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Enhancing peanut plant growth with bio-enzymes derived from kitchen wastes

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ABSTRACT

Solid wastes such as municipal wastes and agricultural wastes can pollute our environment if they are not disposed properly. In contrast, under manipulation, they can be changed into valuable products such as bio-enzymes and bio fuel. Bio-enzymes derived from kitchen wastes and agricultural wastes are cost effective and can be utilized in industry and agriculture with cutting in cost. This research is carried out to provide information about the cost-effective bio-enzymes with emphasis on their effect on germination and growth of seedlings of peanut (*Arachis hypogea* L. subsp. *fastigiata*). For bio-enzyme production, kitchen wastes such as onion-peels, cruciferous and citric fruits were used as solid substrate. Fermentation method was solid state fermentation with and without isolated microbes. The fermented formulation was that the mixture of three substrates at a weight/weight/weight ratio of 1:1:1 was placed into four airtight plastic containers containing the mixture of molasses and water at a volume/volume ratio of 1:10. Isolated bacteria, P1, P2 and P105 were introduced into three formulated mixtures, BE2, BE3, and BE4, respectively. All of them were fermented at 25℃ - 30℃ for three months. From this research, different hydrolytic activities of these four formulated bio-enzymes were investigated and their concentration being likely to impact on the germination and growth of peanut were examined in comparison to Hydro treatment used as negative control. Among BE1, BE2, BE3, BE4 and Hydro treatments, BE3 treatment at 1:200 dilution significantly showed the most effective activity on germination index, vigor index, branches per plant and pods per plant with 6.31 ± 0.29, 44.9 ± 23.63, 6.85 ± 0.15 branches per plant and 28.98 ± 0.07 pods per plant, respectively. This research shed light on the effect of bio-enzymes on peanut plant cultivation, particularly BE3, and it may become a potential for smallholder farmers.

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INTRODUCTION

Enzymes are key players in the various physiological functions of living things as well as in the industrial processes. Moreover, they are also enhancers of soil quality (Sadh et al., 2018). Like other living things, the productivity of higher plants is determined by synthesis of endogenous hydrolytic enzymes in seed during germination. These endogenous hydrolytic enzymes are vital for breaking down complex substances found in the seed (Ali & Elozeiri, 2017). These complex substances are mostly starch, protein and lipid and govern the germination (Alencar et al., 2012; Zhao et al., 2018).

In cereal grains, 50-80% starch, 5-6% proteins and 1-10% lipids of their weight are present (Garutti et al., 2022). During their germination, α-amylase stored in the aleurone layer become activated and hydrolyze the endosperm starch into metabolizable sugars, which are essential for the growth of roots and shoots (Akazawa & Hara-Nishimura, n.d.; Kaneko et al., 2002; Mitsui et al., n.d.).

In legumes, their protein content is about 20-45% by weight and is higher than that of cereal grains. Their starch and dietary fiber content are ± 60% and 5–37%, respectively. Their fat content is generally low with the exception of peanuts (± 45%), chickpeas (± 15%) and soybeans (± 47%) (Maphosa & Jideani, 2017).

Legume proteins are generally classified into storage protein, biologically active protein, and structural protein. Storage proteins are a store of nitrogen for germination (Onyango, 2022). These seed storage proteins can be sub-divided into albumins (soluble in water), globulins (soluble in dilute saline), prolamins (soluble in alcohol/water mixtures), and glutelins (soluble in dilute acids or bases) (Bera et al., 2023; Osborne, 1909). Biologically active proteins such as lectins, enzymes and enzyme inhibitors have the beneficial or adverse effects on the living tissue, while structural proteins are ribosomal, chromosomal and membrane proteins (Onyango, 2022).

Seed storage proteins are used in metabolism of many seeds by the synthesis of protease and peptidase during germination (Shutov & Vaintraub, 1987). As a result, protein changes typically increase by activating these enzymes during germination but protein digestion in legume seeds is still poor (Bera et al., 2023). According to S. Gepstin and Ilan, these enzymes in germinating beans activate and increase during the first 7 days but it is partly influenced by the development of embryonic axis (Ali & Elozeiri, 2017).

