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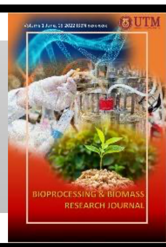

Research Article

Effect of Microbial-Based Plant Biostimulants on the Growth of *Brassica Chinensis* in Acidic Clay Soil

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ABSTRACT

Acidic clay soil is common in Malaysia and is often perceived as problematic soil as its acidic nature and high clay content are not ideal for plant growth. Plant biostimulants are defined as substances or microorganisms applied to plants to enhance nutrition efficiency, abiotic stress tolerance, crop quality, regardless of their nutrient content. This study assessed whether commercial plant biostimulants containing effective microorganism could enhance plant growth in acidic clay soil. A field trial was conducted using *Brassica chinensis var. parachinensis* (Choy Sum), a major vegetable crop in Malaysia. The plants were treated twice a week with T1 (control with distilled water), T2 (Midori Enviro Magic Active Solution) and T3 (Midori Active Organic Solution), with five replicates each (n=5). Plant growth data (leaf length and number, height, stem circumference) were collected weekly, while plant weight and root length were measured at the end of experiment. Soil pH, soil carbon, soil moisture, soil organic matter, microbial count, and contents of N, P, K, Ca, Mg, K, Na, and S contents were analysed. Both T2 and T3 ($P<0.01$) produced plants with larger diameter (32.71±12.43 cm and 59.43±26.05 cm, respectively) compared to the control. Soil treated with T3 exhibited higher pH (4.81± 0.06) and organic matter content (5.94 ± 0.31%) than T2 (pH: 4.51± 0.04, organic matters: 3.51 ± 0.28%) and non-treated soil (pH: 4.53 ± 0.06, organic matters: 4.49 ± 0.25%). The findings demonstrated the microbial-based biostimulants have the potential to improve plant tolerance to acidic clay soil conditions.

INTRODUCTION

Soil acidification in agricultural land affects soil fertility and plant growth. It is often caused by excessive application of ammonium, sulphur and urea-based fertilizers or prolong growing of legumes at the same land (Goulding, 2016). This acidification results from the release of protons (H^+) from reactions of carbon, nitrogen, and sulphur containing compounds. Farmers in Malaysia commonly use limestone ($CaCO_3$) or Dolomitic limestone ($CaMg(CO_3)_2$) to remediate acidic soil before planting, as most crops thrive at a higher pH. However, there is a debate on whether agricultural liming is a sustainable practice due to potential CO_2 emission and uncertainty about its long-term effects on soil (Holland et al., 2018).

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Several technical advancements have been proposed over the past few decades to improve the sustainability of agricultural production systems by significantly reducing synthetic agrochemicals such as pesticides and fertilisers. Plant biostimulants (PBs) are a promising environmentally friendly innovation that has the potential to improve plant growth, flowering, fruit set, crop productivity, nutrient use efficiency and abiotic stress tolerance (Goulding, 2016; Rouphael & Colla, 2020a; Wozniak et al., 2020). Du Jardin (2015) described PBs as substances or microorganisms applied to plants with the goal of improving nutrition efficiency, abiotic stress tolerance, and/or crop quality traits, regardless of nutrient content. Biostimulant products are frequently used in organic vineyards with the aims to reduce the needs for synthetic fertiliser, pesticides, and fungicides. Plant growth promotion after application of PBs has also been reported in a range of harsh environment conditions related to global warming (Rouphael and Colla, 2020b). The PBs help to improve plant growth and resilience to water and abiotic stress while decreasing the demand for fertilisers. They are efficient at low concentrations which favour the functioning of the plant's critical activities and enhance yields and produce quality. PBs affect plant growth via increased nutrient absorption and utilisation, control plants hormone levels. In addition, they promote growth of roots and branching, which may result in higher nutrient and water intake, as well as improved anchoring and stability (Bhupenchandra, et al. 2022). According to Yousfi et al. (2021), rhizogenic biostimulant application increased the soil microbial activity, organic matter, enzymatic activity, calcium, potassium, magnesium, and phosphorus content while decreasing pH and electrical conductivity. The most significant changes were observed in calcium and potassium content in sandy loam soil, with increases of about 77% and 38%, respectively (Yousfi et al., 2021).

