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Potential of *Bacillus toyonensis* and *Paenibacillus alvei* as Plant Growth Promoter on Melon Manis Terengganu (MMT)

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Abstract: *Cucumis melo var inodorus* cv. Manis Terengganu is known as Terengganu's iconic fruits. However, MMT production is still insufficient to satisfy local demand. Over-reliance on chemical fertilizers to MMT has reduced soil fertility, degraded soil quality resistance, and negatively affected the environment. As a result, efficient microorganisms such as *Paenibacillus alvei* and *Bacillus toyonensis* were investigated as possible ecologically compatible biofertilisers for MMT. In this study, *B. toyonensis* and *P. alvei* produced indole-3-acetic acid (IAA) at concentrations of 20.49 $\mu g.mL^{-1}$ and 19.18 $\mu g.mL^{-1}$, respectively. hen, the greenhouse testing was conducted to evaluate the potentiality of effective microbes as plant growth promoter rhizobacteria by introducing the soil with *B. toyonensis*, *P. alvei*, and consortium. The experiment was laid out in a randomized complete block design (RCBD) with three groups of bacteria plus the control and each treatment replicated 15 times. Data were analyzed according to ANOVA procedure. As a result, *B. toyonensis*, *P. alvei*, and consortium significantly increased in all experimental growth parameters such as plant height, leaves, chlorophyll content, root mass, root length, leaf area, fruit weight, macronutrients, and micronutrients compared to control. Overall, the results showed that the effective single microbial strains promoted the development of MMT more effectively than the consortium

Keywords: Biofertilization, *Cucumis melo*, Effective microorganisms.

Introduction

Cucumis melo var inodorus cv. Manis Terengganu 1, also known as Melon Manis Terengganu (MMT), is a new melon cultivar exclusively planted in Terengganu, Malaysia and known as Terengganu's iconic fruits in 2015 (Qian Ong & Khandakar, 2021). Farmers can collect up to RM 20,000 with 10 tonnes of MMT in one season, indicating that MMT has a lot of potentials to help farmers earn more money , However, crop production depends on intensive use of chemical fertilizers and pesticides in agriculture to produce high crop yields (Xu & Kim, 2014; Liu *et al*., 2018). Still, this approach is costly and poses risks to the environment and human health. Furthermore, the public is also concerned that the exclusive use of pesticides and synthetic chemical fertilizers can lead to environmental pollution, contamination of surface and groundwater, environmental change, and adversely affect human health directly or indirectly (Zhang *et al*., 2021).

Many scientists are on board with the idea of utilising microbes as biofertilizers as a means of regulating this problem. Plant growth-promoting bacteria are a class of beneficial microorganisms that aid plant development in a number of ways, including crop production, nutrient absorption, and adaptation to fluctuating biotic and abiotic circumstances (Liu *et al*., 2018; Khan *et al*., 2020; Zerrouk *et al*., 2020).The production of plant growth hormones, biological nitrogen fixation, and improved plant tolerance to abiotic stress due to reduced host ethylene levels are just a few of the ways in which these efficient bacteria boost plant growth and productivity (Spaepen & Vanderleyden 2011).

Paenibacillus alvei and *Bacillus toyonensis* are spore-forming bacteria, and Gram-positive bacteria. This genera were reported to synthesize the plant hormone indole acetic acid (IAA) and cytokinin, solubilizing soil phosphorus, siderophore production, biofilm production, and nitrogen fixation (Liu *et al*., 2018). IAA synthesis is vital because it allows plants to build highly structured root systems that enable more effective nutrient uptake (Ribeiro & Cardoso, 2012).

Previous research found that when *Paenibacillus* spp. and *Bacillus* spp. were applied to tomato, chilli, and cucumber plants, they had a substantial rise in plant height, fresh and dry shoot weight, root augmentation, root length, and fresh and dry root weight (Xu & Kim, 2014; Yanti *et al*., 2019). Therefore, the purpose of this research is to assess whether or not *Bacillus toyonensis* and *Paenibacillus alvei* can effectively stimulate plant development in MMT.

