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EFFECTS OF BIOFERTILIZER AND OLIGOCHITOSAN IN INCREASING NITROGEN USE EFFICIENCY (NUE) IN *BRASSICA JUNCEA*

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ABSTRACT

Brassica juncea is one the most popular vegetables cultivated in Malaysia. The effects of M99 biofertilizer and oligochitosan in increasing nitrogen use efficiency (NUE) in Brassica juncea (mustard) was investigated. Biofertilizer is a mixture of microbes containing major nutrient-providing microorganisms. Oligochitosan on the other hand acts as an elicitor that has been proven to be very effective in controlling disease infections and improving crop productivity. Five treatments namely control (C), farmers practice (FP), biofertilizer (B), oligochitosan (O) and biofertilizer with oligochitosan (BO) were applied on the plants. For FP treatment, nitrogen, phosphorus and potassium (NPK) fertilizer were used. All of the treatments were applied onto plants at week three and four after sowing. In addition to NUE, data on plant yield in tonne per hectare was also collected. From this study, highest NUE (20.02%) was obtained through BO treatment whilst FP treatment was observed to produce the highest yield (40.22%) among other treatments. From this finding, combination of BO and FP applications are suggested to be used. However further investigation is needed in order to see the effect on yield and efficient use of plant nutrients.

Keywords/Kata kunci: Biofertilizer, oligochitosan, *Brassica juncea*, NUE.

INTRODUCTION

Brassica juncea (B. juncea) or mustard is a vegetable that is widely grown and sold in the daily market, supermarket or grocery store. This vegetable is also easy to grow and has short planting period of about 30 days. Mustard can be grown commercially in an open field, under a rain shelter or in polybags using fertigation or hydroponic systems. Besides, it can also be planted in mini vegetable garden using urban farming methods (urban farming) which aims to be marketed to the public to increase family income. High nutrient content in mustard such as omega-3 alpha-linolenic acid is important for cardiovascular and stroke risk reduction (Blondeau, 2016).

The *B. juncea* plant adapts well in hot and cold weather so it can be planted in both highland and lowland areas. The growth of mustard plant is influenced by internal and external factors (Istarofah & Salamah, 2017). The internal factors consist of heredity, enzymes and hormones whilst external factors are light intensity, water adequacy, temperature and minerals. Another factor to be considered for plant growth is nutrients which only can be obtained through fertilization. Fertilization is important to prevent macronutrients and micronutrients deficiency in plants (Zydlik et al., 2022).

There are two types of fertilizer recommended by the Department of Agriculture Malaysia for *B.juncea* growth namely organic fertilizer and NPK fertilizer (farmers practice) (DOA, 1998). Organic fertilizer application is important to enhance the development of root system as well as nutrient absorption in *B.juncea*. Whereas, the chemical fertilizer contains high nutrient content than is essential for plant growth. The release of plant nutrients from chemical fertilizer is faster than organic fertilizer due to their solubility and capability of penetration into the soil (Sharma & Chetani, 2017). Therefore, chemical fertilizer is used at certain stages of growth, for example 10 to 20 days after planting, flowering stage and fruit formation to ensure the nutrients are fully utilized by the plant at each development stage (Jena et al., 2020).

The application of chemical fertilizer such as NPK is obviously the enormous cost associated with the crop production (Fang et al., 2021). Moreover, excessive use of the chemical fertilizer can causes more damage to the environment. For instance, the surplus of nitrogen lost in volatile form which is about 71 to 83% (Zeng et al., 2021), such as ammonia and nitrite or nitrate gases, will pollute the atmosphere, or leach into groundwater and runoff into surface water (Cai et al., 2015). Therefore, it is necessary to improve the nitrogen use efficiency, which will be environmentally and economically beneficial.

