Biocontrol of *Meloidogyne* sp. on Tomato Plants by Selected *Bacillus* spp.

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Abstract. *Meloidogyne* spp. are recognize as the most economically important worldwide. It is difficult to control *Meloidogyne* spp., because they have wide range of hosts. Due to the high toxicity of chemical nematicides, it is necessary to develop new control strategies. Our previous research showed that some *Bacillus* spp. effective as biocontrol agents of plant pests and diseases. They have multifunction, such as plant growth promoter, phosphate dissolving bacteria, etc. The aim of this research was to obtain the selected *Bacillus* spp. to control *Meloidogyne* spp., to enhance growth and yield of tomato. The treatments were 8 strains of *Bacillus* spp., Carbofuran, *Meloidogyne* spp. inoculated tomato and control. The *Bacillus* spp. have inoculated as seed treatment and seedling treatment before transplanting. The *Meloidogyne* spp. have inoculated on 4 weeks tomato plants. The parameters were disease development, multiplication of *Meloidogyne* spp., growth and yield of tomato. The results showed that *Bacillus* spp. reduced the number of galls, egg masses, eggs per egg mass and nematode per 300 g soil compared to the inoculated control, but the nematicide treatments was more effective to control *Meloidogyne* spp. Conversely, three strains of *Bacillus* spp. showed to enhance the growth and yield of tomato than Carbofuran.

Keywords: Multifunction; Inoculated; Carbofuran; Treatment; Transplanting

1. Introduction

*Meloidogyne* spp. are sedentary endoparasites and are among the most destructive agricultural pests. Species of *Meloidogyne* are aggressive to a wide range of crops [1], particularly vegetable crops, and cause dramatic yield losses, mostly in tropical and sub-tropical agriculture [2]. In Indonesia, root-knot disease in tomato caused by *Meloidogyne* spp. are one of an important disease, the losses in carrot 15-95 % at Cipanas, West Java [3], the losses in tomato ranged from 24-38% [4]. Our observation at Batu Bagiriaik Village, Lembah Gumanti District, Solok Region, West Sumatera showed the high infestation of *Meloidogyne* spp., the gall index on tomato root samples were between 2.5-3.4 (gall index 0-4 according Ozdemir *et al.*, 2018) and the number of nematode in the soil of tomato rhizosphere were 5.3-17.6 nematodes/300 g soil. It is very difficult to grow important vegetables such as tomato in tropical or semi-tropical soil infested with root-knot nematodes [5].

Because agrochemicals have poisonous effects on human health and the environment, alternative control methods are needed. Several attempts have been made to utilize biological control of root-knot nematodes. The development of biological control agents is also considered an effective alternative for nematode control on vegetables [6]. Biological control is considered to encompass control that results
from the action of soil microorganisms and the soil microfauna and is mediated through mechanisms such as parasitism, predation, competition and antibiosis [7]. Another strategy used for the biological control of nematodes is based on the introduction of bacteria colonizing the rhizosphere of the host plant or so called rhizobacteria. These microorganisms that can grow in the rhizosphere also provide front line defense for roots against pathogen attack and are considered ideal for use as biocontrol agent [8]. Rhizosphere bacteria have the ability to colonize plant roots [9] and they also have positive effects on plant growth as direct and indirect mechanisms [10]. They have been named plant growth promoting rhizobacteria (PGPR) [11] or plant health promoting rhizobacteria (PHPR) by [12].

Out of numerous plant growth-promoting rhizobacteria (PGPR) genera, Bacillus spp. have a considerable effect as biocontrol agents and root colonization, multiple modes of action [13], and promising ability to sporulate under stressed conditions [14]. Bacillus strains are known as plant growth-promoting bacteria (PGPB). These bacteria use direct mechanisms, such as phosphate solubilization, nitrogen fixation, and phytohormones and siderophores production, or indirect mechanisms, such as induction of systemic resistance to pathogens, production of antibiotic and lytic enzyme, inhibition of plant ethylene synthesis, or competence by nutrients or niche [15; 16; 17], quorum quenching [18]. They also have multiple function to control many plant diseases cause by virus, bacteria, fungi, nematode [13] and insect pest [19].

