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Research Article
Role of Indigenous Rhizosphere Bacteria in Suppressing Root-knot Nematode and Improve Plant Growth Tomato

Rika Alfianny, I. Nyoman Pugeg Aryantha and Tati Suryati Syamsudin

School of Life Sciences and Technology, Institute Teknologi Bandung, Jalan Ganesa 10, Bandung 40132, Indonesia

Abstract
Background: Root-knot nematode caused by *Meloidogyne* spp., is a significant disease in tomato plants in Indonesia, causing yield loss up to 46.2%. The use of rhizosphere bacteria is one alternative method for controlling *Meloidogyne* spp. The mechanism is brought into action, either directly (antagonist) or indirectly through induced systemic resistance. In the tomato central production area of West Java, tomato cultivation is quite intensive in using fertilizers and pesticides. Different method of cultivation will cause differences in characteristics of the local rhizosphere bacteria. **Objective:** The aim of this study is to find out the indigenous rhizosphere bacteria which are able to control root-knot nematodes and to improve growth of tomato. **Materials and Methods:** The experiment was conducted in an agriculture experimental station using a split-root method with 57 treatments of rhizosphere bacteria in the form of single isolates or consortium. **Results:** The consortia of five bacteria could increase of 37.6% in plant height, 58% number of leaves, 100% in the number of bunches, 37.1% in the number of flowers and 30.9% in the yield of tomato fruits. Three consortia belongs to three isolates bacteria and one single isolates decreased in the number of gall and larvae II by 74.8 and 85.6%, respectively. **Conclusion:** The five indigenous rhizosphere bacteria consortium capable to control root-knot nematodes and four indigenous rhizosphere bacteria consortium could improve the growth of tomatoes.

Key words: Indigenous rhizosphere bacteria, *Meloidogyne* spp., tomato

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Corresponding Author: Tati Suryati Syamsudin, School of Life Sciences and Technology, Institute Teknologi Bandung, Jalan Ganesa 10, Bandung 40132, Indonesia

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tomato plants (*Lycopersicum esculentum* Mill) are one of Indonesia's horticultural commodities that have high economic value. However, tomato production is still low (17.6 t ha^{-1}) compare to other country's[1]. One of the causes of low tomato production in Indonesia is root-knot nematode disease due to *Meloidogyne* spp. This disease can reduce yields up to 46.2%[2,3] and an annual yield loss estimated about $ 215.7 billion[4].

Meloidogyne spp., attacks and infects plant root causing the development of a form of gall as a result of abnormal cell growth, which disturbs the absorption and transportation of nutrients. Affected plants will show nutrient deficiency symptoms, such as stunted growth and yellowing and wilted leaves[5].

Various attempts to controls *Meloidogyne* spp. by means of physical, chemical and biological treatment have been made in Indonesia. However up to now, it has not been possible to resolve the problem of nematode growth in tomatoes. In addition, the use of nematicide by tomato growers often cause further problems, such as negative impacts to the environment and human health. Thus, it is necessary to find safe alternative in controlling nematode disease in tomato.

The development of *Meloidogyne incognita* could suppress by using rhizosphere bacteria such as *Paenibacillus polymyxa* NFB7, *Bacillus megaterium* and *B. circulans*[6]. The use of rhizosphere bacteria such as *Pseudomonas* sp. and *Bacillus* sp. for controlling nematodes has been done on commodities such as gerbera, beans, cucumbers, potatoes, okra and tomatoes for controlling nematodes kidney (*Rotylenchulus reniformis*), root knot nematodes (*Meloidogyne* spp.), cyst nematode (*Globodera pallida*)[7-9].

The role of rhizosphere bacteria as growth regulator (biostimulant) capable of being and provides helps the absorption of nutrients by plants (biofertilizer) and suppresses the disease development (bioprotectant) were classified as Plant Growth Promoting Rhizobacteria (PGPR)[10]. The PGPR mechanisms against pathogens may be direct as an antagonist through the competition of space, substrate, production of toxic compounds such as siderophores, hydrogen cyanide (HCN), antibiotics[11] and indirect interaction through induction of systemic resistance[12]. It has been reported that mixtures of PGPR strains either in a two-way or three-way combination gave a greater protection, compared to single-strain treatments of cucumber angular leaf spot disease caused by *Pseudomonas syringae* pv. *lachrymans* under field conditions[13].

