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Effect of agriculture waste materials application on Kenaf (*Hibiscus Cannabinus*) growth and resistancy against root knot Nematode disease

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BRIS soil fertility and the infestation of root-knot nematode are the main challenge in Kenaf (*Hibiscus cannabinus*) plantation at Malaysia. The growth of kenaf is largely depended on the soil nutrient level and its resistancy against the infestation by pests and disease. This study was designed to determine the best soil treatment using agriculture waste materials for kenaf cultivation. The experiment was conducted by using Randomized Complete Block Design (RCBD). Plant was grown at 5 meter x 5 meter plot area for each treatment and replicated 10 times. Soil was treated with biochar, cow dung, organic compost, and commercial fertilizer. The efficacy of those soil treatments was evaluated through the performance of plant growth and its physiological responses, as well as the plant resistance against root-knot nematodes infestation. The results showed that kenaf grown at the field plot treated with cow dung has better plant growth performance compared to the plants grown in other treatments. The application of cow dung was found significantly enhanced the height of the plant, chlorophyll content, stalk diameter, length of the stalk, dry weight and fiber content of the kenaf plant. In addition, the plant roots at the plot treated with cow dung is free from nematode infestation. As a conclusion, kenaf grown at the field plot treated with cow dung has the best growth performance, contains more fiber and free from root-knot nematode infestation.

Keywords: Kenaf, agriculture waste materials, plant performance, root knot nematode

INTRODUCTION

Kenaf (*Hibiscus cannabinus*) is an annual herbaceous plant that belongs to the Malvaceae family. This plant is closely related to cotton, okra, and hollyhocks (Su et al. 2004). Kenaf was planted for its fiber and as the animal feed. Kenaf fiber and core were commonly used to produce environmental friendly products such as paper products, absorbents materials, bio-composites, bio-plastics, building materials such as medium-density fiberboards, textile, and furniture. Kenaf plants is also processed into high-protein animal feed for livestock industries (Ammar et al. 2020). Many researches show that the vegetable oil produce from kenaf's seeds has high omega antioxidant content, which can be used in cosmetics, industrial lubricants, and biofuel (Tan, 2013). Kenaf absorbs more carbon dioxide from the atmosphere than any other crop (Saba et al. 2015). Every hectare of kenaf consumes approximately 30 to 40 tons of carbon dioxide in each growing cycle which is the same amount of carbon dioxide emitted by 20 cars emit in one year (The Asian Post, 2019).

Kenaf was first introduced in Malaysia in the early 70's and its importance were highlighted in the late 90's to replace the tobacco crop. According to the statistic book of the National Kenaf and Tobacco Board (2018), the total kenaf's plantation area in Malaysia is approximately 1,338.4 hectares (ha) with 679 growers. The largest plantation is at Kelantan (507.3 ha), Pahang (422.0 ha) and Terengganu (305.1 ha). The kenaf variety used for seed and fiber production by the National Kenaf and Tobacco Board (NKTB) is V36. This kenaf variety takes approximately 90 to 120 days to reach its maturity with a flowering rate of 10%. Record shows that the total production of kenaf dried stem in 2018 is 10,011.8 ton and 4.342 ton for seed production. To date, the sales price for Kenaf's seed is approximately RM15/kg or RM500/tons.

The kenaf plantation area at Kelantan, Pahang and Terengganu are dominated by BRIS soil. According to Armanto et al. (2013), sandy soils or BRIS (Beach Ridges Interspersed with Swales) soils in Peninsular Malaysia are mostly found near the coastal area in Terengganu with an area of 67,582.61 ha, in Pahang around 36,017.17 ha and in Kelantan about 17,806.20 ha. The characteristics of BRIS soil is a result of excessive accumulation of sediments and sand from the undulating sea during the monsoon seasons that carry along coarse sand particles (Ishaq et al. 2014). BRIS soil is a problematic soil with low physical, chemical and biological properties, limited ability to support crop growth, poorly structured, and low water retention; thus make it unfertile for agriculture purposes. Therefore, this soil should be treated to increase its fertility and productivity before can be cultivated with crop plants.