Materials & Methods

Bacterial culture strains

Two potential bacteria were tested as plant activators to Melon Manis Terengganu: *Bacillus toyonensis* and *Paenibacillus alvei* All strains were provided from the laboratory, where molecular identification and biochemical characterization were performed (Badaluddin *et al*., 2020). Bacterial strains were cultured on Nutrient Agar (NA). These two bacteria were compatible for consortium treatment which showed no antagonistic reaction (Glick *et al*., 2007).

Preparation of Effective Microbial Granules

For the preparation of the pasta granules, small adjustments were made to the method described by Mejri *et al*. (2013). For combination A, we autoclaved 800 g wheat flour and 200g kaolin at 121°C for 20 minutes. Under laminar airflow, 200mL of effective bacteria suspension and 200g of sucrose were added to combination A and stirred until a cohesive dough was formed. The dough was rolled out to a thickness of 1 mm and then air dried in a laminar air flow for 72 hours. The dried sheets were grinded into powder using an electric grinder. The concentration of one gram of pasta granules was then determined by serial dilution.

Indole-3-Acetic (IAA) Production Assay

After a 24-hour incubation at room temperature with gentle shaking, a 1-mL bacterial suspension was added to a 50-mL TSB medium containing $4mL.L^{-1}$ Tryptophan. After 24 hours, the cultures were centrifuged at 4°C for 10 minutes at 10,000 rpm. Using the Salkowski reagent, the concentration of IAA was used to quantify the generation of indolic chemicals (50mL of purified water, 30mL of concentrated sulphuric acid, and 10mL 0.5M ferric chloride). Authentic IAA was used to calibrate a spectrophotometer that measured the colour intensity at 530 nm.

Plant growth promotion assay

Bacteria granules weighing three grams were diluted in 1000 ml with a concentration of 10^7 cfu.ml^{-1}.The suspension was sprayed onto the plant in question while it was growing in a greenhouse. Randomized complete block design (RCBD) was used to set up the experiment with four groups: three spiked with bacteria, and one acted as a control. There are 15 replicates of each group.

To prepare for planting, MMT seeds (Green World Genetic (GWG) Sdn. Bhd.) were soaked in water for a whole night. A seedling tray was filled with potting soil and seeds were planted in them. Individual MMT seedlings were germinated in a greenhouse, and then transplanted to polybags containing a mixture of 1:1 topsoil and cocopeat after 10 days. Every two weeks, the soil was sprayed and drenched with the bacterial inoculum (Xu & Kim, 2014). They were irrigated every day, and after 60 days, they were ready to harvest. The number of leaves and the overall height of the shoot were measured weekly. At the same time, measurements of the roots' fresh weight, dried weight, leaf area, and length were taken. The SPAD-502 chlorophyll metre was also used to assess the level of chlorophyll (Konica Minolta, Tokyo, Japan). A portable inductively coupled plasma optical emission spectrometer (ICP-OES) was also used to assess soil's macronutrient and micronutrient availability. Minitab statistical software was used to do a one-way analysis of variance on the collected data.

Results & Discussion

IAA production assay

A colorimetric assay was used to determine the amount of IAA produced. The results showed that all tested bacteria could enhance IAA production ranging from 20.49 µg/ml and 19.18µg.ml^{-1} (Table 1). IAA synthesis is necessary because it helps plants form a well-organized root system that enables more effective nutrient uptake (Ribeiro & Cardoso 2012; Xu & Kim, 2014; Yaish *et al*., 2015). In the current study, IAA were synthesized in the presence of L-tryptophan by both strains Plant growth-promoting bacteria are known to impact their host's phytohormone signaling and modify phytohormone levels which boost seed germination, enhance root system development, and influence metabolite production and resistance to severe environments like salt and drought (Zerrouk *et al*., 2020).

Table (1): Quantification of indole 3-acetic acid (IAA) on Tryptic Soy Broth (TSB) after 24 hours of inoculation with bacteria strains.

Strains	IAA Concentration (µg.mL^{-1})
B. toyonensis	20.49^{b}
Paenibacillus sp.	19.18^{a}
Control (without inoculum)	9.56^{c}

*The same letter in each row represents no significant difference between treatments, based on the range test at $p \leq 0.05$.