West Java is the largest supplier of tomatoes; cultivation system in tomato central production area is very intensive using fertilizers and pesticides. The effect will respond by different character of rhizosphere bacteria differently from one location to another. The indigenous rhizosphere bacteria have more chances to succeed in controlling the diseases because they are more adaptable to the environment, it has potential to control root-knot nematode.

This study aims to find out the indigenous rhizosphere bacteria which capable in controlling root-knot nematodes and improve the growth performance of tomatoes plant.

MATERIALS AND METHODS

The study was conducted in an agriculture experimental station using a randomized block design. A total of 189 single isolates were found from tomato root surface at Central Production West Java. Eighteen isolates had been tested for their potential as PGPR and six isolates were found to be potential. These six isolates were evaluated further as single and consortium consisting of 56 treatments: 6 treatments single isolates, 15 treatments of consortium from two isolates bacteria, 20 treatments of consortium from three isolates bacteria and 15 treatments of consortium from four isolates bacteria and no treatment as controls (Table 1).

Propagation of nematode inoculum (*Meloidogyne* spp.): Infected tomato roots by *Meloidogyne* spp. (age from 6-12 weeks after planting) in the form of gall were collected and cut into 1 cm length, washed with aquadest and sterilized by 0.5% NaOCl solution. The root was then pulverized using a mortar and pestle and set aside for 4 min. The root juice was then filtered with three different size of sieve i.e., 75, 50 and 35 µm. The sieves were rinsed, eggs and larvae II then collected. Propagation of the eggs and larvae II was done using the Huettel Method[14]. The eggs and larva II were infested in to the Petri dish containing carrot pieces and incubated at 27°C for two months and then used as a source of inoculum.

Test of indigenous rhizosphere bacteria as Induced Systemic Resistance (ISR): An ISR test was conducted using the split-root system[15] in an agriculture experimental station. For one treatment, three plastic pots were set for split root method by placing one pot above the other two pots (Fig. 1) The upper pot was perforated to facilitate split root. Each pot was filled by 3 kg of sterile soil. Tomato seedling (age 2 week) was planted in each pot. After 2 weeks the bottom pot was