Insect infestation and disease infection are common in the BRIS soil plantation area. In the Besut area, sweet corn grown at BRIS soil area reported attacked by at least thirteen species of insect pest and two types of fungi (Sulong et al. 2019). In addition to that, one of the major problems for the crop grown at BRIS soil area is an infestation by nematodes. Root-knot nematode, which are from Meloidogyne species is plant parasite nematode, that commonly found attacking plant at the BRIS soil area. This nematode possesses a stylet for injecting secretions as well as ingesting nutrients from host plant cells. As a result of nematode feeding, large galls or "knots" can form throughout the root system of infected plants. Galled roots have a limited ability to absorb and transport water and nutrients to the rest of the plant. Severe infections result in reduced yields on numerous crops and can also affect consumer acceptance of many plants, including vegetables (Mitkowski & Abawi, 2003).

The Kenaf industry has a great potential that can be developed as a new source of income for Malaysia. However, the kenaf production yield and quality are low as this plant is grown in the BRIS soils area in Malaysia (Basri et al. 2014). BRIS soil is known as unfertile soil and crop planted in this area are easily infected with the nematode (Roslan et al. 2011). Even though kenaf is one of the important commodity crops in Malaysia, not much researches were done to improve the soil fertility for kenaf cultivation; and to solve the nematode infection problem in enhancing plant growth. The effect of organic matter added into the BRIS soil in controlling nematode infestation is unknown. The usage of Effective Microbes (EM) to influence plant growth, yield, quality, and resistancy against pests and disease is also not properly studied in kenaf cultivation. A lot of waste materials from the agriculture industry such as paddy husk and animal dung were dump and not utilized properly (Goodman, 2020). The objective of this study is to determine the best soil treatment using agriculture waste materials through the evaluation in the plant growth performance and its physiological responses. The effect of soil treatment on the incidence of nematode infestation on kenaf was also evaluated.

MATERIALS AND METHODS

Plant materials and experimental design

The seeds of kenaf were supplied by the National Kenaf and Tobacco Board, Merang Station, Terengganu. The experiment was done from November 2019 until February 2020 at the Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Terengganu Malaysia (5.7537° N, 102.6265° E).

This experiment was conducted by using Randomized Complete Block Design (RCBD) with five treatments and replicated 10 times. The total area for experiment is approximately 25 m x 25 m with planting plot is 5 m X 5 cm per treatment. Each plot was divided into 5 rows with four plants were planted per row. The treatments are (1) 100 grams (g) of commercial NPK fertilizer, (2) 35 g of biochar derived from rice husk biochar (RHB), (3) 230 g of cow dung, (4) 235 g of organic compost made of spent mushroom compost treated with commercial effective microbe consortium (BOMO Super), and (5) untreated plot.

Plant preparation

Kenaf seeds were soaked in tap water for 30 minutes in order to select the viable seeds. The unviable seeds that float on the surface of the water were removed. The viable seeds then were sown in the seeding tray contain Superb Agro media (Agropolis UniSZA, Malaysia); watered and kept in closed black plastic to maintain the moisture. After three days, the plastic cover was removed and trays containing kenaf seedlings were put under sunlight for the hardening process before transplant into the field. Ten days old kenaf seedlings were transplanted at the respective plot for the experiment. At the beginning of the experiment, 20 kenaf plants were planted in each plot; but only 10 plant that looks healthy and free from any symptom of disease were selected randomly for analysis. Kenaf plant were harvested after 103 days when reaching its maturity.

Determination of plant traits

Plant height is measured by straightening the plant to its fullest length. The diameter of stalk and chlorophyll content are taken a day before the crop is harvested. The diameter of the stalk was measure by using Vernier caliper while the chlorophyll content data taken by using the handheld chlorophyll content meter (SPAD-502 plus, Japan). The second uppermost collared leaf was selected for chlorophyll content determination. Data were taken from 11 am to 1 pm to avoid wetness effects on the leaf surface (Lah et al. 2011). The age of the plant when start to produce the first flower was counted. After harvested, the diameter and length of the stalk were measured. After that, the fresh plant mass was oven-dried in the laboratory at 52°C until it reached the constant weight.

To measure the fiber content, the kenaf bark was soaked in 0.85% Triton X-100 (wetting agent) at a fiber-to-liquid (w / v) ratio of 1:10 for one hour. The bark was removed and immersed in 7% Sodium hydroxide (NaOH) and 0.5% sodium bisulfite ($NaHSO_3$) (w / v), and the fiber to liquid ratio increased to 1:20. The solution was boiled for one hour, with fibers submerged in it. The fibers were removed and washed in hot water until the water ran clear, then were submerged quickly in a 0.2% acetic acid (v / v) solution for two minutes and again washed thoroughly in hot water. The resulting fibers were air-dried, combed and weighed by using analytical balance (Radwag AS 220.R2, Poland).

