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Endophytic Bacteria from Wet Soil of South Kalimantan as Biological Control Agent for Root Nematodes (NPA) in Celery (*Apium graveolens*)

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ABSTRACT

Endophytic bacteria are microorganisms that are associated with plants, don't cause disease, and mutualism associated with plants. One of the uses of endophytic bacteria in agriculture is as biological agent in overcoming the attack of the root-knot nematode Meloidogyne sp. It is a polyphagous parasite that can attack plants by forming galls on the roots of celery plants (A. graveolens), can lack nutrients, and in chronic conditions causes death of host plants. It's control is important to reduce crop yield loss. Control of the nematode *Meloidogyne* sp., at this time use many chemical pesticides because they quickly kill nematodes, but the continuous use of chemical nematicides will potentially pollute the environment. So, it is necessary to study alternative control using endophytic bacteria. The purpose of this study was to determine the potential of endophytic bacteria from wetlands of South Kalimantan as biological agents of it in celery plants. This research was conducted in Sumber Glagah, Pacet, Mojokerto, using Completely Randomized Design (CRD). The treatment was carried out by immersing the roots of celery plants in a suspension of endophytic bacteria (24 hours) and concentration (108 cfu/ml), then planted in polybags measuring 30 x 30. The polybags were filled with soil that had been divested with 50 juvenile Meloidogyne sp. Observation parameters were the number of galls per g of roots and juvenile population per g of root and per 10 g of soil. The results showed that the treatment of endophytic bacteria reduce: (1) number gall per g celery roots was 19.67–31.00 and control (46.67); (2) population juvenile population per g root was 9.33-25.67 and control 50.67; (3) juvenile population per 10 g of soil was 11.33-17.00 and control 29.33.

Keywords: Corruption Endophytic bacteria, wet soil of South Kalimantan, NPA, celery

Introduction

Celery (*Apium graveolens* L.) is a vegetable that is widely used as food and herbal medicine. This is because celery contains provitamin A, vitamin B, glutamine, choline and fatty acids (danolaeic and palmitate) (Hidayat & Napitupulu, 2015). In Indonesia, celery lives in the highlands because the ideal air temperature for celery plants is 15°C-24°C. The development of celery production in Indonesia cannot be separated from cultivation techniques and pest and disease control. One of the diseases that can reduce the production of celery plants is root cholecystitis caused by the root-knot nematode Meloidogyne sp. this nematode belongs to the type of obligate parasite and is distributed in the tropics and subtropics. Rapid development and high resistance make this nematode species capable of attacking many plants. Nematodes can attack plants by forming galls on the roots of celery plants (A. graveolens), can lack nutrients, and in chronic conditions causes death of host plants. It's control is important to reduce crop yield loss.

Nematode control currently still uses pesticides that are toxic and have a high negative impact on the environment. This triggers the emergence of the concept of alternative control methods that are safer and more efficient, namely by utilizing the potential of microorganisms as biological control

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agents. One of them is endophytic bacteria. It is suspected that endophytic bacteria are bacteria that are beneficial to plants and associate in plant tissues. According to Kurniawati et al. (2020) the role of endophytic bacteria in plant tissues is very important, namely as a tool for plant growth promotion and inducing plant resistance by producing antibiotics and antimicrobial compounds.

South Kalimantan is an area with a fairly wide wetlands. Wetland conditions are thought to have a positive influence on microbes such as endophytic bacteria and fungi that live in plant tissues. Because the types of plants such as trees to plants such as rice can grow well in wetlands. This triggers the suspicion that microbes such as endophytic bacteria can play a good role in plants. Several uses of endophytic bacteria have been reported to control root-knot nematode attacks. According to Khan et al. (2012) reported that endophytic bacteria species Pseudomonas sp. strains CD 38 and CD 62 were able to inhibit egg hatching and gall formation from the nematodes Xiphinema americanum, Hipolaimus indicus and *M. incigonita* on mung bean plants. According to Munif and Harmi (2011) reported that the use of endophytic bacteria on pepper plants was able and effective in suppressing the number of root cavities and the population of *M. incigonita* by 90%. The purpose of this study was to determine the potential of endophytic bacteria from wetlands of South Kalimantan as biological agents of it in celery plants.