Fig. 1: ISR test on tomato plants using the split-root method

Table 1: Indigenous bacterial isolates use for PGPR treatment

No.	Treatment	No.	Treatment
1	*Bacillus* sp.+*Bacterium* sp.	30	*Bacillus* sp.+*Bacillus thuringiensis*+*Ochrobactrum* sp.+*Bacillus cereus*
2	*Bacillus* sp.+*Ochrobactrum* sp.+*Bacillus cereus*+*Staphylococcus sciuri*	31	*Bacillus* sp.+*Ochrobactrum* sp.+*Bacillus cereus*
3	*Bacillus* sp.+*Bacterium* sp.+*Bacillus thuringiensis*+ *Ochrobactrum* sp.	32	*Bacterium* sp.+*Bacillus thuringiensis*+*Bacillus cereus*+*Staphylococcus sciuri*
4	*Bacterium* sp.+*Ochrobactrum* sp.	33	*Ochrobactrum* sp.
5	*Bacillus* sp.+*Ochrobactrum* sp.	34	*Bacillus* sp.+*Bacterium* sp.+*Bacillus cereus*+*Staphylococcus sciuri*
6	*Bacillus thuringiensis*+*Ochrobactrum* sp.	35	*Bacillus* sp.+*Bacillus thuringiensis*+*Ochrobactrum* sp.
7	*Bacillus* sp.+*Staphylococcus sciuri*	36	*Bacterium* sp.+*Bacillus thuringiensis*+*Ochrobactrum* sp.+*Staphylococcus sciuri*
8	*Bacterium* sp.+*Staphylococcus sciuri*	37	*Bacillus* sp.+*Bacillus cereus*+*Staphylococcus sciuri*
9	*Bacillus* sp.+*Bacillus cereus*	38	*Bacterium* sp.+*Bacillus thuringiensis*+*Ochrobactrum* sp.+*Bacillus cereus*
10	*Bacterium* sp.	39	*Bacterium* sp.+*Bacillus thuringiensis*+*Ochrobactrum* sp.
11	*Bacterium* sp.+*Bacillus thuringiensis*+*Staphylococcus sciuri*	40	*Bacillus* sp.+*Bacillus thuringiensis*
12	*Bacterium* sp.+*Bacillus cereus*	41	*Bacillus* sp.+*Bacillus thuringiensis*+*Bacillus cereus*+*Staphylococcus sciuri*
13	*Bacterium* sp.+*Ochrobactrum* sp.+*Staphylococcus sciuri*	42	*Bacillus* sp.+*Bacterium* sp.+*Staphylococcus sciuri*+*Bacillus thuringiensis*
14	*Bacillus* sp.+*Bacterium* sp.+*Bacillus thuringiensis*	43	*Bacillus* sp.+*Bacillus thuringiensis*+*Staphylococcus sciuri*
15	*Ochrobactrum* sp.+*Bacillus cereus*+*Staphylococcus sciuri*	44	*Bacillus thuringiensis*+*Bacillus cereus*+*Staphylococcus sciuri*
16	*Bacillus cereus*	45	*Bacillus thuringiensis*+*Ochrobactrum* sp.+*Bacillus cereus*
17	*Bacillus thuringiensis*+*Staphylococcus sciuri*	46	Ochrobactrum sp.+*Bacillus cereus*
18	*Bacillus* sp.+*Bacillus thuringiensis*+*Bacillus cereus*	47	*Bacillus* sp.+*Bacterium* sp.+*Ochrobactrum* sp.
19	*Bacterium* sp.+*Bacillus thuringiensis*	48	*Bacillus* sp.+*Bacterium* sp.+*Bacillus thuringiensis*+ *Bacillus cereus*
20	*Bacillus thuringiensis*+*Ochrobactrum* sp.+*Staphylococcus sciuri*	49	*Bacillus* sp.+*Bacterium* sp.+*Bacillus cereus*
21	*Bacillus cereus*+*Staphylococcus sciuri*	50	*Bacillus* sp.+*Ochrobactrum* sp.+*Staphylococcus sciuri*
22	*Bacterium* sp.+*Ochrobactrum* sp.+*Bacillus cereus*	51	*Bacterium* sp.+*Bacillus thuringiensis*+*Bacillus cereus*
23	*Bacterium* sp.+*Bacillus cereus*+*Staphylococcus sciuri*	52	*Bacterium* sp.+*Ochrobactrum* sp.+*Bacillus cereus*+*Staphylococcus sciuri*
24	*Bacillus* sp.+*Bacillus thuringiensis*+*Ochrobactrum* sp.+*Staphylococcus sciuri*	53	*Bacillus* sp.+*Bacterium* sp.+*Ochrobactrum* sp.+*Bacillus cereus*
25	*Bacillus thuringiensis*+*Ochrobactrum* sp.+*Bacillus cereus*+*Staphylococcus sciuri*	54	*Bacillus cereus*+*Bacillus thuringiensis*
26	*Bacillus* sp.	55	*Bacillus thuringiensis*
27	*Bacillus* sp.+*Bacterium* sp.+*Staphylococcus sciuri*	56	*Staphylococcus sciuri*
28	*Bacillus* sp.+*Bacterium* sp.+*Ochrobactrum* sp.+*Staphylococcus sciuri*	57	Control
29	*Ochrobactrum* sp.+*Staphylococcus sciuri*		

inoculated with 50 mL suspension of indigenous rhizosphere bacteria isolates with a density of 10^9CFU mL^{-1}. Ten days later, the second pot was inoculated with 1000 nematode larvae II. Two replications were made for every treatment including control.

Incidence of root- knot nematode: The incidence of root-knot nematode examined observed by counting the number of gall and the number of larvae II present. The number of galls found on the roots was counted using hand held tally counter. The number of larvae II present was counted using a modified Bearman funnel method, by taking the soil from the pot and thoroughly mixed. The 100 mL of soil was placed in a plastic basin that had been coated with tissue paper. Water was then added until the soil was submerged. After one night, 10 mL of sediment water using the plate count and then nematodes were counted under a microscope.