Plant growth promotion assay on MMT

Both bacteria were able to improve the growth of Melon Manis Terengganu where it showed an increase in all observed growth parameters compared to control (Figs. 2, 3, and table 2). *B. toyonensis* and *P. alvei* displayed significant growth-promoting in leaf area, at 462.46 cm^2 and 458.37 cm^2 and fruit weight at 777.2 g and 765.0 g respectively compared to the consortium (424.65 cm^2, 670.60 g) and control (398.21 cm^2, 530.00 g). Meanwhile, *B. toyonensis*, *P. alvei*, and consortium showed the most significant increase in plant height, the number of leaves, and chlorophyll content (Figs. 2 and 3, and table 2).

Chlorophyll content in *B. toyonensis* (66.358 nm) was able to secrete more compared with *P. alvei* (54.206 nm), consortium (52.154 nm), and control (24.978 nm). As shown in fig. (1), the leaf color in different treatments varied due to the difference in chlorophyll content. (Fig. 2) showed that *B. toyonensis*, *P. alvei*, and consortium significantly promote plant height at 213.0 ± 4.4 cm, 206.2 ± 13.6 cm, 204.2 ± 27.1 cm respectively, compared with control at 162.4 ± 11.1 cm. The presence of IAA in bacteria supported the production of hormones such as auxins, cytokinins, and gibberellins could be linked to plant height (Jaroszuk-Ściseł *et al*., 2019). Several mechanisms, including the suppression of harmful root microorganisms, the secretion of plant growth hormones, increased nutrient uptake or availability of essential nutrients, and the reduction of compounds in the soil that suppress plant growth have been shown to contribute to improved plan height (Mbarki *et al*., 2017; Nieto-Jacobo *et al*., 2017). In our research, *B. toyonensis* and *P. alvei* showed the highest plant growth-promoting activity compared to control. Shurigin *et al*. (2022) reported that *B. toyonensis* HAPH8, showed high plant growth promoter properties such as nitrogen fixation, phosphate solubilization, production of IAA, and high plant stimulatory activity. *P. polymyxa* has been reported to stimulate growth in crested wheatgrass by producing plant growth stimulating compounds with comparable efficacy to IAA (Holl *et al*., 1988; Lal & Tabacchioni, 2009). In our study both growth-promoting bacteria produce a significant amount of IAA concentration compared to control (Table 2), promoting all plant growth parameters. The result showed that plants treated with single strains are better as plant growth promoters than the consortium. As shown in fig. (2), *P. alvei* and *B. toyonensis* strain reported an increment in plant height at Week 9 (206.2 ± 13.6 cm and 213.0 ± 4.4 cm, respectively) compared with consortium at 204.2 ± 27.1 cm. In addition, *B. toyonensis* has better results in leaf area, root mass, root length, and chlorophyll content compared to the consortium. This increase in root weight could be due to the increase in photosynthesis associated with PGPR, which provides the primary energy to the root. Conversely, the improved roots can absorb more water and nutrients from the soil to support shoot growth. This result is contradicted with Yanti *et al*. (2019 and Shamsudin *et al*. (2021), wherein her study, the *Bacillus* spp. consortium is more profitable growth in Chili seedlings than single strains in term of colonization, domination of the rhizosphere, and roots. However, depending on the circumstances, the effect of a given bacteria on a plant may vary. Moreover, a wide range of mechanisms can be used by bacteria, such as direct mechanisms and indirect mechanisms; hence, different crops may have other modes of action (Glick, 2012).