Lipases, the major hydrolytic enzymes during germination, hydrolyze and catalyze the lipids stored as triacylglycerols in oleosomes of oilseeds and the energy derived from which are crucial for the synthesis of sugars, amino acids (mainly asparagine, aspartate, glutamine and glutamate) and carbon chains required for embryonic growth (Ali & Elozeiri, 2017; Quettier & Eastmond, 2009).

In addition to these endogenous hydrolytic enzymes, other endogenous compounds such as melatonin, antioxidant, tryptophan, phytohormones IAA8 and gibberellic acid play a critical role in seed germination. Although Li et al., (2019), Hussain et al., (2020), Rodrigues de Queiroz et al., (2023) and numerous studies stated that such exogenous compounds influence the seed germination like those endogenous compounds, the study of the role of exogenous hydrolytic enzymes in seed germination were not reported except the investigation of Phitsuwan et al., (2013) so far.

Phitsuwan et al., (2013) stated that supplementation of cellulase improve soil fertility by degrading plant residues in soil. Besides soil enriched with nutrients, cellulase at the right concentration or antagonistic cellulolytic fungi to crops promotes plant growth. Moreover, it enhances seed germination as well as protective effects.

Their unveiling the effect of exogenous cellulase on germination and plant growth causes the interesting about the effect of other exogenous enzymes on germination and plant growth. Thus, we hypothesized that the exogenous application of other hydrolytic enzymes gives a positive effect on peanut growth. Consequently, this study was carried out to compare the effects of four formulated bio-enzymes derived from kitchen wastes on germination and growth of peanut plant with the effect of water. Peanut is rich in protein content and one of the principle crops of Myanmar and its cultivating area is 22 million acres. Among

the principle crops, peanut is an exception because it does not meet the local demand for edible oil (FAO, 2022).

For this research, hydrolytic bio-enzymes were produced cost effectively by fermenting the mixture of onion peels, rough leaves of cruciferous vegetables and rotten citric fruits and its peels with only native microbes or addition of isolated microbes (Hemalatha & Visantini, 2020; Phibunwatthanawong & Riddech, 2019; Sadh et al., 2018). These raw materials being used as substrate can be collected throughout the year because Myanmar produces paddy, maize, beans, mangos, sesame, onions, chillies and others for domestic consumption and export. In addition, cruciferous vegetables and citric fruits are cultivated and produced to meet the demands of domestic consumption all year round. After fermentation, the product bio-enzymes were used in peanut cultivation and their effect on the germination and the growth of peanut were examined by comparing with the result of Hydro treatment used as negative control.

The findings of this research may be useful for future research and smallholder farmers of the developing countries.

MATERIALS AND METHODS

Strain Isolation, Physiochemical Characterization and Inoculum Preparation

Ten bacterial strains were isolated from the oil spilled soils. After that, physiochemical characters of these isolated strains such as cellular morphologies, colony morphologies, biochemical tests, enzyme tests were conventionally examined in Microbiology Laboratory, Department of Biotechnology, Mandalay Technological University. All isolated strains were citrate positive. Moreover, they also showed cellulase activity, except P105. However, among them, three strains, P1, P2 and P105, thrive in low amount of nitrogen and complex nitrogen content such as yeast extract in their culture medium and survived at the wide range of temperature (20℃ - 40℃). According to physiochemical characters, P1 and P2 were *Bacillus* species and P105, *Streptomyces* species. Plants and *Streptomyces* are in a mutualistic relationship (Chater K. F., 2016). Thus, P105 was selected as well as P1 and P2 to ferment kitchen waste mixture, although it lacked in cellulase activity. The two selected strains P1 and P2 were cultured in the media containing 10 g/L starch, 5 g/L peptone, 5 g/L yeast extract and *Streptomyces* species, P105, in 10 g/L starch, 0.3 g/L casein, 2 g/L KNO_3, 2 g/L NaCl, 2 g/L K_2HPO_4, 0.05 g/L $MgSO_4$, 0.02 g/L $CaCO_3$, 0.01 g/L $FeSO_4$. Both selected culture media were neutral (about pH 7) and all isolated cultures were incubated at 37 ℃ by shaking at 250 rpm for two days.