In this study, two commercial plant biostimulants namely Midori Enviro Magic Active Solution and Midori Active Organic Solution were evaluated. According to product description, Midori Enviro Magic Active Solution contains organic acids and metabolites derived from effective microorganisms, along with minerals and trace element enhancers. On the other hand, Midori Active Organic Solution is a biostimulant consisting of effective microorganisms, organic acids and amino acids. Both products are EM certified by EMRO Japan, confirming the presence of beneficial microorganisms such as lactic acid bacteria, yeast and phototrophic bacteria. In addition, both products hold organic certification from the National Association for Sustainable Agriculture, Australia (NASAA). The commercial plant biostimulant product were tested to evaluate their effect on *Brassica rapa var. parachinensis* (Choy Sum) grown in acidic clay soil. *Brassica rapa* is one of the most important green leafy vegetables widely produced and consumed in Asia (Ropi et al., 2020; Teck et al., 2021). The main goal of this study was to examine the effect of the commercial plant biostimulant products on plant growth and acidic soil characteristics.

MATERIALS AND METHOD

Materials

The field trial site was situated at Gelang Patah, Johor, Malaysia with an elevation of 18.79 m above sea level and a tropical climate. The experiment involved the application of two commercial microbial-based plant biostimulants: Midori Enviro Magic Active Solution (T2) and (Midori Active Organic Solution (T3) with distilled water as the control (T1). The treatments were applied twice a week at a 5% concentration (product recommended concentration) on *Brassica chinensis var. parachinensis* with 5 replicates each (n=5).

Planting Media Preparation

The acidic clay soil was collected from a local farm at Kluang, Johor and was double-autoclaved using pressurized steam at 121 °C (Hirayama HVE-50) according to Wolf and Skipper (1994). Sterilization procedures may alter soil chemical or physical properties, therefore, an analysis of soil before and after autoclaving process. **Table 1** shows the physical and chemical properties of the acidic clay soil. The acidic soil contained low organic matter which is not ideal for plant and microbial growth, therefore, rice husks (see **Table 1**) was added as a soil amendment (at 5% of total soil weight).

Table 1 Physical and chemical properties of acidic clay soil and rise husk (composite sampling)

	Before Autoclave	After Autoclave	Rise Husk
pH	3.5	3.5	5.0
Moisture Content, %	3.16	3.08	14.15
Organic matter, %	1.59	1.94	89.04
Carbon, %	0.5482	0.5673	18.61
Nitrogen, %	0.0569	0.0646	0.2000
Sulphur, %	0.5482	0.5673	18.61
Phosphorus, mg/kg	30.15	45.35	240.70
Potassium, mg/kg	5375	7234	4344
Calcium, mg/kg	0	0	2.099
Magnesium, mg/kg	37.3	22.1	4.6
Sodium, mg/kg	165.8	188.3	34.2

Seeding, Transplanting and Treatment

Brassica chinensis var. parachinensis (GWG Caixin Cut flower No.10) was seeded in a seeding tray with peatmoss as the planting media. The seedlings were transplanted into polybags (30 cm x 38 cm) containing 1 kg of acidic clay soil (amended with rice husk) 14 days after seeding. Two *Brassica* seedlings were transplanted per polybag, and a culling process was conducted one week after transplantation to remove weaker seedlings. The plants were grown in polybags under open field condition in a randomised complete design (RCD). Distance between each polybag was approximately 15 cm. One week after transplanting, the plants were treated with treatment T1 (distilled water, as the control), T2 (Midori Enviro Magic Active Solution), and T3 (Midori Active Organic Solution) using a drenching method, with 5 replicates for each treatment (n=5).

Soil Analysis

Double-autoclaved soil was used in this study to eliminate existing microbes and dormant endospores. This ensures a controlled setting for investigating the interactions between plants and microbial-based plant biostimulants. Soil samples from before autoclaving, after autoclaving and rice husk were each collected using a composite sampling method (12 sampling points) for analysis. Furthermore, soil sample (10-15 cm depth) collected at the end of experiment (week 12) was taken once from each replicate and treatment. The soils were analysed for pH, soil moisture, soil organic matter according to Ropi et al., (2020).