The use of microbial inoculants or biofertilizers has the potential to reduce the use of conventional chemical fertilizers (Saeed et al., 2015). Many of theese biofertilizers can efficiently use available nitrogen and help to access nutrients such as phosphorus (P) and nitrogen (N) from organic fertilizers and soil stocks, which subsequently help improve plant health and increase its yield (Schütz et al., 2018). In previous study by Ajeng et al. (2020), they found that the use of 30% biofertilizer and 70% of chemical fertilizer increased the growth of palm oil seedlings and soil nutrient status. According Kumar et al. (2022), biofertilizer also plays a crucial role in enhancing plant biomass and cropyield under greenhouse and field conditions. The mechanism of plant growth improvement include increased avaibility of nutrients, phytohormone modulation, biocontrol of phytopathogens and amelioration of biotic and abiotic stresses.

The M99 biofertilizer is a new local liquid biofertilizer as an addition to the fast-growing biofertilizer enterprises worldwide. It is a plant and soil microbes friendly. It contains one type of beneficial microorganism namely *Pseudomonas putida*. It can fix atmospheric nitrogen, solubilize phosphate and potassium thus making the macro nutrients available for plant uptake. Previous study showed that combination of M99 biofertilizer and radiation processed chitosan has increased the potential of nutrients uptake by brassica plant in greenhouse condition (Abdullah et al., 2019).

Oligochitosan is an alternative plant supplement which acts as an elicitor that is proven to be very effective in controlling disease infections and improving yield productivity (Yin et al., 2010). It is derived from the degradation of chitosan using gamma radiation. Chitosan is a type of elicitor that attach to special receptor proteins located on plant cell membranes in order to trigger bioactivities mechanism for plant benefits (Li et al., 2020). The raw material of oligochitosan is chitosan which is produced from the exoskeleton of crustaceans (shrimps, crabs), insect and also the cell wall of fungi and algae. Chitosan application in some vegetable plants resulted in an increase in yield by 20% (El-Tanahy et al., 2012).

In this study, we conducted a quantitative evaluation of the pertinent literature in the form of a meta-analysis. The objective was to quantify the effect of M99 biofertilizer and oligochitosan on the crop performance indicators such as yield and NUE.

MATERIALS AND METHODS

Plant Materials

B. juncea or mustard seeds were used in the study. The seeds were sown on filter papers in laboratory until emerged. The two week old seedlings were transplanted into pots filled with soil mixture containing topsoil, compost and sand with a ratio of 2:1:1. Plants were allowed to acclimate for a month in a glass-house with a 12 hour photoperiod, 32 ± 4 °C day temperature and 28 ± 4 °C night temperature.

Fertilizer Application

Five different fertilizer treatments were applied in this study namely control (C), farmers practice (FP), biofertilizer (B), oligochitosan (O) and biofertilizer and oligochitosan. The labelled were T1, T2, T3, T4 and T5 respectively. There was no fertilizer application for the control whilst NPK fertilizer with a ratio of 15:15:15 was used for farmers practice treatment. The amount of NPK fertilizer, biofertilizer and oligochitosan applied in the study were 0.59 tonne/ha, 10 litre/ha and 4 litre/ha respectively. Treatments were applied at week 2 and week 3 after sowing. For nitrogen use efficiency analysis, additional fertilizer which is ^{15}N-labelled fertilizer was used in each treatment group with the rate of 20 kg N per hectare. The labelled fertilizer was used as a tracer to calculate the nitrogen use efficiency (NUE) at the end of the study.

Measurements

Plant Yield

In each treatment, ten plants were harvested at the end of experiment. Total plant biomass were weighed to obtain the overall fresh weight. The plant yield was recorded in the form of mean value for further analysis. The plant vegetative parts were separated and the fresh weight of each was measured. Stem, leaves and roots were oven-dried at 70°C until constant weights were obtained. The dry weight of each component was determined and the parts were finely milled to reduce the particle size and to ensure the homogeneity of the samples.

Nitrogen Use Efficiency (NUE)

The samples were ground to a fine powder and kept in an airtight container prior to analysis. The N content and ^{15}N isotope were determined by using Elemental Analyzer Isotope Ratio Mass Spectrometer (Sercon, United Kingdom). Powdered samples (2 mg) were weighed into tin capsules, folded and compressed to contain the sample and dropped into the autosampler of the analyzer for the analysis. The final result is automatically calculated by the analyzer software and the values were rounded to two decimal places.