Preparation and Production of Bio-enzymes

Different kitchen wastes such as onion-peels, cruciferous and citric fruits were collected separately and cut into small pieces. Its size was about 2 cm or 3 cm. Molasses, onion-peels, cruciferous, citric fruits and water at a volume/weight/weight/weight/volume ratio of 1:1:1:1:10 were used as fermentation media (Hemalatha & Visantini, 2020; Phibunwatthanawong & Riddech, 2019). The fermentation media was divided into four portions; (1) formulation BE1 was the media only and used as positive control, (2) formulation BE2 was the media with the inoculated *Bacillus* species, P1 strain, (3) formulation BE3 was the media with the inoculated *Bacillus* species, P2 strain, and (4) formulation BE4 was the media with the inoculated *Streptomyces* species, P105 strain. The ratio of inoculum to fermentation media was a volume/volume of 1:200.

Fermentation Condition

The four formulated kitchen waste mixtures were fermented in four airtight plastic containers at room temperature (25℃ - 30℃) for three months and their covers were open for a while once a day to release gas in the first 10 days. Their initial pH was 5.5 - 6.3. The temperature and pH of media were check once a week. Mode of fermentation was solid state fermentation (Hemalatha & Visantini, 2020; E. Oriol et al., 1988; Phibunwatthanawong & Riddech, 2019; Sadh et al., 2018). Water content in this fermentation was about 75% of total mass. During fermentation, fermentation temperature was about 2℃ higher than the room temperature. However, the nearer the end of the fermentation process becomes, the closer the fermentation temperature to the room temperature. After three months, fermentation was complete.

Physiochemical Characteristics of Four Bio-Enzymes

Physical and chemical properties of those four three-month-mature bio-enzymes were analyzed at National Analytical Department of Research and Innovation, Yangon.

Element Contents and Their Concentration of Four Bio-Enzymes

Element content and their concentration of those four three-month- mature bio-enzymes were tested at Material Science Research Division, Kyaukse in Mandalay Division. The method used in this test was Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES).

Screening Tests for Hydrolysis Activities of Bio-Enzymes

Hydrolysis activities of these four bio-enzymes were examined like the method of Zhang et al. (2021). Their method was a simple method for the analysis of the protease activity in colored sample using agar well diffusion method. For each screening, 20 ml of agar medium containing respective substrate were prepared and poured into sterile 90 mm Petri dishes. After the agar plates had been cooled, 7 mm × 5 mm agar cylinders were cut aseptically from the agar plates using agar punch. For screening tests, 5 ml of three-month- mature bio-enzyme were collected from each container and filtered using Whatman grade 1 filter paper. The filtrates were centrifuged at 4000 rpm for 10 min. After centrifugation, 150 μL of each bio-enzyme were used to examine their hydrolysis properties by filling them into agar wells. The prepared agar plates for each screening test were incubated at 35 ℃ for 18 hours (Zhang et al., 2021).

Screening Test for Protease Activity

Protease activity of these bio-enzymes was examined using 1% skim milk agar plate (Dogan & Taskin, 2021). Papain and water were used as positive and negative controls. Clear-zone around the well indicated protease activity and no clear zone was negative activity of protease.

Screening Test for Cellulase Activity

Screening test for cellulase activity was performed using 1% CMC agar plates (Dogan & Taskin, 2021). In this case, positive and negative controls were dilute hydrochloric acid and water. Cellulase activity was visually investigated by 0.1% congo red flooding the incubated plate for 20 minutes. A clear zone around the well appeared distinctly during flooding time and then the plate was washed using 1 M NaCl for 10 minutes. Clear zone was not shown around the well if any bio-enzyme or enzyme was lack of cellulase activity.