One milligram of soil sample (in triplicate) was weighed and analysed for C, N, and S using a CHNS/O elemental analyser (Elementar Vario Micro Cube, Germany) based on standard method ISO 16948:2015 (Ropi et al., 2020). The elemental composition of the sample was determined by acid digestion according to EPA method 3052, followed by the determination of P and K by ICP-OES and Ca, Mg, Na using atomic absorption spectroscopy (AAS) (Perkin Elmer PinAAcle 900T Flame). A 3.5-4.0 g of soil sample was digested by adding 2.5 mL of HNO_3 and 7.5 mL of HCl in a clean Teflon tube and mixed. The sample mixture was then heated using a Berghof microwave at 180 °C for 20 min follow by heating at 50 °C for 5 min (Abou Seedo et al., 2017).

About 20 g of soil sample for each treatment and replicate was sent to Innovation Centre in Agritechnology for Advanced Bioprocessing, Universiti Teknologi Malaysia-Pagoh for total bacterial plate count (in-house method LAB-STP-4001) to determine bacterial colony forming units per gram of soil (CFU g^{-1}).

Plant Growth Data

Plant growth data (length of third leaf, leaf number, plant height, stem circumference) were recorded weekly. At the end of the experiment, root length, root weight, and plant weight were measured.

Statistical Analysis

All statistical analyses were performed using a student's t-test. A p-value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Brassica chinensis var. parachinensis grown in acidic soil treated with biostimulants showed variation in growth particularly plant diameter and third leaf length, compared to control plants (**Table 2**). Both commercial biostimulants T2 and T3 ($P<0.01$) resulted in larger plant stem circumference of 32.71 ± 12.43 cm and 59.43 ± 26.05 cm, respectively, while control plants shrank in size compared to their initial diameter at transplanting (-13.03 ± 27.79 cm). This suggests acidic soil has negative impact towards plant growth if no remediation is applied. Similar trend was observed for third leaf length, whereby plants treated with commercial biostimulants have longer third leaf length of 15.17 ± 0.95 cm and 37.11 ± 8.84 cm for T2 and T3, respectively, while the average third leaf length of control was 9.69 ± 1.58 cm. Several studies reported biostimulant affects the physical, chemical and morphological properties of plant but the effects vary according to the types of plant and biostimulant used (Drobek et al., 2019). A study on tomato plant showed that biostimulant containing plant growth promoting bacteria and arbuscular mycorrhizal fungi enhanced the fruit quality such as biomass, length and dimeter but no difference was found on plant collar diameter compared to untreated plant (Bona et al., 2018). Parađiković et.al (2018) suggest the benefits of biostimulant application on horticultural plant especially under stress conditions such as during plant transplanting, reduced fertilization, or abiotic stress (salinity, drought and others). In this study, the plants were grown in acidic soil which is an abiotic stressor and the plant biostimulants T2 and T3 were able to enhance the plant resilience. This supports the theory whereby the biostimulants helps plants cope in unfavourable conditions such as extreme temperatures and water scarcity (Parađiković et al., 2019).

Table 2 Plant growth of *Brassica chinensis var. parachinensis growth in acidic clay soil* treated with different biostimulant (n=5). Different letters signify statistically significant difference between the treatment ($P<0.05$)

	Control (T1)	Midori Enviro Magic Active Solution (T2)	Midori Active Organic Solution (T3)
Plant Height, cm	54.85 ± 53.59^a	67.51 ± 37.55^a	104.79 ± 18.34^a
Stem Circumference, cm	-13.03 ± 27.79^a	32.71 ± 12.43^a	59.43 ± 26.05^a
3rd Leaf length, cm	9.69 ± 1.58^a	15.17 ± 0.95^b	37.11 ± 8.84^c
Leaf Number	19.20 ± 16.31^a	38.69 ± 4.80^a	35.71 ± 13.81^a
Root Length, cm	12.84 ± 1.60^a	8.36 ± 2.68^a	8.72 ± 2.05^a
Root Weight, g	6.60 ± 2.23^a	7.20 ± 3.75^a	6.40 ± 2.14^a

Interestingly, the root length of control plants was longer compared to that of biostimulant-treated plants. The root length for T1, T2, and T3 were, 12.84 ± 1.60 cm, 8.36 ± 2.68 cm, and 8.72 ± 2.05 cm. The root weight of control plant is 6.60 ± 2.23 g, while those of T2 and T3 were 7.20 ± 3.75 g and 6.40 ± 2.14 g, respectively. Longer root lengths often suggest the plants were trying to increase its ability to find and absorb nutrients from the soil. Nutrient availability in the soil is known to influence root growth. Previous studies show a strong correlation between nitrogen accumulation and root biomass (Liu et al., 2024), while higher nutrient concentrations might lead to reduced root length (Ostonen et al., 2007). This suggests the beneficial microorganism in biostimulants T2 and T3 might enhance the nutrient availability for plants.