Plant growth: A total of 57 treatments was set for ISR testing, for each treatments growth performance of the plant was observed through several parameters: Plant height at 6 Weeks After Planting (WAP), the number of leaves at 6 WAP, the

number of bunches at age 7 WAP, the total number of flowers at 8 WAP and the total harvest at 10-13 WAP.

Data analysis: To test the effect of PGPR on root-knot nematode incident and plant growth, univariate analysis was used with a randomized block design and advanced test (different test) using the Scott Knott test at the 5% significance level[16]. Relationships of plant growth and root- knot nematode incident were analysis biplot with using software Microsoft® Excel 2007/XLSTAT Version[17]. The maximum distance from the center point defined the character of a common identifier (stated by Hotelling test) was used to determine the parameters affected by the treatments using formula:

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

Where:

r_i	=	Radius ellipse for PC
p	=	PC components used
n	=	Number of treatments in trials
(α, p, n-p)	=	Distribution F at odds with df 5% p and n-p
S^2PC_1	=	Variance score PCI

RESULTS AND DISCUSSION

The split-root trial showed 44 of 56 single and consortium isolates indigenous rhizosphere bacteria were significantly differ than control and could suppressed gall formation from 51-74.8% than control (Fig. 2). However; observations on survival of larva II showed that 48 consortia isolates and one single isolates of indigenous rhizosphere bacteria decreased the number of larva II from 57.6-85.56% compared to controls (Fig. 3). It is clear that indigenous rhizosphere bacteria acted as an antagonistic agent against *Meloidogyne* spp. by reducing nematode populations significantly. According to Hallmann *et al.*[18], nematodes can be controlled by rhizosphere bacteria through various antagonistic mechanism, such as production of toxins, enzymes, secondary metabolites, nutrients competition and plant growth promotion and induced systemic resistance. The results also show that consortium treatments more effective than single treatments showing by reducing the number of gall and larvae II. The decrease in the number of larvae II and gall is one proof of reduction of infection that means bacterial treatments suppress penetration and reproduction of *Meloidogyne* spp.[8].

Growth and yield of tomato: The inoculation of indigenous rhizosphere bacteria on tomato roots resulted in different effects on plant growth. From 57 treatments, the plant height 46 treatments was higher than control. The single isolates and consortium could increase the plant high up to 26.6-37.67% (Fig. 4). This phenomenon confirm that the indigenous rhizosphere bacteria act as PGPR. According to Kloepper[19] and Timmusk and Wagner[20] the ability of rhizosphere bacterial isolates as plant growth promoter was shown by the ability to provide and mobilize the absorption of various nutrients in the soil as well as the changed of phytohormone concentration of plant growth promoters.

The same trends was found on the number of leaves that was from 57 treatments, 41 treatments significantly higher compared to the controls (Fig. 5) and could increase the number of leaves up to 37-58%.

The effect of treatments on number of bunches, that was from 57 treatments, 24 treatments single isolate and consortium significantly higher compared to the controls and could increase the number of bunches up to 63.6-100% (Fig. 6).

From 57 treatments, the number of flower of 27 treatments significantly was higher compared to control. They were two consortia of 8 isolates, three consortia of 7 isolates, four consortia of 10 isolates and two of single isolates. It could increase the number of flowers from 3.2-37.1% than control (Fig. 7).

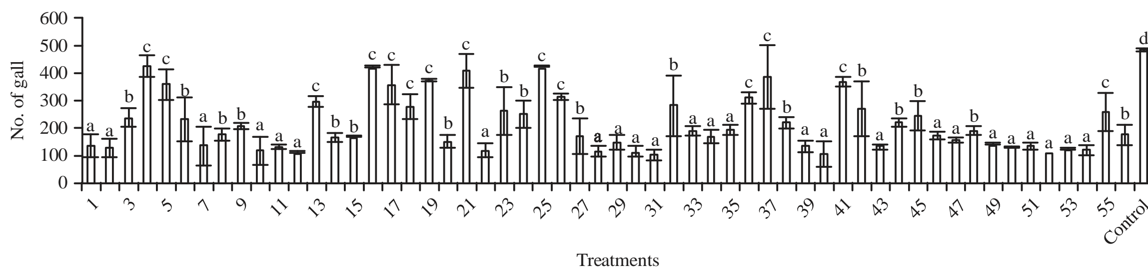

Fig. 2: Effect of indigenous rhizosphere bacterial isolates on gall formation on split root experiment