Screening Test for Lipase Activity

According to the method stated by Carrazco-Palafox et al. (2018), 1% tributyrin agar plates were used to perform screening test for lipase activity of these formulated bio-enzymes. Liquid detergent and water were used as positive and negative controls. Clear-zone around the well indicated lipase activity and no clear zone was negative activity of lipase.

Screening Test for Phosphate Solubilizing Activity

For P-solubilizing ability test, 0.5% calcium phosphate agar plates were used (Chen & Liu, 2019). Clear-zone around the well indicated phosphate solubilizing activity and no clear zone was negative activity of phosphate solubilizing.

Seed Priming Treatment

For this study, peanut (*Arachis hypogea* L. subsp. *fastigiata*) seeds (about 0.5 g per seed) which were collected from a peanut cultivating farmer were soaked with various concentrations such as dilution 1:0 (no dilution), 2:1, 1:1, 1:2, 1:50, 1:100, 1:200 and 1:400 for 4 hours and effect of concentration on germination was

examined by placing the treated seeds into the moist piece of folded cotton bandage under sterile condition at room temperature. According to the result of the effect of concentration on germination, the dilution ratio 1:200 was chosen to study the effect of steeping time on germination. The investigation into steeping duration was performed for 4 hours, 8 hours and 12 hours. Hydro priming was used as a negative control for all studies. All studies were performed two times, each in triplicate.

Soil Sampling and Analysis

Five soil samples were collected from 15 cm depth of the centre and four corners of the sown area by using clean garden trowel to analyse nitrogen (N), phosphorous (P) and potassium (K) of soil also known as soil NPK (M Hughes et al., 2008; Zerbato et al., 2017). The sown area was the unplanted area of the Department of Biotechnology in Mandalay Technological University (21° 58′ 08″ N, 96° 11′ 16″ E, 90.93 m about sea level). Soil NPK analysis was carried out by applying Wet Digestion Method for total nitrogen, Olsen Method for available phosphorous and Flame Photometer FP-640 for available K_2O and exchangeable K^+. Electrical conductivity of soil was measured with HI-98331 Groline Direct Soil Conductivity and Temperature Tester and soil pH for Fisherbrand AB 150 pH Benchtop Meter. Soil texture was determined by following FAO guidelines. All analyses were done at the Department of Agriculture (Land Use Division) in Mandalay.

Soil Preparation

The weed-free soil used for planting peanuts was loose up to a depth of 30 cm. After that, the loose soil was raised to a height of 15 cm to form a soil bed that was 40 cm wide and 200 cm long. The distance between two soil beds was 45 cm apart. Total planted soil area was 0.00264 hectare.

Determination of Germination

Their germination rate and index and vigor index were calculated as the following formulae (Thongtip et al., n.d.; Tian et al., 2023).

$$\text{Germination Rate} = \frac{\text{Number of Germinated Seed on Day 5}}{\text{Total Number of Seeds}} \times 100\%$$

$$\text{Germination Index (GI)} = \sum \frac{Gt}{Tt}$$

Where,
Gt = the number of germinated seeds on Day t
Tt = time corresponding to Gt in day

Vigor Index (VI) = Germination Index (GI) × Mean 10 days old seedling Length

Screening Test for the Growth of Germinated Seed in Bio-enzyme Treated Soil

Before being cultivated on the soil beds, ten germinated peanut seeds from each treatment were sown in the cups filled with the respective bio-enzyme treated soil. In treating the soil with the respective bio-enzymes, dilution ratio 1:200 was employed like in seed priming. To ensure the soil moisture, only water was utilized through the screening test. Once a week, the respective bio-enzyme dilutions were fed to the seedlings under bio-enzyme treatments. Screening the growth of germinated seeds was ended in day 15 after sowing the germinated seeds.