Bacillus spp. as biocontrol agent for different plant pathogens have been reported, such as B. amyloliquefaciens BLB369 efficiently protected wheat against Fusarium graminearum [20]. Five strains of B. subtilis: B46, B209, B211, B298 and B315 inhibit the growth of Colletotrichum capsici and C. gloeosporioides [21]. B. megarhizium TRS 4 showed the antagonist against Fomes lamaeensis, Poria hypobrumea, Sphaerostilbe repens, and Sclerotium rolfsii [22]. Six strains of Bacillus reduced the Fusarial wilt disease on tomato: B. thuringiensis strain R1.2.AB.1.1, B. cereus strain R1.2.AB.2.1, B. cereus strain R1.2.AB.2.2, strain RBKDA2.2, B. subtilis strain R1.1.BP1.2.3, Bacillus sp strain RBKDA1.2 [23]. The other 6 strain of Bacillus reduced the bacterial wilt disease in chili: B. subtilis strain RZ.2.1.AP3, Bacillus sp strain RZ.2.1.AP1, Bacillus sp strain RZ.2.2.AG2, B. cereus strain RZ.2.1.AG1, B. cereus strain RZ.2.1.AP1, B. subtilis strain RZ.1.1.AP1 [24]. B. subtilis strain RZ.2.1AP3 reduced anthracnose disease by C. capsici on chili [25].

To control nematode have been reported by many authors, the rate of M. incognita on Vigna mungo was significantly reduced by P. fluorescens and B. subtilis [26]. This reduction was reflected by increasing plant growth parameters as indicated by certain plant growth criteria and number of bacterial nodules per plant compared to the untreated control. Also, P. fluorescens and B. subtilis inhibited the number of galls by 42.79 galls per plant. B. pumilus and B. mycoides reduced the number of gall 333 % and egg mass 39 % on coffee roots cause by M. incognita [27]). The aim of this experiment was to obtain the selected Bacillus spp. to control Meloidogyne spp., to enhance growth and yield of tomato.

2. Methods

The research methode was designed as complete randomized, it consisted of 11 treatments and 6 replicates The treatments were the inoculation of 8 strains of Bacillus as seed treatment and seedling treatments (B. cereus strain R12A2B.1; B. cereus strain R12A2B.2; B. cereus strain RBKDA2.2; B. mycoides strain IR.1.3.4; B. subtilis strain R111BP1.23; Bacillus sp. strain RBKDA1.2; Bacillus sp. strain RZ21AP1; Bacillus sp. strain RZ22AG2; Carbofuran 10 % as nematicide treatment, Meloidogyne spp. inoculated tomato and water control.

2.1. Preparation of the root-knot nematode inocula

The roots of tomatoes naturally infected with the root-knot nematodes were collected from open fields at Batu Bagiriah village, Lembah Gumanty District Solok Regency, West Sumatera Indonesia. Multiplication of nematodes as follow: the egg masses were collected from tomato roots. Five egg masses were inoculated on 4 weeks old tomato cv. Warani in a greenhouse. Tomato roots showing galls and egg masses were collected 45 days post inoculation (dpi) for nematode egg extraction (Figure 1). Egg masses of infected roots were extracted by using Baermann funnel (modified by Winarto). The funnel is placed in a suitable support and almost filled with tap water. Plant material containing
nematodes is chopped into small pieces of about 1 cm length, placed in a square of nylon gauze, which is folded to enclose the material, and then gently submerged in the water in the funnel. Nematodes emerge from the tissues and sink to the bottom of the funnel stem. After 24–48 h, fully open the clamp to rapidly withdraw 5–10 ml of water containing the nematodes and transfer it to a shallow viewing dish for examination [28]. The inoculum concentration was adjusted with the aid of a Peters chamber to 1.000 eggs mL⁻¹.

