Malaysian Applied Biology (2023) 52(4): 19-25
https://doi.org/10.55230/mabjournal.v52i4.i058

Research

Performance of Okra and Soil Using Indigenous Microorganisms Inoculants

Rubini Devi Selvarajoo and Nurul 'Azyyati Sabri*

Industrial Biotechnology Program, Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang Al - Sultan Abdullah, 26300 Gambang, Kuantan
*Corresponding author: azyyatis@umpsa.edu.my

ABSTRACT

Microbial inoculants are beneficial microorganisms applied to plants or the soil to promote plant growth and control pest disease and weeds. Microbial inoculants isolated from local surroundings are indigenous microorganisms (IMO) inoculants. The performance of the IMO inoculants is varied depending on the sources and the local environment. Therefore, it is important to identify the right sources to enhance the efficiency of the IMO inoculants. This research aims to study the performance of okra and soil by mixing potential yeast sources for indigenous microorganisms (IMO) inoculants. Longan and mango were chosen as the sources of yeasts. The IMO inoculants were fermented for a week, and the microorganisms group was identified. Then, the IMO inoculants were applied to the okra and tested for physical and mineral content analysis. IMO inoculants with mango and longan showed a higher yeast population than the control. However, IMO inoculants with mango showed the best plant growth and harvesting time performance. The soil treated with both IMO inoculants also showed higher potassium and calcium. To conclude, plants treated with both IMO inoculants performed better than the control. Thus, IMO inoculants with longan and mango may potentially enhance the yeast community in IMO inoculants, indirectly improving okra growth and benefiting the agriculture field in the future.

Key words: Biofertilizer, effective microorganisms, indigenous microorganisms, yeast

Article History
Accepted: 24 July 2023
First version online: 31 October 2023

Cite This Article:
Sevarajoo, R.D. & Sabri, N.A. 2023. Performance of okra and soil using indigenous microorganisms inoculants. Malaysian Applied Biology, 52(4): 19-25. https://doi.org/10.55230/mabjournal.v52i4.i058

INTRODUCTION

Fertilizers, such as chemical fertilizers and biofertilizers, are used by farmers to provide different nutrients to increase the plants' productivity and soil quality (da Costa *et al*., 2013). Chemical fertilizers are the most implemented in agriculture. However, the long-term application of chemical fertilizer may lead to an imbalance of nutrients and degrade the soil quality (Ahmad *et al*., 2016), decline the bacterial community's richness (Cui *et al*., 2018), and impact the environment (Yin *et al*., 2017).

Compared to chemical fertilizers, biofertilizers can provide nutrients to crops, improve growth performance and health, and improve the soil's biological activity, leading to better nutrient uptake and transfer from organic and chemical sources and the decomposition of toxic substances (Mącik *et al*., 2020). However, the composition of each nutrient in biofertilizers cannot be designed. Therefore, there are possibilities that biofertilizers lack the major nutrients required by crops and fail to meet the plant nutrient requirements (Han *et al*., 2016). In addition, biofertilizers are decomposed slowly, and in long-term applications, they might accumulate heavy metals, harming the environment (Wei *et al*., 2020).

Therefore, microbial inoculants can offer an alternative solution to overcome this problem. Microbial inoculants composed of beneficial microorganisms may help enhance the microbial community and indirectly improve aspects of nutrient decomposition, productivity, and crop health (Suyal *et al*., 2016). They also protect against various pathogens and effective herbicides (Babalola & Glick, 2012). The beneficial microorganisms include fungi, bacteria, and microalgae, isolated from various locations, such as soil, water, plants, and organic materials or composted manure

(Gamez *et al*., 2019), which makes them environmentally friendly.

Effective Microorganisms (EM) are well-known microbial inoculants that contain a variety of beneficial microorganisms (Higa & Parr, 1994). These microorganisms were classified into yeasts, photosynthetic bacteria, lactic acid bacteria, fungi, and actinomycetes. To date, EM has been applied in different fields, such as agriculture (Joshi *et al*., 2019), eutrophication (Sharip *et al*., 2020), composts (Abu Bakar & Ibrahim, 2013), and water (Firdaus & Azman, 2018).

Similar to EM, indigenous microorganisms (IMO) inoculants are also microbial inoculants, except these beneficial microorganisms are isolated in the local environment. The microbes, including bacteria, fungi, and yeasts, are naturally adapted to the current weather and live in mutual interaction with growing plants (Jan *et al*., 2020). It can be done directly by immersing fermentation culture with molasses solution for IMO inoculums culture or adding various organic materials (Abu Bakar & Ibrahim, 2013).