Cultivation of Germinated Seeds

After screening the growth of germinated seeds, the vigor index and the growth of seedling were studied. Ten germinated peanut seeds from each treatment were prepared in triplicate and sown evenly on each soil bed. The space between two germinated seeds was 20 cm apart. There were fifteen soil beds for five treatments. Cultivating seasons were monsoon and post monsoon of 2023. During growing, soil moisture was regularly checked in two days interval and water was applied if necessary. Unlike Hydro treatment used as a

negative control, the seedlings under bio-enzyme treatments were fed their respective enzymes at a consistent dilution ratio utilized in the screening test once a week.

Determination of Peanut Plant Growth

After cultivation, 90 days old peanut plants with respective treatment systems were harvested separately and examined number of branches per plant and number of pods per plant.

Statistical Analysis

IBM SPSS Statistics 19 software was applied for data analysis. To determine whether there were the statistical significances among five treatments or not, thirty means observed from five treatments were compared by conducting Duncan's multiple range tests on one-way analysis of variance (ANOVA) at the significant level of 0.05 ($p \leq 0.05$). Multiple comparisons showed which treatments differed from each other. This statistical analysis was performed for each experiment. The analyzed data was visualized as bar graphs using MS Excel 2019.

RESULTS AND DISCUSSION

Results

Physiological Characteristics of Inoculums

The results of physiological characteristics of three species, P1 and P2 and P105, used as inoculums in this study, were shown in Table 1. According to cultural, cellular and biochemical characters, two *Bacillus* species, P1 and P2, are facultative aerobes and *Streptomyces* species, P105, is a facultative anaerobe.

Table 1. The physiological characteristics of inoculated strains

Biochemical test	Inoculated Strains		
	P1	P2	P105
Gram stain	+	+	+
Cell shape	small rod	small rod	filamentous
Spore	+	+	+
Catalase	+	+	-
Oxidase	+	+	-
Voges-Proskauer test	+	+	-
Methyl red test	-	-	-
Citrate utilization test	+	+	+
Indole test	+	+	-
Protease	+	+	+
Lipase	+	+	+
Cellulase	+	+	-

Note: + : positive, - : negative

Soil NPK Result

Soil analytical data showed that electrical conductivity, total nitrogen and available phosphorous of soil were low. This loamy soil is composed of high available K_2O and exchangeable K^+. The analytical data was detailed in Table 2.

Table 2. Soil analytical data

Moisture (%)		2.41
pH		8.52
Electrical conductivity (ms/cm)		0.12
Total Nitrogen (%)		0.13
Available Phosphorus (ppm)		2.18
Available K_2O (mg/100g)		31.35
Exchangeable K+		0.67
Texture	Sand (%)	48.93
	Silt (%)	32.98
	Clay (%)	18.09

Properties of Bio-Enzymes

Element content, their concentration and physical and biochemical characteristics of these four bio-enzymes are shown in Figure 1 and Table 3. Those four bio-enzymes, three-month-mature fermented liquids, had protease, cellulase, lipase and phosphate solubilizing activities. Besides, those bio-enzyme liquids contained some macronutrients for plant growth such as potash (K), calcium (Ca), sulphur (S) and magnesium (Mg), micronutrients such as iron (Fe), zinc (Zn), boron (B) and manganese (Mn) and non-essential element for most plants such as sodium (Na). Sodium (Na) concentration of BE4 was 631.5 ppm, and it was the lowest amount among four bio-enzymes. The highest sodium concentration among them was 830 ppm and it was BE2.

Effects of Concentration of Bio-Enzymes and Steeping Time on Germination

Peanut seeds treated with BE1, BE2 and BE3 at dilution ratios 1:0 (no dilution), 2:1, 1:1 and 1:2 did not germinate. In treated with BE4, peanut seeds germinated till the dilution ratio 2:1, but germination rate was below 50%. Germination rates were not significantly different at dilution ratio 1:100 and 1:200. (Data was not shown.) According to this result, dilution ratio 1:200 was chosen to carry out the whole study. At this concentration, effect of steeping times on germination was studied. It was observed that there was no impact on the germination of peanut seeds in the duration of steeping lasting up to 12 hours.