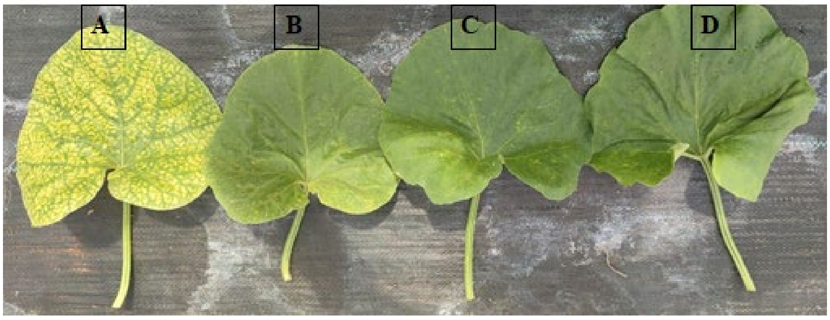

Fig. (1): The comparison of leaf when soil treated with bacteria compared untreated. (A) Control; (B) *Bacillus toyonensis*; (C) *Paenibacillus alvei* and (D) Consortium.

Table (2): Effect of bacteria-treated soil on leaf area, fruit weight, chlorophyll content, root mass and root length of Melon Manis Terengganu (MMT).

Parameters	Treatments			
	Control	*Bacillus toyonensis*	*Paenibacillus* sp.	Consortium
Leaf area (cm^2)	398.21^a	462.46^b	458.37^b	424.65^a
Fruit weight (g)	530.00^a	777.20^b	765.00^b	670.60^a
Chlorophyll content (nm)	24.978^a	54.206^b	66.358^b	52.154^b
Root mass (g)	0.907^a	1.165^b	1.430^b	1.380^b
Root length (cm)	23.5^a	32.2 ^{b}	37.0^b	35.0^b

*The same letter in each row represents no significant difference between treatments, based on the range test at $p \leq 0.05$.

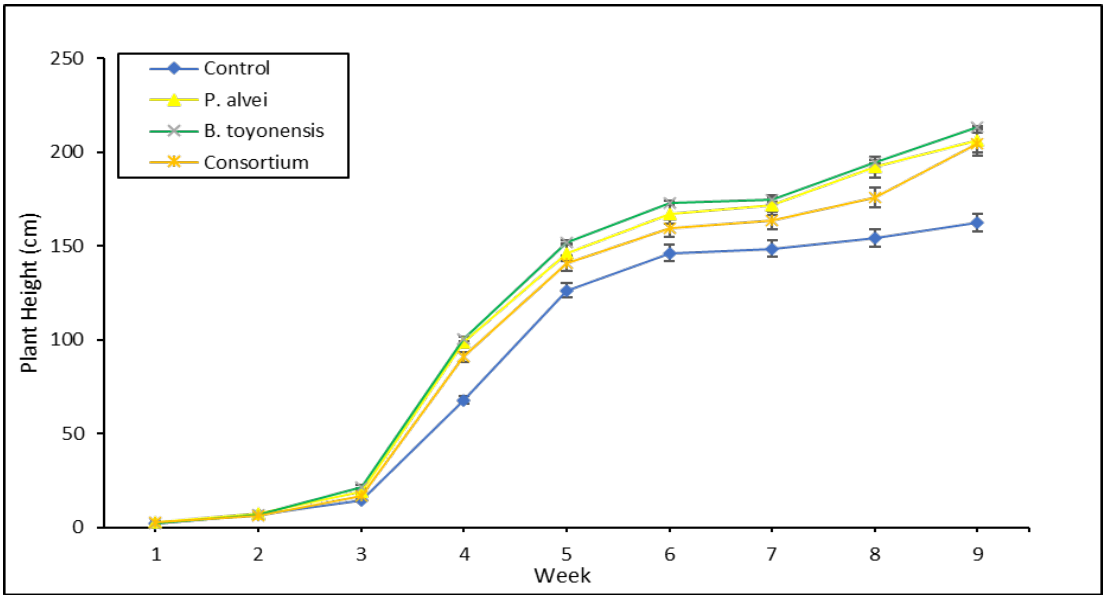

Fig. (2): Effect of bacteria-treated soil on plant height of Melon Manis Terengganu (MMT).