Yeasts are one of the beneficial microorganisms in IMO inoculants. They generate antimicrobial compounds that eliminate destructive pathogens by utilizing amino acids and sugars secreted by plant roots, photosynthetic bacteria, and organic matter (Al-Sahlany *et al*., 2020). However, suitable sources of yeast, particularly for IMO inoculants, have not been discovered yet. Moreover, the population of yeast in the existing method, using only rice and molasses, is low (Sabri, 2011). Therefore, this study aims to study the performance of okra and soil by adding potential yeast sources for the IMO inoculants. These additional sources may enhance the yeast community in the IMO inoculants. These IMO inoculants may also contain some other microorganisms, which can be used as biofertilizers to indirectly improve plant growth.

MATERIALS AND METHODS

Preparation of Indigenous Microorganisms (IMO) Inoculants

Two fruits, longan (*Dimocarpus longan* spp. *malesianus Leenh*) and mango (*Mangifera indica*) were chosen as yeast sources to enhance the yeast community in the IMO inoculants due to their highest leavening ability (Ghani *et al*., 2011). Both fruits were bought at a night market in Gambang, Kuantan. Five formulations were designed, namely, IMO L1, IMO L2, IMO M1, IMO M2, IMO C (positive control), and negative control (organic fertilizer only) (Table 1). The formulations were based on different amounts of longan and mango added into the standard formulation (IMO C). Hereafter, the IMO inoculants formulations in Table 1 are referred to as IMO inoculants and the type of formulations, to enhance the comprehension of the manuscript. The IMO inoculants cultivation methodology was adapted from Ismail *et al*. (2019) and performed in Taman Pertanian, Kuantan.

Table 1. Formulation for each formulation

Indigenous microorganisms (IMO) inoculants	Ingredients
IMO L_1	1 kg rice + 1 kg brown sugar + 0.25 kg longan
IMO L_2	1 kg rice + 1 kg brown sugar + 0.5 kg longan
IMO M_1	1 kg rice + 1 kg brown sugar + 0.25 kg mango
IMO M_2	1 kg rice + 1 kg brown sugar + 0.5 kg mango
IMO C	1 kg rice + 1 kg brown sugar

Preparation of Indigenous Microorganisms (IMO) inoculants identification of yeast group

The IMO inoculants were diluted in sterile 0.9% NaCl solution until 10-5. Then, the dilution was plated on yeast glucose chloramphenicol (YGC) agar (Chemolab). The number of individual colonies growing on each agar plate was quantified and characterized by the colony's color, size, texture, and surface.

Experimental design

The study was conducted in a randomized complete block design with five formulations, as described above. Each formulation was replicated 10 times at Universiti Malaysia Pahang, Pahang, Malaysia. The soil was mixed with 10 g of organic fertilizer and dilutions 1:1000 of inoculants IMO L1, IMO L2, IMO M1, IMO M2, IMO C. For the negative control, only organic fertilizer was added to the soil. Two seeds of *Abelmoschus esculentus* (L.) Moench (Malvaceae), or okra, was sown per hole in each polybag and watered twice daily. All plants, except negative control, were sprayed with the respective 1000× diluted IMO inoculants once a week. The growth variables were monitored and measured before, during, and after the planting experiments. The yield of okra was harvested after 55 - 60 days. On day 70, the whole okra was harvested, and growth parameters and chemical analyses for the soil were recorded.

Growth parameters of plants

The plants were measured for plant height, root length, okra pods, and harvesting time. Plant height and root length were measured weekly for 60 days, every Monday evening.

Mineral content analysis of soil

Before and after harvesting, the soil samples were sent for the mineral content analysis of Potassium (K) and calcium (Ca) using atomic absorption spectrometry (Perkin Elmer).

Statistical analysis

One-way between-groups ANOVA and T-test were performed using the GraphPad Prism 5 software. Tukey was used for the post hoc test.