Study on Germination

In this study, germination rates of five treatments were not significantly different. Among them, the germination rate of BE4 treatment was the least with 93.33%. In contrast, its germination index, 6.18 ± 0.36, was nearly equal to that of BE3 treatment, whose results were the best among the treatments. Means of germination rate, germination index and vigor index were 96.67%, 6.31 ± 0.29 and 44.92 ± 3.63. Germination rate of BE1 treatment was 95.33% and it was nearly equal to that of BE2, 95%. The results of germination index and vigor index of BE1 treatment followed that of BE2 and BE4 but their mean numbers of branches and pods per plant were nearly equal (Figure 2a, 2b, 3a and 3b).

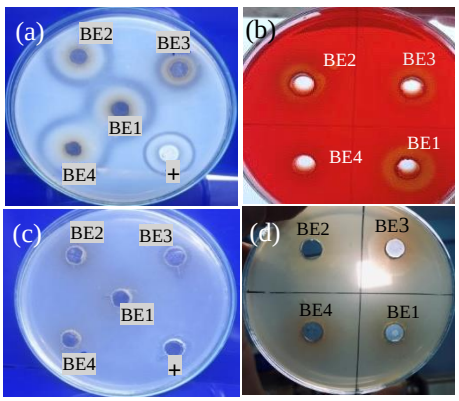

Figure 1. Protease activity (a), cellulase activity (b), lipase activity (c) and phosphate solubilizing activity of bio-enzymes on 1% skim milk, 1% CMC, 1% tributyrin and 0.5% calcium phosphate agar plates, respectively.

Table 3. Element content, their concentration and physical and biochemical characteristics of bio-enzymes

Physical and biochemical characteristics	Bio-enzyme			
	BE1	BE2	BE3	BE4
Color	dark brown	dark brown	dark brown	dark brown
Smell	Pungent	pungent	pungent	pungent
Ca (ppm)	32.7	35.0	35.2	32.7
Mg (ppm)	85.0	85.0	87.5	82.5
Na (ppm)	731.5	830.0	731.5	631.5
S (ppm)	132.0	124.5	114.5	112.0
Fe (ppm)	50.5	38.0	25.5	30.5
Mn (ppm)	0.837	0.562	0.487	0.662
Zn (ppm)	0.40	0.35	0.27	0.25
Si (ppm)	9.50	8.00	6.25	7.50
B (ppm)	0.52	0.40	0.65	0.27
K (ppm)	983.0	948.0	873.0	848.0
Total phosphate (ppm)	176.5	165.0	261.9	262.7
Electrical conductivity (mS/cm)	8.49	11.72	12.47	10.41
pH at 25℃	5.68	5.68	5.68	5.74
Clear-zone diameter of protease activity	34 mm	30 mm	17 mm	37 mm
Clear-zone diameter of cellulase activity	18 mm	14 mm	14 mm	-
Clear-zone diameter of lipase activity	-	9 mm	11 mm	-
Clear-zone diameter of P-solubilizing activity	-	14 mm	33 mm	32 mm

Note: + : positive, - : negative

Effects of Bio-enzymes on Peanut Plants

In the investigation of branches per mature plant, the means of branches per plant of BE1, BE2, BE3, BE4 and Hydro (negative control) treatments were 6.48 ± 0.31, 6.48 ± 0.16, 6.85 ± 0.15, 6.58 ± 0.1 and 5.2 ± 0.18, respectively. BE3 was the highest in branch number followed by BE4, BE1, BE2 and control. The number of branches of BE3 was significantly different from other treatments (Figure 3c).

In the study of number of pods per peanut plant, BE3 treatment was the best in pod yield: 28.98 ± 0.07 pods per plant, the second was BE2: 28.12 ± 0.35 pods per plant, followed by BE1: 28.07 ± 0.42 pods per plant and BE4: 28.02 ± 0.59 pods per plant. The control is the least in pod yield: 23.52 ± 0.55 pods per plant. The pod yield in BE3 treatment was significant among treatments (Figure 3d).