The formula to calculate the NUE is shown below (IAEA, 2008):

$$^{15}\text{N yield} = \frac{\text{yield (kg per ha) x total N (\%) of yield}}{100}$$

$$\text{\% Nitrogen derived from fertilizer } (Ndff) = \%Ndff = \frac{^{15}N_{grain}}{^{15}N_{Fertilizer}} \times 100$$

$$Ndff = \%\, Ndff \times N\ taken\ up\ by\ crop$$

$$\text{Nitrogen Use Efficiency (NUE)} = \text{FNUE} = \frac{Ndff}{\text{Total fertilizer N}_{\text{applied}}} \times 100$$

RESULTS AND DISCUSSION

B. juncea Yield for Different Fertilizer Application

The highest yield for the *B. juncea* plant was obtained from T2 treatment which was FP application ($p<0.05$). The calculated average yield in tonne per hectare (t/ha) is approximately 40.2 t/ha. This was followed by T5, T3, T4 and T1 treatment with average yield of 34.34, 28.90, 25.70 and 24.56 t/ha respectively. Figure 1 shows the average yield in different treatment. The results were in accordance with Fonge at al. (2015) who reported NPK treatments resulted in best yield in leafy vegetables. Besides, BO treatment (T5) also generated plant with high yield (34.3 t/ha) significantly similar with FP ($p<0.05$). Previous study by Berger et al. (2013) found that biofertilizer and chitosan product treatment increased the yield in cowpea plant. Chitosan has shown great efficacy in combination with other fertilizers without affecting the soil's beneficial microbes. Besides, it is helpful in reducing the fertilizer losses and disease infestation due to its coating ability (Sharif et al.,2018). Thus, the availability of sufficient fertilizer combined with less stress factor from plant pathogens will help increase the yield production in the plant (Liliane & Charles, 2020). Furthermore, BO and FP treatments show significant higher than control in the study. This result is in accordance with Schütz et al. (2018) and Saidou et al. (2012) who stated that biofertilizer and farmer's recommended fertilizer application practice are important for sustainable soil fertility.

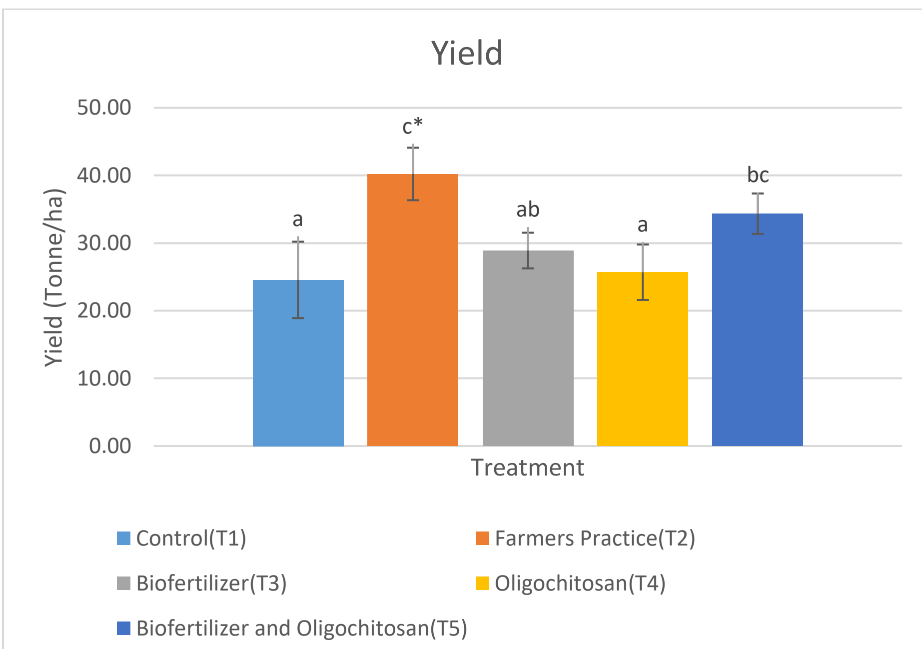

Figure 1: Average plant yield in different treatments. c* indicates significant difference ($p<0.05$) compared to other treatments.