RESULTS AND DISCUSSION

Yeast community

Yeast was detected in all IMO inoculants. The morphological characteristic of the yeast is spherical and smooth, and the color is off-white. Figure 1 shows the yeast colony morphology on the YGC agar. The highest yeast colony was found in the IMO M1 (8×10^5 CFU/mL), followed by IMO L2 (3×10^5 CFU/mL), IMO M2 (1×10^5 CFU/mL), IMO C (3×10^4 CFU/mL), and IMO L1 (2×10^4 CFU/mL) ($p<0.05$). A higher yeast colony was found in mango than in longan. This is in line with Ghani *et al*. (2011), which reported that mango strains possess the budding ability, which denotes a better growth rate than strains from longan. Besides that, the total sugar (glucose, fructose, & sucrose) content in mango and longan may enhance the yeast's growth since yeast utilizes sugar for energy. Yeasts grow well in solutions containing 40% sugar and are relatively tolerant of high sugar concentrations (Battcock & Azam-Ali, 1998). The sugar content of mango is approximately 15 – 30% (Wongkaew *et al*., 2021) and 60 - 70% of longan (Cheng *et al*., 2018). Ramírez-Castrillón *et al*. (2019) also identified a few genera of yeast isolated from mangoes, such as the genus *Hanseniaspora*, genus *Clavispora*, and genus *Meyerozyma*. Therefore, the yeast colony was higher in the IMO inoculants with mango and longan than in the controls.

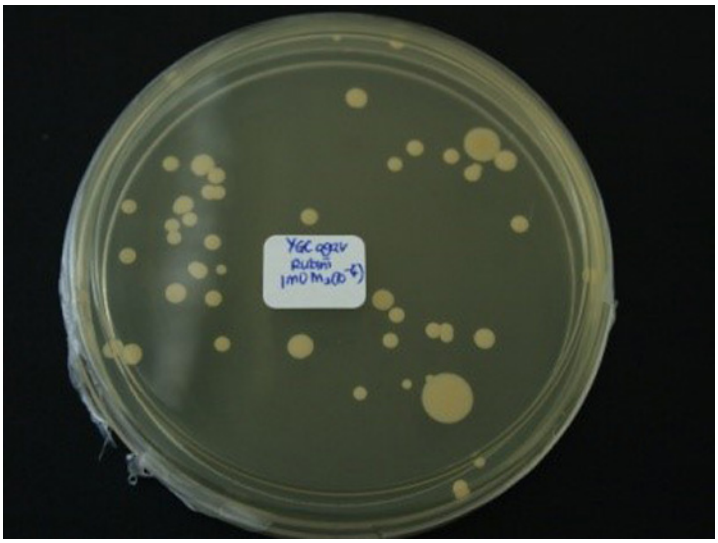

Fig. 1. Yeast colony on the YGC agar plates.

Effects of IMO inoculants on physical analyses of okra

Plants treated with IMO M_1 significantly showed the tallest (64 cm) among all other treated plants (Figure 2(a)). This was followed by the plants treated with IMO L_2 (55 cm), IMO M_2 (50 cm), IMO C (44 cm), negative control (40 cm), and IMO L_1 (28 cm). For root length, plants treated with IMO M1 also showed the longest (58 cm), followed by IMO C, IMO L_2, negative control, IMO L_1 and IMO M_2 (Figure 2(b)). Similar observations were reported by Suryantini and Rahmiana (2021). Okra harvested from plants treated with IMO M_1 was the highest (24 okra pods), followed by IMO L_2 (22) pods, IMO M_2 (14 pods), IMO C (13), negative control (13), and IMO L_1 (10 pods). The first okra was produced by plants treated with IMO M_1 and was harvested in week 7, followed by all plants treated with other IMO inoculants in week 8 and negative control in week 10. Typically, okra plants produce large flowers about 8 weeks after planting, and three to four days after such period, the okra pods are ready to be harvested.