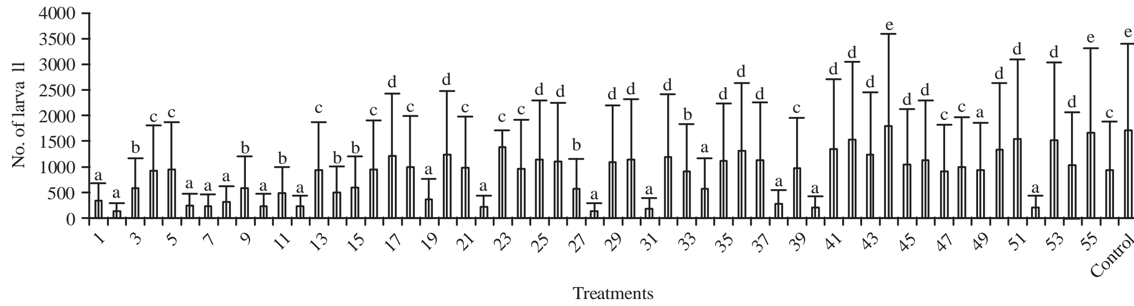

Fig. 3: Effect of indigenous rhizosphere bacterial isolates on the number on larvae II of split root experiment

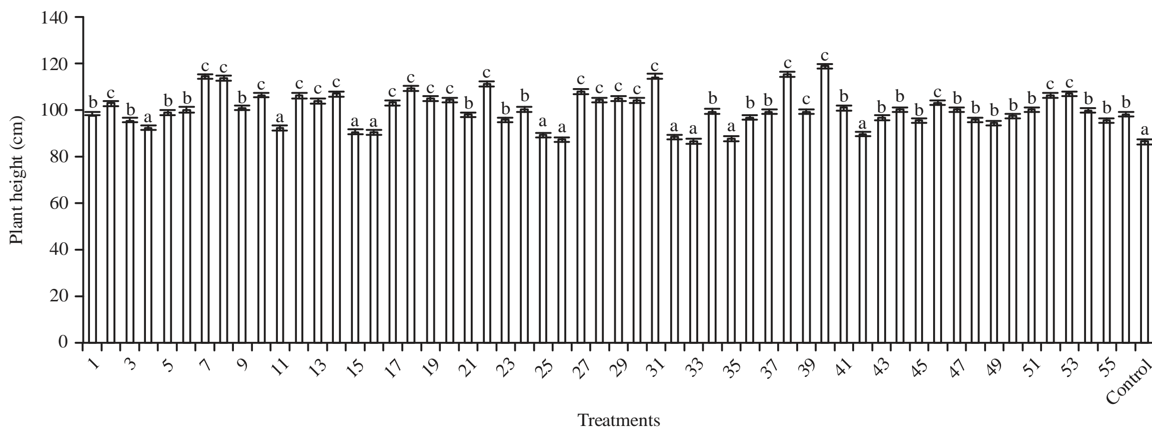

Fig. 4: Effect of indigenous rhizosphere bacteria on tomato plant height on split root experiment