Nitrogen Use Efficiency (NUE) in *B.juncea*

Figure 2 shows the average NUE in different treatments. The highest NUE was observed in T5 treatment (BO treatment) with an average of 20.02% (p<0.05), followed by T3, T4, T2 and T1 treatment with average of 15.07, 11.98, 11.18 and 10.16% respectively. M99 biofertilizer contains of *Pseodomonas putida* which is a beneficial microbe that has the ability to fix atmospheric nitrogen and enhances the availibility of the nitrogen for plant usage (Nur et al., 2020). The advantages possesses by this biofertilizer allow the plant to use the nitrogen efficiently and minimizing potential losses into the environment (through leaching, volatilization, surface runoff or denitrification) as well as sustain high yield production (Qiu et al., 2022). Combination with oligochitosan improved NUE value in *B. juncea*. Chitosan treatment enhances the physiological response that influence plant ability to absorb and utilize nutrients under various environmental and ecological conditions (Hidangmayum et al., 2019). Furthermore, the BO also is significantly show significant higher than control among all of the treatments used in the study. This is in accordance with Qiu et al. (2022) and Javed et al. (2022) who stated that the application of biofertilizer and oligochitosan is effective in improving nitrogen use efficiency (NUE) with lower soil degradation.

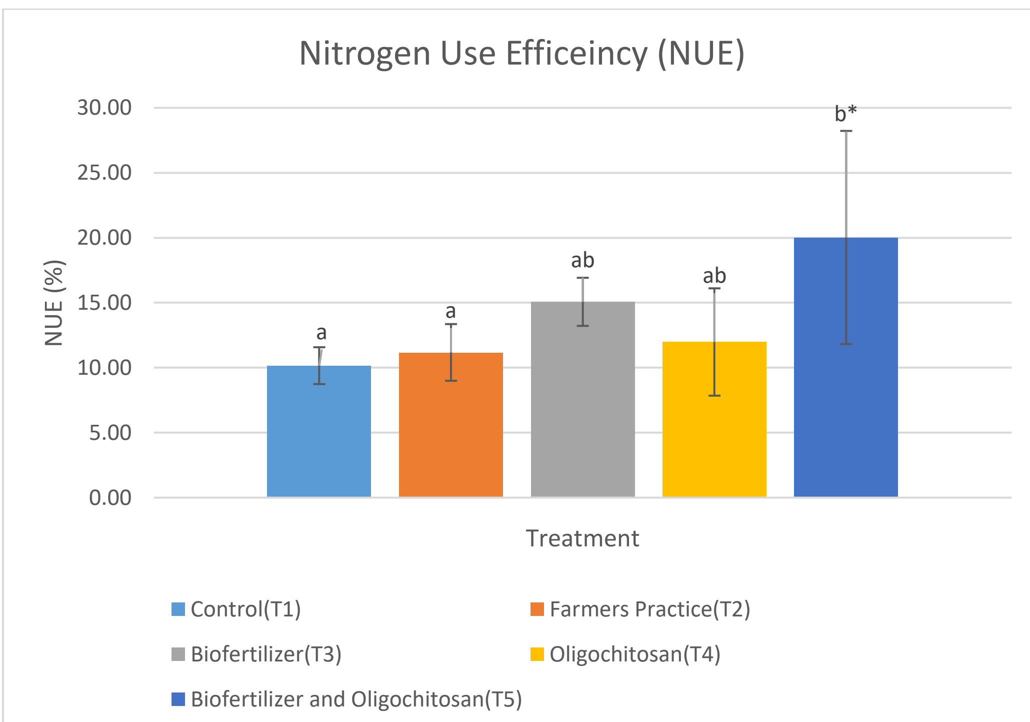

Figure 2: Average plant NUE in different treatments. b* indicates significant difference (p<0.05) compared to other treatments.

CONCLUSION

Based on the findings from this study, the FP treatment was clearly increased the yield of *B. juncea* plant whilst BO treatment gave the highest NUE value. The BO treatment was recommended to be used for the plant development since it has shown great potential in increasing both factors, yield and NUE. Furthermore the synergistic effects by BO were also able to minimize the over-use of N fertilizers that leads to severe pollution of the environment as well as reducing farmer's income.

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