Figure 2. Germination (day 2) (a) and seedlings of peanut seeds (day 7) (b) under treatments with four diluted bio-enzymes in the dilution ratio of 1:200 and water alone

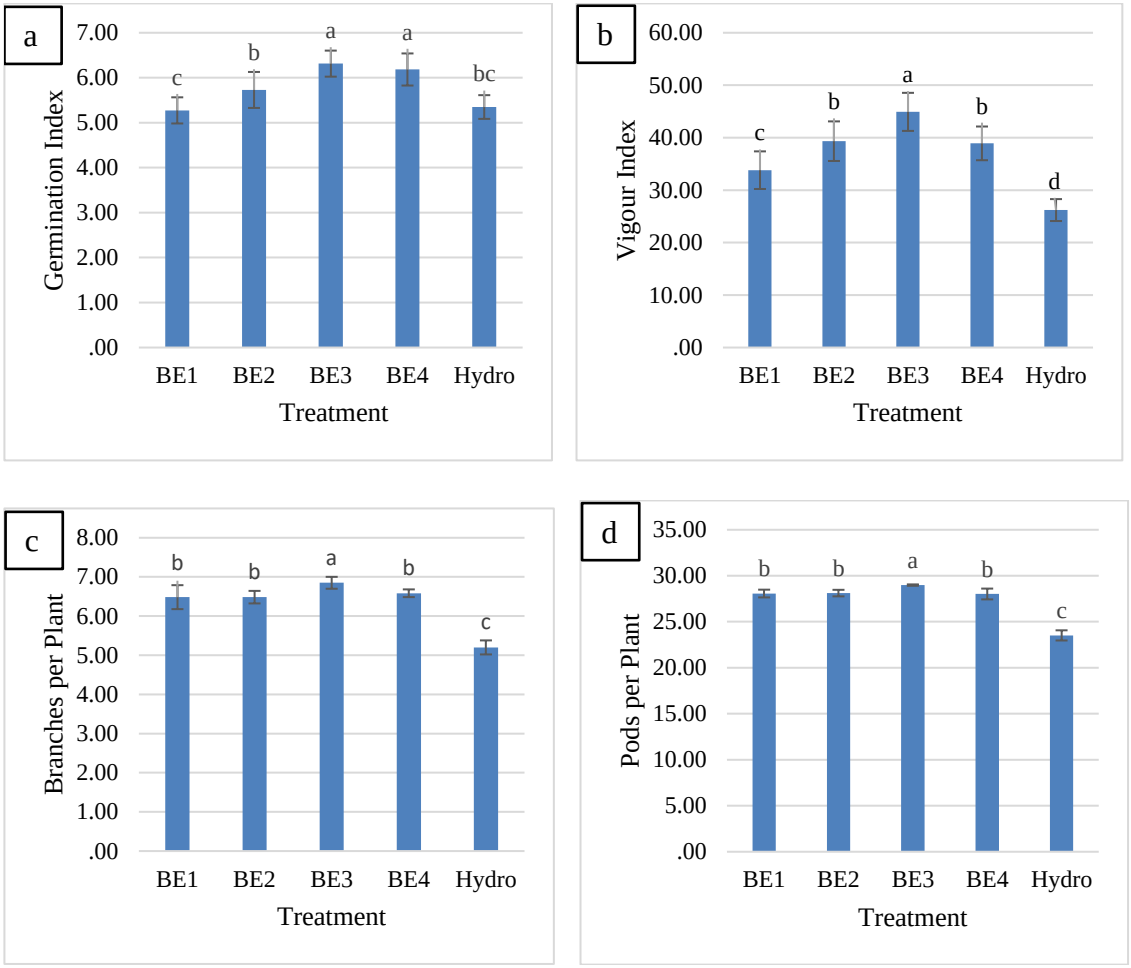

Figure 3. Germinating index (a), vigour index (b) of peanut seeds (Arachis hypogea L. subsp. fastigiata) treated with four diluted bio-enzymes in the dilution ratio of 1:200 and water alone for 4 hours, number of branches per peanut plant (c) and pods per peanut plant (d). Bars indicate standard deviation (n = 6). Different lowercase letters over the bars indicate statistically significant differences among the treatments at $p \leq 0.05$.