2.2. Preparation of Bacillus spp. inocula.
Bacillus spp. from culture collection were grown on Nutrient Agar and incubated for 3 days. One colony was added to 25 mL of NB in culture flasks (50 mL) and incubated in rotary shaker for 24 hrs (preculture). For mainculture, 1 mL of preculture was transferred to 150 mL of sterile coconut water in Erlenmeyer flask and incubated for 2 x 24 hrs [29]. Bacterial suspensions were diluted with sterilized water and compared to McFarland scale 8 (bacterial density estimated 10⁸ cell/mL) [30].

2.3. Inoculation of Bacillus spp.
Germination rate test of tomato seeds were done by common methods using paper [31]. The Bacillus spp. from main-culture were applied as seed treatment, soaked for 15 minutes and shade-dried for 30 minutes then sowed with 2 seeds/pot at pot tray. Seedings and planting were all use the agronomical practices recommend for tomato in Indonesia.

2.4. Inoculation of Meloidogyne spp.
Meloidogyne spp. were inoculated on 4 weeks old tomato after transplanting. The nematode eggs were applied at the rate of 1000 eggs/pot using soil drench method. The plants were up-rooted after 45 and 60 dpi then number of nematodes in soil, root galls, egg masses, number of egg/egg masses and gall index were determined. Gall index was determined based on infection rate 1, 2, 3, and 4 [32].

2.5. Growth and yield of tomato
The role of Bacillus spp. in the improvement of the plant growth parameters, viz. plant height (centimeter), number of leaves/plant, flowering stage (days after transplanting, DAT), fruit yield (grams/plant), was recorded 5 months after transplanting.

2.6. Statistical analysis of data
All data obtained from pots experiments were analyzed using analysis of variance (ANOVA). The significant differences among treatments were determined according to the least significant differences (LSD) at P < 0.05 level of probability, using the CoStat software.

3. Results and Discussion

3.1. Development of Meloidogyne spp. on tomato roots
The results showed that all strains of *Bacillus* and Carbofuran introduced as seed treatment and a root dip, reduced the number of number of *Meloidogyne* spp. in soil, egg masses, and number eggs/egg masses of *Meloidogyne* spp. of on tomato roots significantly (Table 1). Nematicide (Carbofuran 10 %) reduced the number of nematodes in the soil (100%), number of egg masses/plant (100 %), and the number of eggs/eggmass (100 %) compared with the controls. However, significant differences were observed between the *Bacillus* strains, they does not control *Meloidogyne* spp. to the same degree. It was found three strains as the most effective to control *Meloidogyne* spp., they were *B. cereus* strain RBIKDA2.2, *B. cereus* strain RB12AB2.2 and *B. subtilis* strain RBIBPL2.3.

### Table 1. Development of *Meloidogyne* spp. on root of *Bacillus* inoculated tomato at 6 weeks after inoculation.

| *Bacillus* spp. strains | Number of nematode* | Number of egg mass | Number of egg/ eggmass |
|-------------------------|----------------------|--------------------|------------------------|
|                         | Reduced (%)          | Reduced (%)        | Reduced (%)            |
| *Bacillus* sp. strain   |                      |                    |                        |
| RZ2.2AG2                | 17.67                | 49.52              | 13.33b                 |
| *B. cereus* strain      |                      |                    |                        |
| RB12AB2.1               | 23.67                | 32.38              | 14.00b                 |
| RB12AB2.2               | 15.67                | 55.24              | 11.00b                 |
| *Bacillus* sp. strain   |                      |                    |                        |
| RZ2.1AP1                | 16.33                | 53.33              | 12.00b                 |
| *B. thuringiensis* strain|                      |                    |                        |
| RB12AB1.1               | 18.00                | 48.57              | 10.33b                 |
| *B. subtilis* strain    |                      |                    |                        |
| RBIBPL 2.3              | 24.00                | 31.43              | 10.00bc                |
| *Bacillus* sp. strain   |                      |                    |                        |
| RBIKDA1.2               | 20.00                | 42.86              | 14.67c                 |
| *B. cereus* strain      |                      |                    |                        |
| RBIKDA 2.2              | 14.33                | 59.05              | 11.67b                 |
| Nematicide              | 0.00                 | 100.00             | 0.00c                  |
| Water control,          |                      |                    |                        |
| *Meloidogyne* spp.      |                      |                    |                        |
| infection               | 35.00                | 0.00               | 25.00a                 |