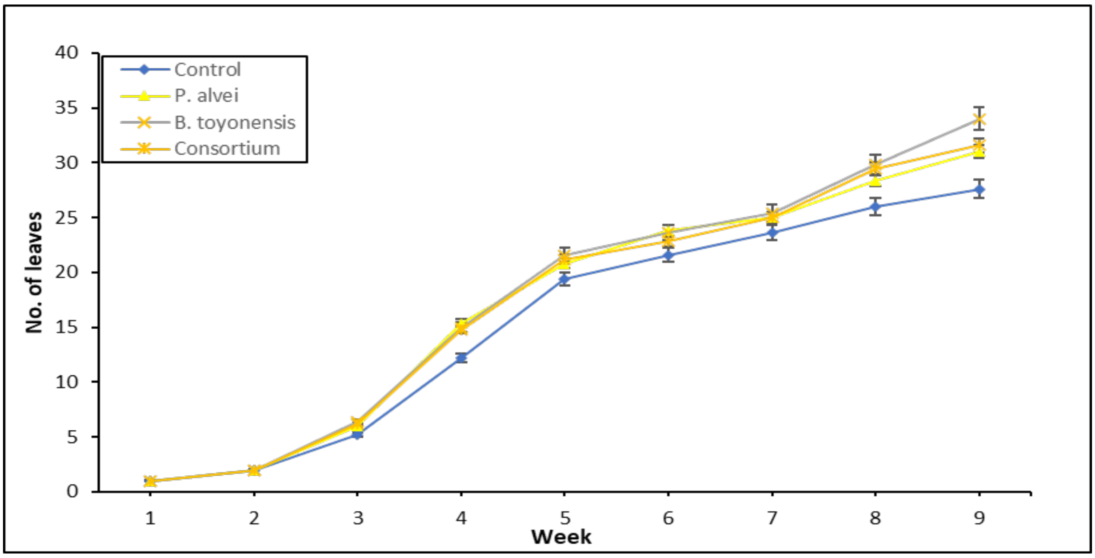

Fig. (3): Effect of bacteria-treated soil on the number of leaves of Melon Manis Terengganu (MMT).

Mineral availability in effective microbes treated soil

Soil is a highly complex ecosystem and a precious resource. Soil is undoubtedly one of our most essential and strategic resources, as it fulfills many important functions, including growing many crops (Yang *et al*., 2020). The increased nitrate (NO_3^-) production in the soil promotes nitrification (Mandal *et al*., 2016). Nitrogen is one of the essential nutrients for plants since it is involved in nucleotides, amino acids, and proteins, which are the basic building blocks of life (Dechorgnat *et al*., 2011). Table (3**)** clearly showed that NO_3^- content in the soil treated with *B. toyonensis* (175474.67 ppm), *P. alvei* (167177.56 ppm), and consortium (161164.19 ppm) significantly boosted up compared to control (83061.22 ppm).

Nutrient content increment in treated soil possibly due to the *B. toyenensis* and *P. alvei* are denitrifying bacteria that can maintain their metabolic activity in moist soil by switching to nitrate respiration (Knox *et al*., 2000). Furthermore, the nitrification process was also influenced by the increase in the number of ammonium-oxidizing bacteria (AOB), which are mainly responsible for ammonium oxidation to nitrate and are driven by rhizospheric bacteria species (Saeid *et al*., 2018).

The phosphorus in treated soil with bacteria reported a significant increase as shown in table (3). The presence of phosphorus in the soil is aided by synthesizing organic acids, one of the most important processes (Oteino *et al*., 2015; Cheng *et al*., 2017). The use of phosphate-solubilizing bacteria such as rhizospheric bacteria increases soil fertility due to their ability to convert insoluble phosphorus into soluble phosphorus by releasing organic acids, chelation, and ion exchange (Saied *et al*., 2018). The soluble potassium (K) content in soil treated with *P. alvei* (3627.00 ppm), consortium (1977.08 ppm), and *B. toyonensis* (1861.30 ppm) was substantially higher compared to the control (1286.66 ppm). A type of *Pseudomonas* and *Bacillus* spp., abbreviated PSB, is crucial to the regulation of potassium homeostasis (Narula *et al*., 2005). Some bacteria, known as potassium solubilizing bacteria (PSB), may release potassium (K) from potassium (K) minerals, hence promoting greater plant development and output (Ali *et al*., 2021). Moreover, they boost crop quality, promote tolerance to stress circumstances like pests and diseases, and activate enzymes that improve nutrient content and cell turgor (Meena *et al*., 2015).