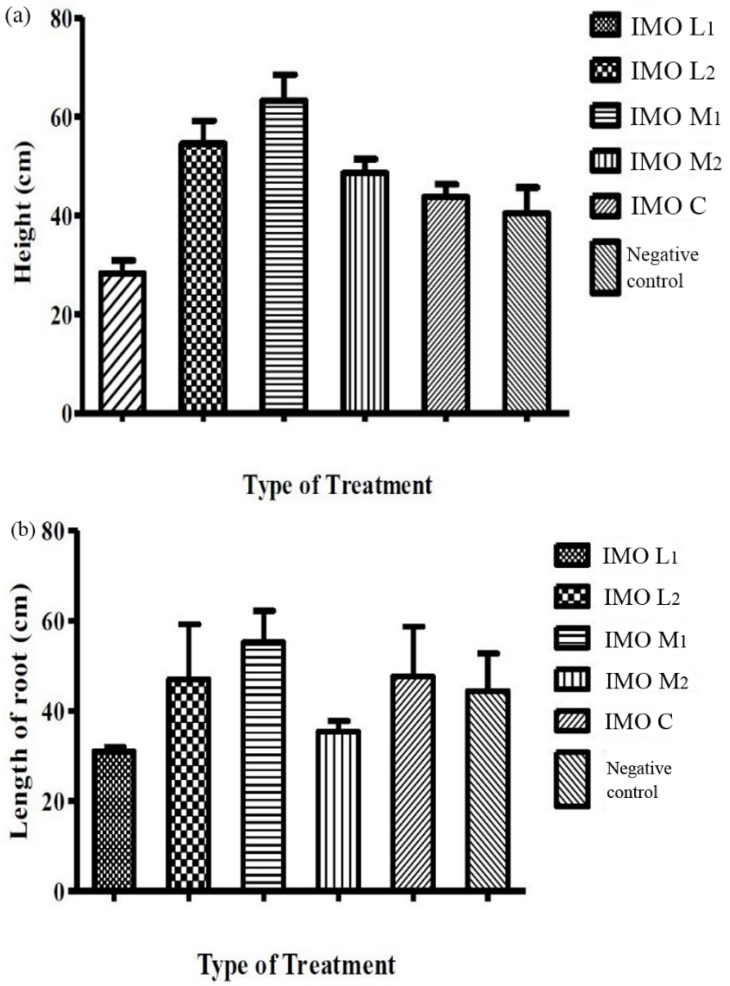

Fig. 2. Physical analysis of okra (a) Plant height (b) Root Length.

Taller plants, longer roots, and faster harvesting time treated with IMO inoculants may result in synergistic effects from the different groups of beneficial microorganisms in the IMO inoculants that can accelerate the breakdown of organic substances into nutrients used by plants. For example, yeasts can work as bio-agents of plant growth-promoting by elevating the production of indole acetic acid, cytokine, and 1-aminocyclopropane-1-carboxylate deaminase, and preventing the growth of plant-pathogen (Gava *et al*., 2018; Mukherjee *et al*., 2020). As a result, yeast improved plant vigor and nutrition during the early growth phase (Lonhienne *et al*., 2014). In addition, lactic acid can solubilize phosphate (Lamont *et al*., 2017). Meanwhile, photosynthetic bacteria produce various chemicals that can induce plant systemic resistance (Su *et al*., 2017). Therefore, okra treated with IMO inoculants, especially IMO M_1, showed taller plants and longer roots than the control.

Effects of IMO inoculants on mineral content properties of soil

The mineral content of the soil before and after the IMO treatment is presented in Figure 3. Even though not all treatments were significant, the potassium and calcium were increased after the treatment of IMO inoculants. However, no potassium changes were observed in the IMO C, and a 50% decrease was experienced in the negative control. Meanwhile, the calcium content increased by 50% in IMO C and did not change in the negative control. Potassium and calcium are part of the cation exchange capacity to indicate soil fertility and soil cations for plant growth. Therefore, inoculants, such as IMO inoculants, favored an accumulation of metal ions, such as potassium and calcium (Talaat *et al*., 2015), which can be observed in the study's result. This observation is also in line with Hashem *et al*. (2012), who stated that the application of inoculants would improve soil conditions for microbial propagation, soil amendment, and increasing potassium and calcium in the soil, even though the effect may depend on the soil type in question (Olle & Williams, 2013).