Material and Methods

Research location

This research was conducted in January-March 2022 in Sumber Glagah, Pacet, Mojokerto. Obervations on nematode at the Plant Phytopathology Laboratory, Faculty of Agriculture, UPN Veteran East Java.

Experimental design

This study use a Completely Randomized Design (CRD) with 9 isolates of endophytic bacteria that had been tested for biosafety throught hypersensitivity testing and 1 control (without using endophytic bacteria). The treatment was carried out by immersing the roots of celery plants in a suspension of endophytic bacteria (24 hours) and concentration (10^8 cfu/ml), then planted in polybags measuring 30 x 30. The polybags were filled with soil that had been divested with 50 juvenile *Meloidogyne* sp. Observation parameters were the number of galls per g of roots and juvenile population per g of root and per 10 g of soil. Observations were made after the celery plant entered the generative phase. Calculation of the number of galls is carried out after the harvesting process by counting the number of galls using a hand counter. According to Pangaribuan and Liestiany (2020), the root gall index uses a scale of 0 -10 with the following categories:

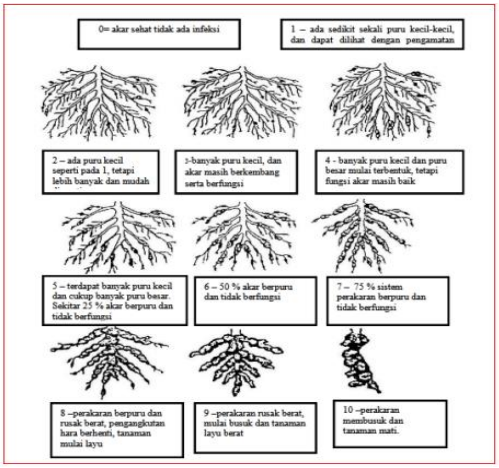

Figure 1. Root gall index

The nematode population analysis was carried out by extraction-isolation of a modified Baerman funnel. The nematode population was observed at the end of the study by counting the number of larvae in 10 ml from the extraction of the Baerman funnel soil and root samples. Nematode population calculation formula (Thomas et al., 2005)

P=n/ (10 ml extracted)

Information:
P = Nematode population
n = Number of nematodes found

Sampling, and extraction and isolation of Nematodes (Meloidogyne sp.)

Exploration of root-knot nematodes (*Meloidogyne* sp.) was carried out by taking root and soil samples on celery plantations in Bumiaji District, Batu City. Soil sampling was carried out in the root area of symptomatic plants of about 300 grams of soil. Sampling of roots was carried out by Purposive sampling on plants that have symptoms of root-knot nematode attack such as wilted plants and yellow leaves, stunted plants and gall formation or swelling of plant roots. Extraction of root-knot nematodes in root and soil samples was carried out using the modified Baerman Funnel extraction-isolation method by taking juvenile nematode 2. Then the nematode extraction results were filtered using 100 mesh and 250 mesh nematode sieves.

Propagation of endophytic bacterial

Isolates Endophytic bacteria were explored and isolated from the roots of the water Lakum plant (*Ludwigia octovalvis* (Jacq.) P.H.Raven) in the wetland paddy field in Marabahan Barito Kuala, South Kalimantan. The propagation of endophytic bacteria was carried out on Nutrient Agar (NA). Endophytic bacteria in abundance are bacteria that have been tested to be safe and have the potential as biological control agents for plants using a hypersensitivity test, namely the isolate code BLR 1.2, BLR 1.3, BLR 2.1, BLR 2.2, BLR 2.3, BLR 3.2, AKL 2.1, AKL 2.2, AKL 3.1 for 24 hours.

Data analysis

The data obtained from each test were analyzed for variance at the 95% confidence level. Significantly different results were further tested with Duncan's test at 5% level using Excel 2010.

Results and Discussion

Number of gall

The number of galls per gram of root in control was 46.67 and the lowest value was in the AKL 2.1 treatment, which was 19.67 per gram root (Table 1). Overall, the treatment with endophytic bacteria proved to be able to reduce the number of galls per gram of root.