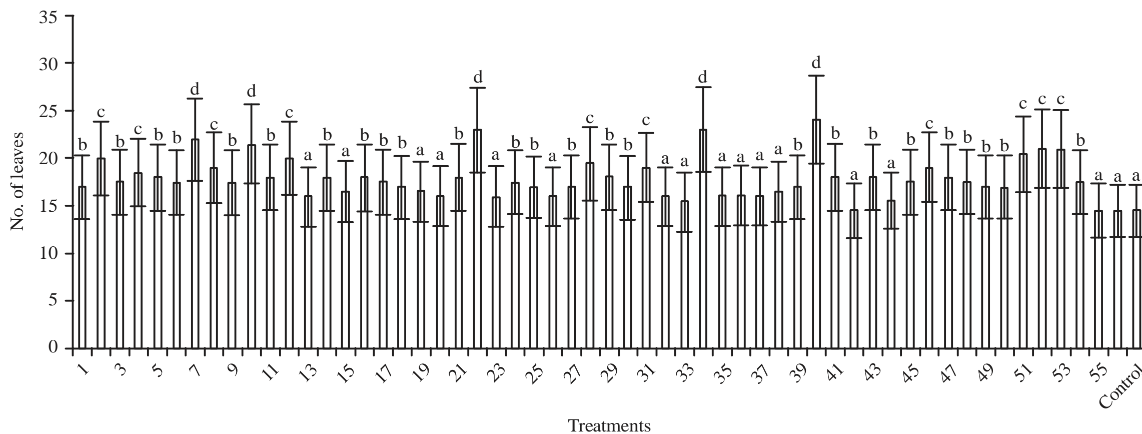

Fig. 5: Effect of indigenous rhizosphere bacteria on tomato number of leaves on split root experiment

The effects of indigenous rhizosphere bacteria on the plant yield showed by the number of tomatoes fruits. Twenty six isolates could increase the number of fruits 15.3-30.9% than control. That was two single isolates, 8 isolates of two consortia, 6 isolates of three consortia and 10 isolates from four consortia (Fig. 8).

The relationship of plant growth and root knot nematode incident was very strong (96.1%), that were described by dimension 1 were 88.2% and dimension 2 were 10.33%. The correlation between plants growth and nematode incident showed by the small angle formed between the treatments parameter (Fig. 9). The results showed number of leaves is highly correlated with plant height and number of galls while the number of gall is highly correlated with the number of larvae II.

Ballot analysis was done to find out the parameters which has greatest variation in each group, the results showed: First group has dominant effect on the yield (1), second group has

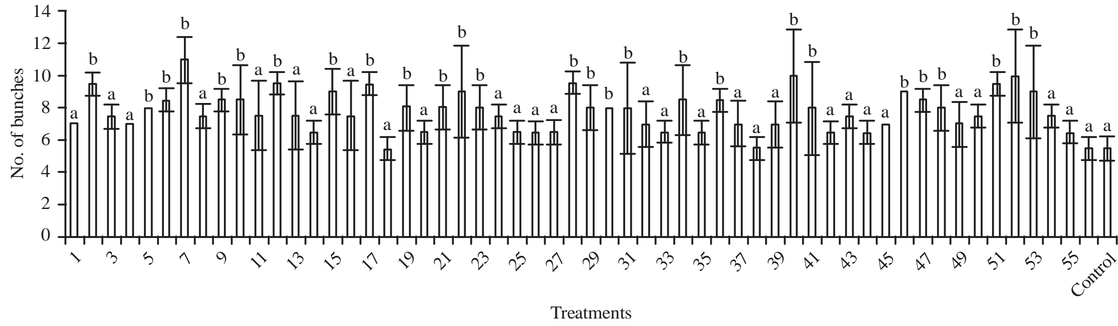

Fig. 6: Effect of indigenous rhizosphere bacteria on tomato number of bunches on split root experiment

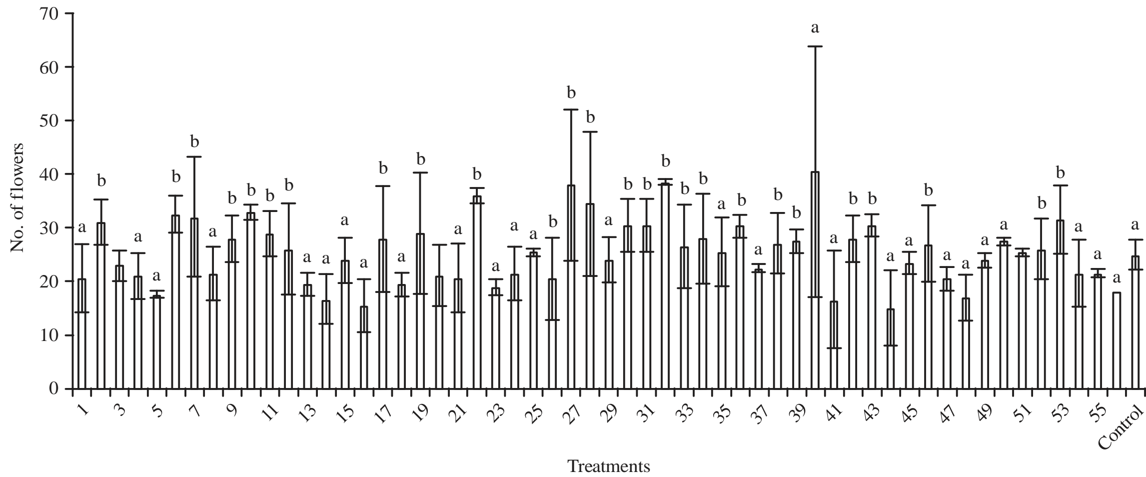

Fig. 7: Effect of indigenous rhizosphere bacteria on tomato number of flower on split root experiment