Nematode disease Incidence and Severity

Roots of the kenaf plant were collected and clean to observe the nematode gall. The quantification of nematode infestation was modified according to Tahery et al. (2011). The severity of nematode infestation was determined by calculating the number of root galls and scored according to the Table 1.

Table 1: Index score on gall presence on the root of kenaf

Score	Roots galls	Score	Roots galls
0	No galls	11	501-550
1	1-50	12	551-600
2	51-100	13	601-650
3	101-150	14	651-700
4	151-200	15	701-750
5	201-250	16	751-800
6	251-300	17	801-850
7	301-350	18	851-900
8	351-400	19	901-950
9	401-450	20	951-1000
10	451-500	21	>1001

Statistics Analysis

The R software was used to analyze all the data. The data obtained were analysed by using one-way ANOVA. The significance difference between treatments was compared by using LSmeans and separation by post-hoc Tukey.

RESULTS AND DISCUSSION

Evaluation on the plant growth performance and physiological responses.

Effect of different soil treatments on plant height.

The result obtained showed that the height of plants is significantly differs between treatments ($F= 89.227$, $P<0.001$). The age of plants also significantly influenced the plant height

(F=364.762, P<0.0001). Overall, the plant height in all treatments gradually increased from week 1 to week 13 (Fig. 1). Based on the result, plants grown at the plot treated with NPK and cow dung are the highest (>200 cm) while the shortest is a plant grown at the untreated plot (<50 cm). Plant at the plot treated with compost fertilizer has the rapid growth from week 2 to week 8; however, the growth becomes slower after week 9. This finding are supported by Zaman et al. (2017) that cow dung application positively influenced the plant growth as it increases the total N, available P, exchangeable K, Ca, Mg, available of S, Zn and B content in the soil. In addition, Dolorima et al. (2018) show that the application of vermicompost as organic manure on the BRIS is recommended for better growth and yield of watermelon. Results obtained show that plants grown at the plot treated with cow dung can produce almost the same yield as inorganic fertilizer NPK.

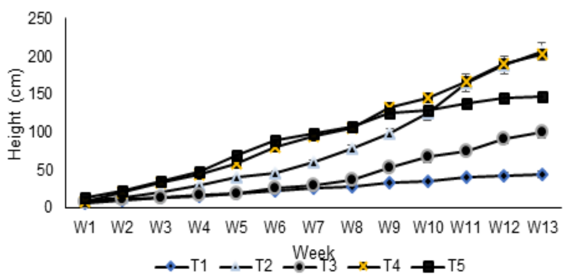

Figure 1: Height of kenaf (±SE) treated with waste materials. (T1) untreated soil; (T2) NPK fertilizer; (T3) Biochar; (T4) Cow dung; (T5) Organic compost as the number of weeks increase

Determination of chlorophyll content in kenaf leaves

Different types of soil treatment significantly influenced the chlorophyll content of leaves (F=16.36, P<0.001). However, there were no significant difference was observed between the plant in T3 and T5 (Fig. 2).

The plant grown at the plot treated with NPK has the highest chlorophyll content followed by the plant grown at the plot treated with cow dung. Plant grown at the plot treated with organic compost and biochar has no significant difference in term of amount of chlorophyll content. The plant grown at the untreated plot has the lowest chlorophyll content; this is because BRIS soil has a low amount of nitrogen (Lah et al. 2011). Photosynthesis is a complex process that is influenced by macronutrient and micronutrient availability in soil (Morgan and Connolly., 2014). Macronutrients and micronutrients that produce from organic fertilizer absorbed by plants; worked in coordination and contribute to chlorophyll structure synthesis (Hossner, 2008).