The content of Magnesium (Mg) in soil that was treated with *P. alvei* (500.32 ppm) and the consortium (344.485 ppm) greatly increased. Magnesium and other nutrient elements might have helped chlorophyll synthesis which in turn increased the rate of photosynthesis. (Dalorima *et al.,* 2019). Chlorophyll synthesis requires magnesium because it forms a compound with Rubisco activase, which in turn activates ribulose-1,5-bisphosphate carboxylase (Rubisco) (Portis, 2003; Masuda, 2008). Soil amended with *P. alvei, B. toyonensis* and consortium had significantly higher concentrations of both sulphur (S) and calcium (Ca) compared to the control. Soil sulphate is the primary form of S taken up by plants, and it is transported to the plastids in the leaves, where it is incorporated into organic compounds. According to (Aziz *et al.,* 2016), the growth-promoting rhizobacterium *Bacillus amyloliquefaciens* (GB03) increases *Arabidopsis*'s sulphur absorption and accumulation through an as-yet-unknown mechanism involving transcriptional activation of genes important for sulphur assimilation. Moreover, the capability of bacterial microorganisms to enhance plant sulphur digestion is the complex integration of microbial signals in plant defence (Aziz *et al*., 2016).

Table (3): Effect of bacteria-treated soil on macronutrients of Melon Manis Terengganu (MMT).

Treatment	Macronutrients					
	Nitrate (ppm)	**Phosphorus (ppm)**	**Potassium (ppm)**	**Magnesium (ppm)**	**Calcium (ppm)**	**Sulphur (ppm)**
Control	83061.22^{a}	120.61^{a}	1286.66^{a}	125.69^{a}	825.41^{a}	440.34^{a}
Paenibacillus alvei	167177.56^{b}	876.79^{b}	3627.00^{b}	500.32^{b}	2745.81^{b}	983.28^{b}
Bacillus toyonensis	175474.67^{b}	830.00^{b}	1861.30^{b}	333.90^{a}	1756.93^{b}	499.53^{b}
Consortium	161164.19^{b}	682.70^{b}	1977.80^{b}	344.49^{b}	1465.72^{b}	506.165^{b}

*The same letter in each row represents no significant difference between treatments, based on the range test at $p \leq 0.05$.

Manganese (Mn), Iron (Fe), Boron (B), Copper (Cu), and Zinc (Zn) are essential micronutrients for the biological growth of plant development. The concentration of Mn, Cu, and B in bacteria treated soil where *P. alvei, B. toyonensis*, consortium showed a significant increase compared to control (Table 4). However, in this study, the bacteria-inoculated soil exhibited no significant difference ($p<0.05$) in Fe concentration than controls (Table 4). The concentration of nickel (Ni) showed that *B. toyonensis* and consortium are significantly different ($p<0.05$) compared to *P. alvei* and control **(**Table 4**)**. In contrast, the concentration of Zn showed *P. alvei* (41.66 ppm) and *B. toyonensis* (30.72 ppm) are significantly different ($p<0.05$) compared with the consortium (23.42 ppm) and control (21.61 ppm) (Table 4). Mn and Cu are essential in redox systems because they function as numerous enzymes, including those involved in superoxide radical detoxification and lignin production. Zn is involved in superoxide radical detoxification, membrane integrity, protein synthesis, and the phytohormone IAA. Ni is a metal component of the enzyme urease, which is involved in nitrogen metabolism, B is essential for cell wall and membrane integrity (Broadly *et al.,* 2012). Based on this result, we can conclude that *B. toyonensis* and *Paenibacillus* sp. correlate with different mechanisms needed by some elements to enhance plant growth and development.

Table (4): Effect of bacteria-treated soil on micronutrients of Melon Manis Terengganu (MMT).