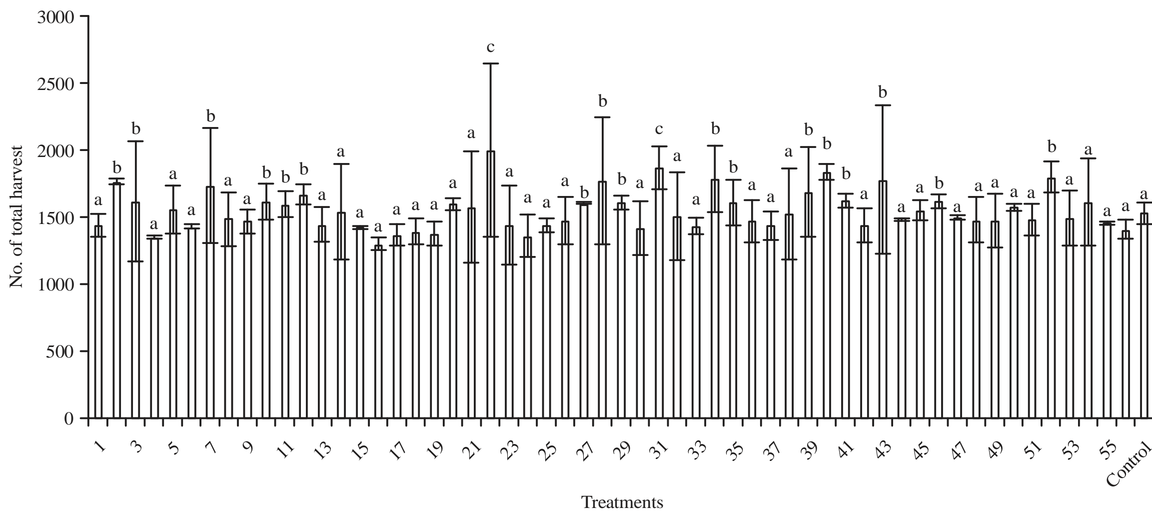

Fig. 8: Effect of indigenous rhizosphere bacteria on tomato number of total harvest on split root experiment

dominant effect on the number of leaves and number of bunches (2), third group has dominant effect on plant height and number of flower (3), the fourth group had dominant effect on the number of gall (4), the sixth group had dominant effect on the number of larvae II (6), the fifth and seventh group didn't have effect on any parameter (5,7).

Indigenous bacterial isolates used as treatments had been tested and proven as PGPR. According to Ramanathan *et al.*[21],