Values within a column followed by the same letter are not significantly different according to Least Significant Difference ($p$ $\geq$ 0.05). *Number of nematode/300 g soil

This result showed that *Bacillus* strains more effective to control *Meloidogyne* spp., compare than other biocontrol agent, such as *Hypocrea lixisii* (*Trichoderma harzianum*) the $10^7$ cfu/mL solution produced the greatest reduction in the number of galls/plant (49.0), the number of egg masses/plant (32.8), and the number of eggs/eggmass (399.8) compared with the controls [31]. The high suppressive effect of the bacteria against *Meloidogyne incognita* may be attributed to the distinctive properties for these genera. As concerning as *B. thuringiensis* it is known that this bacteria produce chitinolytic enzymes, i.e., chitinases which is responsible for degrading chitin in cell walls of the nematode eggs also in nematode eggmasses [33]. The results of the present findings fall in line with the report of suppression of phytonematodes including *Meloidogyne* spp. in different crops due to the application of *Bacillus* spp as made by earlier workers [34; 35; 36; 37]. The relationship between number of gall and egg mass of *Meloidogyne* spp. on root of *Bacillus* strains inoculated tomato at 45 dpi showed that generally the high number of gall followed also the high number of egg mass, especially on water control treated roots. On root of *Bacillus* strains inoculated tomato showed no significant different between number of gall and
egg mass of *Meloidogyne* spp. On nematicide treated root showed very low number gall without egg mass (Figure 2).

![Figure 2. The relationship between number of gall and egg mass of *Meloidogyne* spp. on root of *Bacillus* strains inoculated tomato at 45 dpi](image)

The development of gall, gall index and infection rate of *Meloidogyne* spp. reduced on root of *Bacillus* strains inoculated tomato at 45 dpi and 60 dpi (Table 2). The performance of gall after different treatments on tomato roots showed also different gall index (Figure 3). Nematicide showed the lower gall index (1) at 45 dpi and 60 dpi. Four strains of *Bacillus* spp. showed the same rate of gall index (2) at 45 dpi and 60 dpi, although the number of gall increased on tomato roots. They were *Bacillus* sp. strain RZ2.2AG2, *Bacillus* sp. strain RZ2.1AP1, *B. thuringiensis* strain RBI2AB1.1 and *Bacillus* sp. strain RBIKDA1.2.

![Figure 3. Gall performance cause by *Meloidogyne* spp. on *Bacillus* spp. inoculated tomato roots at 45 dpi. a) water control (score 0), b) control, *Meloidogyne* spp. inoculation (score 3), c) nematicide (Carbofuran 10 %) (1), d) *B. subtilis* strain RBIBPL2.3 treatment (score 2).](image)

**Table 2.** The development of gall, gall index and infection rate of *Meloidogyne* spp. reduced on root of *Bacillus* strains inoculated tomato (45 and 60 dpi).

|        | 45 dpi | 60 dpi |
|--------|--------|--------|
| Gall number |        |        |
| Number of egg mass |        |        |
| Pi | 5 | 5 |
| 10 | 10 |
| 20 | 20 |
| 30 | 30 |
| 40 | 40 |
| 50 | 50 |
| 60 | 60 |
| 70 | 70 |
| 80 | 80 |