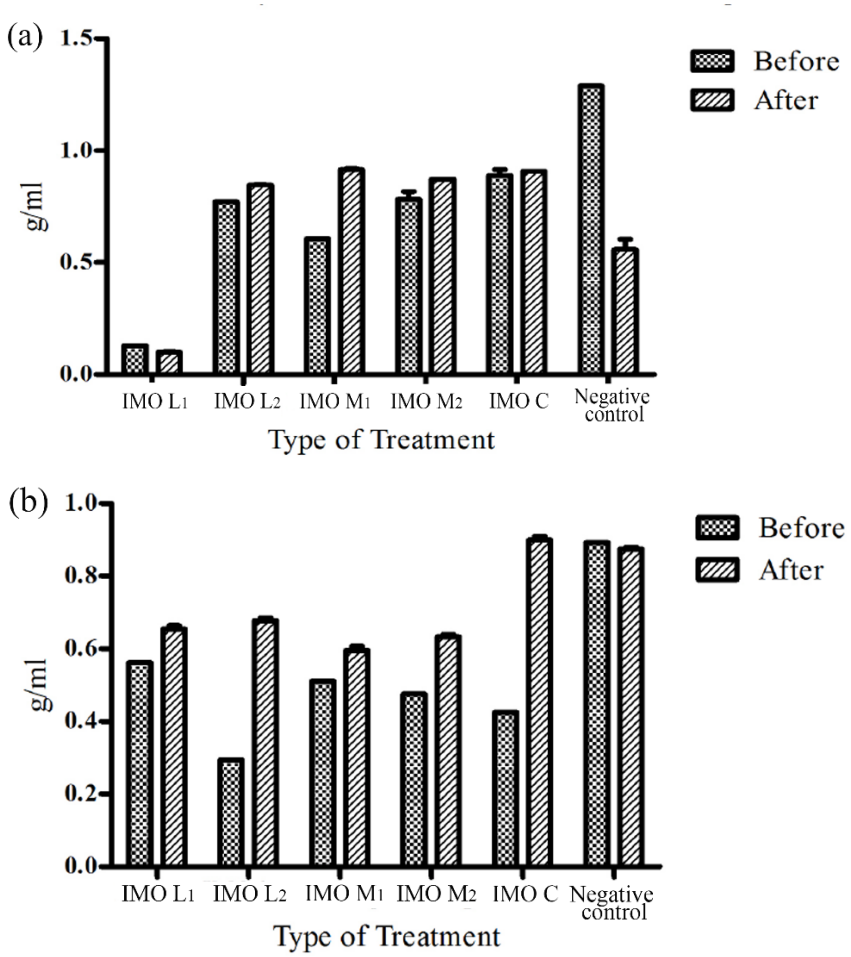

Fig. 3. Chemical analysis in the soil before and after planting okra (a) Potassium (b) Calcium.

CONCLUSION

In this study, the performance of IMO L1, IMO L2, IMO M1, IMO M2, IMO C, and negative control was evaluated in physical analyses of okra and mineral analyses of soil. IMO M1 showed the potential as the suitable source of yeast for IMO inoculants. The yeast population in IMO inoculants, particularly in IMO M1, was higher than the others. This is reflected in the performance of physical analysis, where IMO M1 showed the tallest plant, the longest root, and the shortest harvesting time. After treatment, the potassium and calcium in the soil were improved. Therefore, adding the mango or longan as the alternative source of yeast for IMO inoculants indirectly improved the growth of okra and mineral content. However the IMO inoculants may also contain some other microorganisms that may have a synergistic effect on the growth of plants. Therefore, more studies should be done to fill the gap and understand the mechanisms behind it.

ACKNOWLEDGEMENTS

The research is funded by the RDU200718 Fundamental Research Grant, IIUM-UMP-UiTM Sustainable Research Collaboration Grant 2020 (SRGC), International Islamic University Malaysia, Malaysia.

ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abu Bakar, N. & Ibrahim, N. 2013. Indigenous microorganisms production and the effect on composting process. AIP Conference Proceedings, 1571: 283–286.

Ahmad, A.A., Radovich, T.J.K., Nguyen, H.V., Uyeda, J., Arakaki, A., Cadby, J., Paull, R., Sugano, J. &

Teves, G. 2016. Use of organic fertilizers to enhance soil fertility, plant growth, and yield in a tropical environment. In: Organic fertilizers - from basic concepts to applied outcomes. M.L., Larramendy & S. Soloneski (Eds). IntechOpen. https://doi.org/10.5772/62529

Al-Sahlany, S.T.G., Altemimi, A.B., Al-Manhel, A.J.A., Niamah, A.K., Lakhssassi, N. & Ibrahim, S. A. 2020. Purification of bioactive peptide with antimicrobial properties produced by saccharomyces cerevisiae. Foods, 9(3): 324. https://doi.org/10.3390/foods9030324

Babalola, O.O. & Glick, B.R. 2012. The use of microbial inoculants in African agriculture: Current practice and future prospects. Journal of Food Agriculture and Environment, 10(3&4): 540-549.