Treatment	Micronutrients					
	Manganese (ppm)	Iron (ppm)	Nickel (ppm)	Boron (ppm)	Copper (ppm)	Zinc (ppm)
Control	42.1985[a]	17835.42[a]	5.43[a]	53.57[a]	8.70[a]	21.614[a]
Paenibacillus alvei	141.8055[b]	28159.70[a]	5.13[a]	150.40[b]	20.26[b]	41.658[b]
Bacillus toyonensis	83.2055[b]	27161.20[a]	4.70[b]	100.90[b]	19.82[b]	30.72[b]
Consortium	79.025[b]	21593.03[a]	4.20[b]	84.16[b]	14.35[b]	23.42[a]

*The same letter in each row represents no significant difference between treatments, based on the range test at $p \leq 0.05$.

Conclusions

To sum up, the results demonstrated that *Bacillus toyonensis* and *Paenibacillus alvei* are beneficial bacteria for Melon Manis Terengganu plant development. Intriguingly, our research showed that the foliar spray and soil soaking of these two efficient bacteria significantly increased all growth metrics. It was also shown that individual bacterial treatments were more beneficial to MMT plant development than the ensemble. By providing an alternative to using synthetic chemical fertilizers, this research will help the melons sector in Malaysia remain sustainable into the future.

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Contributions of authors

M.M: Experimentation, data collection, wrote and revised the manuscript.

N.A.B: Suggestion the proposal of the article, revised the manuscript & supervision

M.M.K: Reviewed the manuscript and supervision.

E.A.A: Data collection and reviewed the manuscript.

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Conflicts of interest

The author declares that there are no conflicts of interests regarding the publication of this paper.

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الإمكانات الكامنة لبكتيريا *Bacillus toyonensis* و *Paenibacillus alvei* كمحفزات لنمو النباتات في البطيخ Manis Terengganu (MMT)

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المستخلص: البطيخ *Cucumis melo* var inodorus cv. Manis Terengganu يعتبر أحد الفواكه الرمزية لولاية ترغكانو. ومع ذلك، فإن إنتاج بطيخ Manis T لا يزال غير كافٍ لتلبية الطلب المحلي. أدت الاعتماد المفرط على الأسمدة الكيميائية في زراعة بطيخ Manis T إلى تقليل خصوبة التربة وتدهور جودة التربة وتأثيرات سلبية على البيئة. نتيجة لذلك، تمت دراسة استخدام كائنات دقيقة فعالة مثل بكتيريا *Paenibacillus alvei* و *Bacillus toyonensis* كمحسنات زراعية بيئية ممكنةلانتاج البطيخ .Manis T**.** الدراسة الحالية أنتجت البكتريا *B. toyonensis* و *P. alvei* منظم النمو حمض الإندول الثلاثي الحلقي (IAA) بتركيزات تبلغ 20.49 مايكروغرام.مل$^{-1}$ و 19.18 مايكروغرام.مل$^{-1}$ على التوالي. كما تم إجراء اختبار الزراعة في البيوت المحمية لتقييم قدرة الكائنات الدقيقة الفعالة كمحفزات لنمو النباتات من نوع rhizobacteria عن طريق خلطها مع التربة والبكتريا *B. toyonensis* و *P. alvei*. تم تصميم التجربة بتصميم القطاعات العشوائية التامة (RCBD) مع ثلاث تراكيز من البكتيريا بالإضافة إلى معاملة المقارنة وتم تكرار كل معاملة 15 مرة. تم تحليل البيانات وفقًا لإجراء تحليل التباين. وكنتيجة لذلك، زادت *B. toyonensis* و *P. alvei* والتاثير المشترك بشكل ملحوظ في جميع المعايير النمو التجريبية مثل ارتفاع النبات وعدد الأوراق ومحتوى الكلوروفيل وكتلة الجذر وطول الجذر ومساحة الورقة ووزن الثمار والعناصر الغذائية الكبرى والعناصر النزرة بالمقارنة مع معاملة السيطرة. بشكل عام، أظهرت النتائج أن المعاملات المفردة للبكتريا المستخدمة اثرت بشكل معنوي وساهمت في تطوير نبات البطيخ Manis T بشكل أكثر فاعلية من التاثير المشترك.

الكلمات المفتاحية: الاسمدة الحيوية ، البطيخ (الشمام)، الكائنات الدقيقة الفعالة.