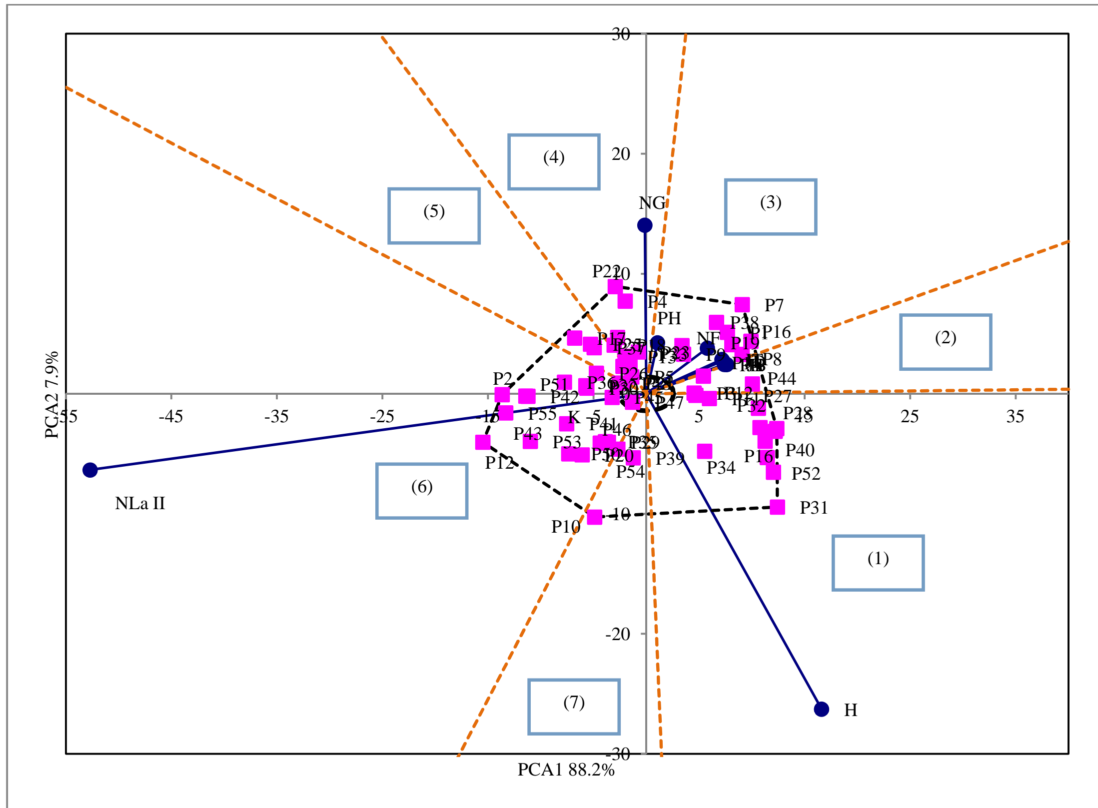

Fig. 9: Biplot analysis results between indigenous rhizosphere bacteria isolates with growth parameters and the incidence of nematodes that affect, P1...P57: Treatment, PH: Plant height, Nle: Number of leaves, NB: Number of bunch, NF: Number of Flower, H: Harvest, NG: Number gall, Nla: Number of larvae II)

a blend of beneficial microorganisms, such as PGPR, can increase crop yields, plant growth and protect plants from pathogens. This occurs because the indigenous rhizosphere bacteria increase the availability of certain nutrients and produce growth hormones, such as auxin and cytokine. The increase of growth parameters (plant height, number of leaves, number of bunches, number of flowers, harvest) is caused by the suppression of nematode population by the rhizosphere bacteria therefore reduced root damage. In addition, the rhizosphere bacteria can stimulate the formation of lateral roots and increase the number of roots so that plants can expand their nutrient absorption[22]. Better nutrient absorption results in a better growth. When *Meloidogyne* spp. infest the roots, it cause root damage because its stylets secrete enzymes during feeding. These enzymes are cellulose and pectinase, which are capable of degrading the cell so that root tips become wounded and ultimately degraded and auxin become inactive. This eventually cause an inhibition on the plant growth.

CONCLUSION

Five consortium of bacteria (*Bacillus* sp.+*Staphylococcus sciuri*), (*Bacterium* sp.+*Ochrobactrum* sp.+*Bacillus cereus*), (*Bacillus* sp.+*Bacterium* sp.+*Ochrobactrum* sp.+ *Staphylococcus sciuri*), (*Bacillus* sp.+*Bacillus thuringiensis*) and (*Bacterium* sp.+ *Ochrobactrum* sp.+*Bacillus cereus*+*Staphylococcus sciuri*) gave an increase of 37.6% in plant height, 58% in number of leaves, 100%, number of bunches, 37.1% the number of flower and 30.9% in yield. Three Consortium of bacteria (*Bacillus* sp. *Ochrobactrum* sp.+*Bacillus cereus*+*Staphylococcus sciuri*), (*Bacterium* sp.+*Bacillus cereus*), (*Bacillus* sp.+ *Ochrobactrum* sp.+*Bacillus cereus*) and single isolates (*Bacterium* sp.) were able to reduce the number of gall and larvae II up to 74.8% and the number of larvae II 85.6%, respectively. Biplot analysis showed a high variations between treatment with growth parameters and the incidence of nematodes.

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