Battcock, M. & Azam-Ali, S. 1998. Fermented fruits and vegetables: A global perspective. In FAO Agricultural Services Bulletin No. 134. Food and Agriculture Organization of the United Nations, Rome.

Cheng, Y., Lan, H., Zhao, L., Wang, K. & Hu, Z. 2018. Characterization and prebiotic potential of longan juice obtained by enzymatic conversion of constituent sucrose into fructo-oligosaccharides. Molecules, 23: 2596. https://doi.org/10.3390/molecules23102596

Cui, X., Zhang, Y., Gao, J., Peng, F. & Gao, P. 2018. Long-term combined application of manure and chemical fertilizer sustained higher nutrient status and rhizospheric bacterial diversity in reddish paddy soil of central south China. Scientific Reports, 8(1): 16554. https://doi.org/10.1038/s41598-018-34685-0

da Costa, P.B., Beneduzi, A., de Souza, R., Schoenfeld, R., Vargas, L.K. & Passaglia, L.M.P. 2013. The effects of different fertilization conditions on bacterial plant growth promoting traits: Guidelines for directed bacterial prospection and testing. Plant and Soil, 368(1): 267-280. https://doi.org/10.1007/s11104-012-1513-z

Firdaus, A. & Azman, S. 2018. Improvement of Sungai Sebulung water quality using effective microorganism. International Journal of Engineering and Technology(UAE), 7: 59-61. https://doi.org/10.14419/ijet.v7i3.9.15275

Gamez, R., Cardinale, M., Montes, M., Ramirez, S., Schnell, S. & Rodriguez, F. 2019. Screening, plant growth promotion and root colonization pattern of two rhizobacteria (pseudomonas fluorescens ps006 and bacillus amyloliquefaciens bs006) on banana cv. Williams (musa acuminata colla). Microbiological Research, 220: 12-20. https://doi.org/10.1016/j.micres.2018.11.006

Gava, C.A.T., de Castro, A.P.C., Pereira, C.A. & Fernandes-Júnior, P.I. 2018. Isolation of fruit colonizer yeasts and screening against mango decay caused by multiple pathogens. Biological Control, 117: 137-146. https://doi.org/10.1016/j.biocontrol.2017.11.005

Ghani, M.A., Asyikeen, Z.N., Mutalib, S. & Ayob, M. 2011. Leavening ability of yeast isolated from different local fruits in bakery product. Sains Malaysiana, 40: 1413-1419.

Han, S.H., An, J.Y., Hwang, J., Kim, S.B & Park, B.B. 2016. The effects of organic manure and chemical fertilizer on the growth and nutrient concentrations of yellow poplar (liriodendron tulipifera lin.) in a nursery system. Forest Science and Technology, 12(3): 137-143. https://doi.org/10.1080/21580103.2015.1135827

Hashem, M., Agami, R. & Alamri, S. 2012. Effect of soil amendment with yeasts as bio-fertilizers on the growth and productivity of sugar beet. African Journal of Agricultural Research, 8: 46-56.

Higa, T. & Parr, J.F. 1994. Beneficial and effective microorganisms for a sustainable agriculture and environment. Atami, Japan: International Nature Farming Research Center.

Ismail, M.F., Sabri, N.A., Tajuddin, S.N., Lee, C.M., Mortan, S. & Hairul, M. 2019. Isolation and identification of denitrifying bacteria from indigenous microorganisms. Malaysian Journal of Biochemistry and Molecular Biology, 22: 17-21.

Jan, U., Feiwen, R., Masood, J. & Chun, S.C. 2020. Characterization of soil microorganism from humus and indigenous microorganism amendments. Mycobiology, 48(5): 392-398. https://doi.org/10.1080/12298093.2020.1816154

Joshi, H., Bishnoi, S., Choudhary, P. & Mundra, S. 2019. Role of effective microorganisms (EM) in sustainable agriculture. International Journal of Current Microbiology and Applied Sciences, 8: 172-181. https://doi.org/10.20546/ijcmas.2019.803.024

Lamont, J.R., Wilkins, O., Bywater-Ekegärd, M. & Smith, D.L. 2017. From yogurt to yield: Potential applications of lactic acid bacteria in plant production. Soil Biology and Biochemistry, 111: 1-9. https://.doi.org/10.1016/j.soilbio.2017.03.015

Lonhienne, T., Mason, M., Ragan, M., Philip, H., Schmidt, S. & Paungfoo-Lonhienne, C. 2014. Yeast as a biofertilizer alters plant growth and morphology. Crop Science, 54: 785. https://.doi.org/10.2135/cropsci2013.07.0488

Mącik, M., Gryta, A. & Frąc, M. 2020. Chapter two - biofertilizers in agriculture: An overview on concepts, strategies and effects on soil microorganisms. Advances in Agronomy, 162: 31-87.