DISCUSSION

In this research, 10 bacterial strains were isolated and conventionally identified accordance with Bergey's manual. *Bacillus* species (P1 and P2) and *Streptomyce* species (P105) were applied in bio-enzyme formulations. During bio-enzyme fermentation, temperature and pH were checked regularly. The pH of all vessels dropped in the first month and the flocculation were observed in the fermentation period. Having completed the bio-enzyme, these bio-enzymes were used as plant growth promoters for peanut plants. Before plantation, germination tests were performed and peanut plants were cultivated with each bio-enzyme treatments.

Germination rate of peanut seeds of all treatments were not significantly different. As described above, this study was carried out in the bio-enzyme dilution ratio 1:200 at 25 ℃ - 36 ℃. Peanut seeds treated with the bio-enzymes, BE1, BE2 and BE3, at dilution ratios 1:0 (no dilution), 2:1, 1:1 and 1:2 did not germinate at all. These three bio-enzymes had cellulase activity. Peanut seeds under BE4 treatment were able to germinate at dilution ratio 2:1 although its protease activity was the highest among them in screening test. Apart from the others, it did not show cellulase activity. Phitsuwan et al.(2013) stated that applying cellulase or antagonistic cellulolytic fungi to crops enhance plant growth, seed germination and protective effects (Phitsuwan et al., 2013). Peanut seeds are rich in protein and fat (Maphosa & Jideani, 2017). Storage protein of peanut kernels is a store of nitrogen for germination (Onyango, 2022). Globulins, one of storage protein, dissolve in dilute saline (Osborne, 1909). Salinity, also described as electrical conductivity, of these four bio-enzymes were 8.49 mS/cm, 11.72 mS/cm, 12.47 mS/cm and 10.41 mS/cm respectively. These may be causative that length of radicle and high of seedlings under bio-enzyme treatments were extreme compared to Hydro treatment. Maria

Balota reported that the right soil pH range for peanut growing is 5.8 – 6.2 (Balota, n.d.). In this study, the soil pH for growing peanut seedlings was 8.52. pH of these four bio-enzymes was above 5.5 and thus their pH could not impact on the peanut plant growth on high soil pH.

According to ICPOES result, sodium (Na) concentration of BE1, BE2, BE3 and BE4 were 731.5 ppm, 830.0 ppm, 731.5 ppm and 631.5 ppm, respectively and their potassium (K) concentration were 983.0 ppm, 948.0 ppm, 873.0 ppm and 848.0 ppm, respectively. Sodium potassium concentration ratio of all of them was nearly equal to 1:1. Mary and Emmanuel (2007) reported that at the equal value of sodium potassium concentration (1:1), the content of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) in the shoot was increased (Kemi Idowu & Adote Aduayi, 2007). Sodium concentration at very low amount is considered beneficial to plant growth although it is non-essential element for most plant (White & Brown, 2010). Nonetheless, from this research, peanut plants under bio-enzyme treatments being able to bear early flowers and secondary shoots were also studied.

Numbers of branches and pod yields of BE3 was significant in comparison of the other treatments. Therefore, BE3 combination gave the best results and potential effect on peanut plants.

CONCLUSION AND RECOMMENDATION

According to this study, enzymatic activities and concentration of bio-enzyme had a discernible impact on the germination and seedling growth of peanut. BE3 which had multiple enzyme activities at the right concentration gave the best result among the treatments. The finding that peanut plants under bio-enzyme treatments bore early flowers and secondary shoots may support peanut cultivating smallholder farmers in the developing countries. Finally, we can also recommend that bio-enzymes which had sodium potassium concentration ratio greater than one should not be applied in plant cultivation on sodic soil.

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