Soil Characteristic

Statistically, there were no differences in terms of element content between each treatment except for soil treated with Midori Active Organic Solution (T3) which has a significant higher nitrogen (0.107 ± 0.003%) and sulphur (0.11 ± 0.03%) compared to other treatments (**Table 3**). The higher nitrogen and sulphur likely from the biostimulants product itself as the analysis showed it contains 2.60% w/v of nitrogen and 0.63% w/v of sulphur. In addition, soil treated with T3 had higher pH (4.81± 0.06) and organic matters (5.94 ± 0.31%) compared to T2 (pH: 4.51± 0.04, organic matters: 3.51 ± 0.28%) and non-treated soil (pH: 4.53 ± 0.06, organic matters: 4.49 ± 0.25%). A study showed the application of pig and rabbit manure fertilizer reduced the relative abundance of soil *Acidobacteria* compared to chemical fertilizer, which leads to increased soil pH (Zhang et al., 2023). Higher soil microbial necromass might be one of the contributing factors for the higher organic matter content in T3. A quantitative assessment based on published papers indicates more than half of soil organic carbon in ecological systems originates from microbial necromass (Liang et al., 2019). This suggest that the biostimulants T3 might be able to further remediate the acidic soil by changing the soil microbial community. This is supported by the CFU count for T3 which is about 2.06 x$10^8 \pm$ 5.97 x10^7 CFU/g which is slightly higher than that of T1 (1.85x$10^8 \pm$ 8.34 x10^7 CFU/g) and T2 (8.21 x$10^7 \pm$ 7.08 x10^7 CFU/g), however, there were no significant differences between treatments (P>0.05). Further research on the soil microbial profile should be conducted to confirm this hypothesis.

Table 3 The characteristic of acidic clay soil after growing *Brassica chinensis var. parachinensis* and treated with different biostimulants using drenching method (n=5). Different letters signify statistically significant difference between the treatment (P<0.05)

	Control (T1)	**Midori Enviro Magic Active Solution (T2)**	**Midori Active Organic Solution (T3)**
pH	4.53 ± 0.06^a	4.51 ± 0.04^a	4.81 ± 0.06^b
Moisture Content, %	0.38 ± 0.02^a	0.45 ± 0.02^b	0.25 ± 0.01^c
Organic matter, %	4.49 ± 0.25^a	3.51 ± 0.28^b	5.94 ± 0.31^c
Carbon, %	2.38 ± 0.32^a	2.03 ± 0.07^a	2.10 ± 0.18^a
Nitrogen, %	0.083 ± 0.005^a	0.086 ± 0.002^a	0.107 ± 0.003^b
Sulphur, %	0.04 ± 0.02^a	0.00 ± 0.00^a	0.11 ± 0.03^b
Phosphorus, mg/kg	250 ± 3^a	317 ± 13^a	288 ± 59^a
Potassium, mg/kg	5023 ± 416^a	5141 ± 94^a	4982 ± 302^a
Calcium, mg/kg	0.022 ± 0.017^a	0.58 ± 0.338^a	0.00 ± 0.004^a
Magnesium, mg/kg	28 ± 6^a	34 ± 5^a	52 ± 14^a
Sodium, mg/kg	138 ± 12^a	123 ± 12^a	178 ± 28^a
Total Microbial Count, CFU	1.85x$10^8 \pm$ 8.34 x$10^{7\,a}$	8.21 x$10^7 \pm$ 7.08 x$10^{7\,a}$	2.06 x$10^8 \pm$ 5.97 x$10^{7\,a}$

CONCLUSION

The commercial microbial-based plant biostimulants show promising potential in supporting plant growth in unfavourable conditions, such as acidic soil. Plants treated with Midori Enviro Magic Active Solution (T2) and Midori Active Organic Solution (T3) had significantly greater leaf length and stem diameter. In addition, Midori Active Organic Solution (T3) increased soil pH and organic matters after one planting cycle, suggesting its possible use as acidic soil remediation. However, further research is recommended to study the changes in microbial diversity to investigate the fundamental mechanism behind the changes in soil characteristics.

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Conflicts of Interest

The author declares that there is no conflict of interest regarding the publication of this paper.

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