Mukherjee, A., Verma, J.P., Gaurav, A.K., Chouhan, G.K., Patel, J.S. & Hesham, A.E.-L. 2020. Yeast a potential bio-agent: Future for plant growth and postharvest disease management for sustainable agriculture. Applied Microbiology and Biotechnology 104(4): 1497-1510. https://doi.org/10.1007/s00253-019-10321-3

Olle, M. & Williams, I.H. 2013. Effective microorganisms and their influence on vegetable production – a review. The Journal of Horticultural Science and Biotechnology, 88(4): 380-386. https://doi.org/10.1080/14620316.2013.11512979

Ramírez-Castrillón, M., Usman, L.M., Silva-Bedoya, L.M. & Osorio-Cadavid, E. 2019. Dominant yeasts associated to mango (*Mangifera indica*) and rose apple (*Syzygium malaccense*) fruit pulps investigated by culture-based methods. Anais da Academia Brasileira de Ciências, 91(4): e20190052. https://doi.org/10.1590/0001-3765201920190052

Sabri, N.A. 2011. Production and evaluation of locally produced effective microbes on okra cultivation and composting (Master). Universiti Teknologi Malaysia.

Sharip, Z., Abd. Razak, S.B., Noordin, N. & Yusoff, F.M. 2020. Application of an effective microorganism product as a cyanobacterial control and water quality improvement measure in Putrajaya lake, Malaysia. Earth Systems and Environment, 4(1): 213-223. https://doi.org/10.1007/s41748-019-00139-4

Su, P., Tan, X., Li, C., Zhang, D., Cheng, J.E., Zhang, S., Zhou, X., Yan, Q., Peng, J., Zhang, Z., Liu, Y. & Lu, X. 2017. Photosynthetic bacterium rhodopseudomonas palustris gj-22 induces systemic resistance against viruses. Microbial Biotechnology, 10(3): 612-624. https://doi.org/10.1111/1751-7915.12704

Suryantini & Rahmiana, A.A. 2021. Effectiveness of plant growth promoting microorganisms as biofertilizer for soybeans under oil palm plantations on tidal land. IOP Conference Series: Earth and Environmental Science, 743(1): 012022. https://doi.org/10.1088/1755-1315/743/1/012022

Suyal, D.C., Soni, R., Sai, S. & Goel, R. 2016. Microbial inoculants as biofertilizer. In: Microbial inoculants in sustainable agricultural productivity: Vol. 1: Research perspectives. D.P. Singh, H.B. Singh & R. Prabha (Eds.). Springer India, New Delhi. pp 311-318.

Talaat, N.B., Ghoniem, A.E., Abdelhamid, M.T. & Shawky, B.T. 2015. Effective microorganisms improve growth performance, alter nutrients acquisition and induce compatible solutes accumulation in common bean (*Phaseolus vulgaris* L.) plants subjected to salinity stress. Plant Growth Regulation, 75(1): 281-295. https://doi.org/10.1007/s10725-014-9952-6

Wei, B., Yu, J., Cao, Z., Meng, M., Yang, L. & Chen, Q. 2020. The availability and accumulation of heavy metals in greenhouse soils associated with intensive fertilizer application. International Journal of Environmental Research and Public Health, 17: 5359. https://doi.org/10.3390/ijerph17155359

Wongkaew, M., Kittiwachana, S., Phuangsaijai, N., Tinpovong, B., Tiyayon, C., Pusadee, T., Chuttong, B., Sringarm, K., Bhat, F.M., Sommano, S.R. & Cheewangkoon, R. 2021. Fruit characteristics, peel nutritional compositions, and their relationships with mango peel pectin quality. Plants, 10(6): 1148. https://doi.org/10.3390/plants10061148

Yin, H., Zhao, W., Li, T., Cheng, X. & Liu, Q. 2017. Balancing straw returning and chemical fertilizers in China: Role of straw nutrient resources. Renewable and Sustainable Energy Reviews, 81(2): 2695-2702. https://doi.org/10.1016/j.rser.2017.06.076