Tabel 1. Number of galls/gram root in celery plant

No	Treatment (Endophytic Bacteria)	Number of galls/gram
1	Control	46,67 d
2	BLR 1.2	28,33 c
3	BLR 1.3	26,00 bc
4	BLR 2.1	28,00 c
5	BLR 2.2	31,00 c
6	BLR 2.3	28,67 c
7	BLR 3.2	27,00 c

To be continued…

8	AKL 2.1	19,67 a
9	AKL 2.2	20,67 ab
10	AKL 3.1	29,00 c

Note: Numbers in the same column followed by the same letter indicate that there is no significant difference in the DMRT test at the 95% confidence stick.

The number of galls is related to the nematode population. the more nematode population the higher the number of galls. According to Himawan et al. (2018), the roots of host plants experience swelling of different sizes due to the presence of female nematodes, eggs and larvae in the roots. The population density of plant parasite nematodes in the soil around the roots greatly affects the level of damage to plant roots (Wibowo, 2015).

Population of Juvenil- II nematode Meloidogyne sp. in Soil and Roots

The population of juvenile II in the highest root tissue was found in the treatment of endophytic bacteria with treatment without biological agents or control with a value of 50.67/g, while the treatment had low value was obtained in the isolate AKL 2.1 was 9.33/g (Table 2). The observation of juvenile II population in root tissue was positively correlated with the number of galls formed, the more root cavities formed, the higher the number of juvenile II populations. The development of the nematode population is influenced by the growth and development of the host. Mechanism of endophytic bacteria to plant roots is thought to result in interactions between plants and bacteria as well as plants and nematodes. Bacteria induce plants by producing secondary metabolites such as chitinase and protease that play an important role in controlling nematodes. According to Siddiqui and Shaukat (2005) and Tian et al. (2007) the production of protease by bacteria is one of the bacterial mechanisms as controlling agents for the nematode *Meloidogyne* sp.

Tabel 2. The population of Juvenile-II *Meloidogyne* sp. on roots and soil

No	isolate code	Population J-II/g Roots	Population J-II/10 gr Soil
1	Kontrol	50,67 f	29,33 d
2	BLR 1.2	19,00 cde	17,00 bc
3	BLR 1.3	14,67 abc	14,67 abc
4	BLR 2.1	25,67 e	17,67 c
5	BLR 2.2	19,33 cde	13,33 abc
6	BLR 2.3	18,00 cd	16,00 bc
7	BLR 3.2	24,67 de	16,67 bc
8	AKL 2.1	9,33 a	11,33 a
9	AKL 2.2	11,00 ab	12,33 ab
10	AKL 3.1	24,33 de	15,00 abc

Note: Numbers in the same column followed by the same letter indicate that there is no significant difference in the DMRT test at the 95% confidence stick.

The juvenile II population of the nematode *Meloidogyne* sp on the soil showed significantly different results in the treatment of endophytic bacteria with the treatment of plants that were not given biological agents or controls. The highest juvenile-II population in the soil was 29.33% in the control treatment and the lowest juvenile-II population was 11.33 in the treatment of endophytic bacteria with AKL isolate code 2.1. In juvenile phase II, nematode are very effective in causing attacks on plants because the larvae move to the roots to penetrate the stylet in order to enter the plant root tissue and absorb plant nutrients. According to Nugrohorini (2011) states that in optimal environmental conditions will support growth so that juvenile II larvae emerge and move in the soil to the tips of growing roots. In this case, giving endophytic bacteria to plants makes it difficult for

nematodes to breed. According to Pangaribuan and Liestiany (2020) resistant hosts will inhibit reproduction even though the initial population is quite high. Giving endophytic bacteria to the roots of celery plants makes celery plants resistant to nematode attacks. The presence of HCN compounds which are secondary metabolites produced by bacteria play an important role in suppressing the development of nematodes. The availability of nutrients that are not good enough for the development of nematodes causes the sex ratio to vary. The more limited the availability of nutrients, the male nematode population was more than the female nematode. This is because female nematodes need more nutrients to reproduce than male nematodes. This condition is one of the efforts to maintain his life by increasing the number of male reproductive females can be suppressed so that food is available even though it is limited. Unfavorable environmental influences (lack of food supply and extreme temperatures) can play a role in the development of male nematodes.

Conclusion

The results showed that the treatment of endophytic bacteria reduce: (1) number gall per g celery roots was 19.67–31.00 and control (46.67), (2) population juvenile population per g root was 9.33-25.67 and control 50.67, (3) juvenile population per 10 g of soil was 11.33-17.00 and control 29.33.

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