is non toxic on plants, and antagonist to pathogens, as well as providing the plant with high resistance to the diseases during plantation period. During this utilization, the bacteria consortium produces specific compound i.e. growth hormones, and nutrients therefore it roles as both biofertilizer and bioprotectant which supports the development of sustainable agriculture.

The observation in number of gall and II-larva after induction provided significantly ($p = 0.05$) vary results compare to the control. The treatment P31 resulted in the lowest number of gall on roots at 38.1 units, and the second lowest was in P52 at 41 units with percentage of decrease were calculated about 49.48 and 45.65% less than control respectively. In the same time, both treatments provided much lower number of II-larva as much as 566.22 and 557.56 units with the decrease were calculated about 45.32 and 46.16% for P31 and P52 respectively.

Every treatment inhibited the presence of -larvae II with range of repressing about 1.56 – 46.16 %. From the results it was suggested that the bacteria consortiums were able to repress and inhibit the presence of pathogen organisms. One of the proposed mechanisms was by generating antibiosis i.e. bulbiformin, a very toxic compound to the plant pathogens (Arwiyanto, 1997)

Biplot analysis provides the total variation as much as 99.9% due to the relation between parameters to the treatments. The first biplot component (PC1) contributes the variation at 95.3% and PC2 at 4.6%. In biplot visualization, harvest result has the longest parameter vector line that corresponds to the highest variation, the next are number of larvae II, gall, leaves, and height of the plants.

The general characteristic parameters included in the ellipse zone are height, number of leaves, and number of gall, with the treatments are P7, P10, and P40. Whereas the specific characteristic parameter includes harvest result and number of larvae II, with P2, P12, P22, P28, P31, P52, and control as the treatments. These calculated results conclude that only harvest result and number of larvae II are able to differentiate between the treatments applied.

It is pointed that the optimum treatments are placed on the corners out of ellipse including P2, P12, P22, P28, P31, and control. Additionally, the specific characteristics as responses for each treatment are classified into several sector as follow:

1. Sector I, consisting treatments P22, P31, and P40 provided the optimum harvest results.
2. Sector II and III, there was no treatment influencing any parameter.

3. Sector IV, consisting P28, P52, and control had low ability to repress the number of II larvae.
4. Sector V, P7 had low ability to repress the number of gall.
5. Sector VI, consisting P2, P10, and P12 provided favorable effects height, and number of leaves parameters.

Findings

1. It was emphasized that P7, P52 consortiums only had the potency in repressing *Meloidogyne* spp, whereas P40 only resulted in proper growth. While P31 led to optimum parameters in *Meloidogyne* spp repression, growth, and harvest result.
2. Biplot analysis provided that sector I was recommended as bioprotectant due to negative relation on II larva repression. Sector IV was recommended as biofertilizer because it indicated the optimum growth.

References

- Arwiyanto T. 1997. Pengendalian hayati penyakit layu bakteri tembakau: isolasi bakteri antagonis. Jurnal perlindungan tanaman Indonesia, 3(1), 54-60.
- Estrada, J. D., Rossi, M. S., Andreas, J. A., Rovera, M., Correa, N. S., dan Rosas, S. B. (2004): Greenhouse evaluation of *Pseudomonas aurantica* formulated as inoculation for the biocontrol of plant pathogen fungi, cited from website: <http://www.ag.anburu.edu/argentina/pdfmanuscripts/estrada.pdf>. Downloaded at October 12th 2016.
- Fitri, S. N. A., Gofar, N. (2009): Increasing of rice yield by using growth promoting endophytic bacteria from swamp land. Journal tropical soil, 15(3), 271-276.
- Huettel R.N. 1985. Carrot disc Culture. In : Zukermant B.M., Mai W.F. and Harrison, Editor. Plant Nematology Laboratory Manual. Massachusetts. The University of Massachusetts Agricultural Experiment Station. Hal. 153-154.
- Johnson, R., and D. Wichern. 2014. Applied Multivariate Statistical Analysis. Sixth. Pearson Education, Inc., Edinburgh.
- Kloepper, J. W. (2003): A review of mechanisms for plant growth promoting by PGPR. Six international workshop on plant growth promoting rhizobacteria, Calcut, India, October 5-10, 2003.
- Schmidt, F. H., dan Ferguson, J. H. (1951): Rainfall types based on wet and dry period, ratio for Indonesia with Western New Guinea. Verhandelingen.

Timmusk, S., dan Wagner, E. G. H. (2004): The plant growth promoting rhizobacterium *Paenibacillus polymyxa* induces changes in *Arabidopsis thaliana* gene expression- a possible connection between biotic and abiotic stress respons,cited from website: <http://www.ag.auburn.edu/argentina/pdfmanuscript/timmmusk.pdf>. Downloaded at October 28th2015.