![Figure 3. Gall performance cause by *Meloidogyne* spp. on *Bacillus* spp. inoculated tomato roots at 45 dpi. a) water control (score 0), b) control, *Meloidogyne* spp. inoculation (score 3), c) nematicide (Carbofuran 10 %) (1), d) *B. subtilis* strain RBIBPL2.3 treatment (score 2).](image)
It is evident that the suppression of root knot nematode population/incidence followed by the application of *Bacillus* spp. as biocontrol agent resulted in increase in terms of plant height, earlier generative phase and yield of tomato. In the present study the most effective isolate of *B. subtilis* strain RBIBPL 2.3 against *Meloidogyne* spp. also improved the plant growth characters viz. height (43.98 %), accelerate the generative phase (13.22 %), and fruit yield (206.89 %) compare than control plant (Tabel 3). The nematicide as the best control for *Meloidogyne* spp., but the treated plant showed also improve the plant growth and yield, but only at medium range viz. height (31.12 %), accelerate the generative phase (9.50 %), and fruit yield (127.58 %) compare than control plant. The other *Bacillus* strain, *B. cereus* strain RB12AB2.1 showed the effectivity to control *Meloidogyne* spp. in medium range (Tab. 1 and Tab. 2), but this strain could improve the growth and yield of tomato viz. height (58.50 %), accelerate the generative phase (10.74 %), and fruit yield (213.79 %) compare than control plant. This due to the ability of this strain to increase tolerance of tomato against *Meloidogyne* spp., or disease tolerance [39].

### Table 3. The growth and yields of Bacillus strains inoculated tomato

| Bacillus spp. strains | Plant height | Generative phase | Yield |
|-----------------------|--------------|------------------|-------|
|                       | cm | Enhance (%) | Days | Accelerate (%) | g/plant | Tons/ha | Enhance (%) |
| *Bacillus* sp. strain | 57.00 ab | 41.90 | 33.00 bc | 18.18 | 223.33abc | 14.89 | 131.03 |

3.2. Plant growth and yield
| Strain Type | Strain Code | Temperature 1 | Temperature 2 | Temperature 3 | Temperature 4 |
|-------------|-------------|---------------|---------------|---------------|---------------|
| B. cereus  | RBI2AB2.1   | 63.66 a       | 58.50         | 36.00 abc     | 10.74         |
|             |             |               |               |               | 303.33 a      | 20.24         | 213.79        |
| B. cereus  | RBI2AB2.2   | 42.83 bc      | 6.63          | 32.00 c       | 20.66         |
|             |             |               |               |               | 166.67bcd     | 11.08         | 72.41         |
| Bacillus   | RZ2.1AP1    | 46.83 bc      | 16.59         | 35.66abc      | 11.73         |
|             |             |               |               |               | 120.00cd      | 8.01          | 24.13         |
| B. thuringiensis | RBI2AB1.1 | 54.83abc      | 36.51         | 36.66abc      | 9.09          |
|             |             |               |               |               | 226.67abc     | 15.09         | 134.48        |
| B. subtilis| RBIBPL 2.3  | 57.83 ab      | 43.98         | 35.00 bc      | 13.22         |
|             |             |               |               |               | 296.67a       | 19.77         | 206.89        |
| Bacillus   | RBIKDA1.2   | 55.33 bc      | 37.75         | 34.66 bc      | 14.04         |
|             |             |               |               |               | 143.33 cd     | 9.55          | 48.27         |
| B. cereus  | RBIKDA 2.2  | 50.66 bc      | 26.14         | 32.67 bc      | 9.09          |
|             |             |               |               |               | 273.33ab      | 18.23         | 182.75        |
| Nematicide |             | 52.66abc      | 31.12         | 36.50abc      | 9.50          |
|             |             |               |               |               | 220.00abc     | 14.69         | 127.58        |
| Meloidogyne spp. inoculation | 40.16 c | 0.00          | 40.33 a       | 0.00          |
|             |             |               |               |               | 96.66 d       | 6.41          | 0.00          |
| Water control | 51.50 bc | 28.21         | 38.00 ab      | 5.78          |
|             |             |               |               |               | 190.00abc     | 12.69         | 96.55         |

Values within a column followed by the same letter are not significantly different according to Least significant Difference (p = 0.05).

4. Conclusion

In conclusion, biocontrol agents can be used as an alternative approach of the chemical nematicides for controlling various nematodes. B. cereus strain RBI2AB2.1 and B. subtilis strain RBIBPL 2.3 are recommended for the management of root-knot nematode cause by Meloidogyne spp. However, further investigations are required to determine the formulation that can achieve the best results.

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