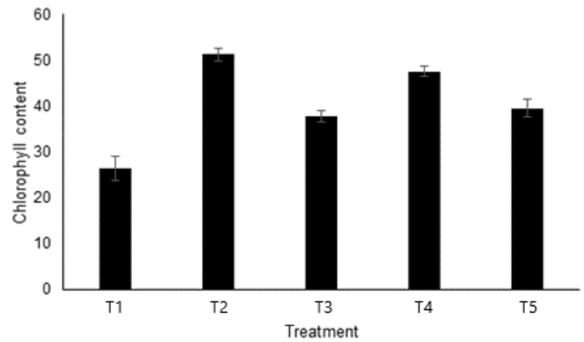

Figure 2: Chlorophyll contents (±SE) in leaves of Kenaf grown on different soil treatments. (T1) untreated soil; (T2) NPK fertilizer; (T3) Biochar; (T4) Cow dung; (T5) Organic compost

Determination of stalk diameter of kenaf plant.

Result shows that the types of soil treatment influenced the stalk diameter of the plant (F= 69.81, P<0.0001). However, no significant difference in stalk diameter was observed between the plant in T2 and T3; and T2 and T4 (Fig. 3).

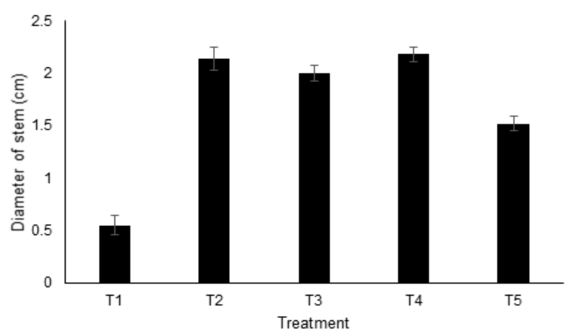

Figure 3: Diameter of kenaf (±SE) treated with different waste materials. (T1) untreated soil; (T2) NPK fertilizer; (T3) Biochar; (T4) Cow dung; (T5) Organic compost

The stalk diameter of kenaf plant influence the yield of kenaf fiber (Hossain et al. 2011). The bigger the size of the stalk, the higher the amount of fiber could be harvested. Commonly, at the age of 3-month-old, the base diameter of kenaf stalk may achieved 3 to 5 cm (Akil et al. 2011). However, it is reported that the environment condition and the soil fertility will influence the performance of kenaf plant (Abdul Hamid, 2009).

Determination of length of kenaf's stalk

Types of soil treatment influenced the stalk diameter of the plant (F=76.83, P<0.0001). Most of the treatments showed significant differences between treatments. However, there is no significant difference was observed between T2 and T4 (Fig. 4).

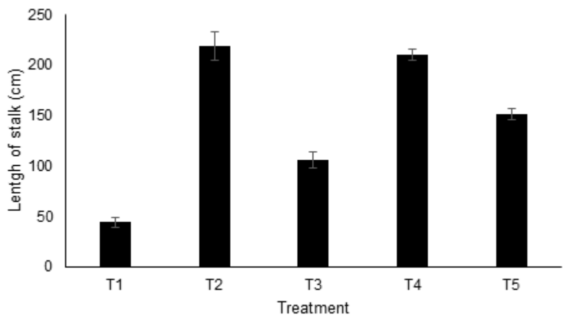

Figure 4: Length of kenaf's stalk (±SE) grown on different soil treatment. (T1) untreated soil; (T2) NPK fertilizer; (T3) Biochar; (T4) Cow dung; (T5) Organic compost

Stalk diameter and its length is important criteria in producing a high quality of kenaf fiber. Both criteria will influence the quantity of kenaf fiber. Results obtained show that the length of Kenaf's stalk at the plot treated with cow dung has a significant effect compared to the plant grown at the plot treated with biochar and organic compost. The plant at the NPK treated plot has the longest stalk (2119.3 cm) followed by the plant grown at the plot treated with cow dung (210.39 cm), organic compost (151 cm) and biochar (106.25). The plant grown at the untreated plot has the shortest stalk (44.26 cm). The plants grown at the plot treated with cow dung produce has a nearly similar length of the stalk with the plants grown at the plot treated with NPK fertilizer. This finding is supported by a previous study by Blanchet et al. (2016) that the addition of manure increased nutrient content C, N, and P in soil. The addition of manure has been proved to increase the population of ammonifying and nitrifying bacteria in soil (Shukla et al. 2008). The integrated application of organic and inorganic amendment on crops could effectively gain a high yield of production (Khairuddin et al. 2018).

Determination of dry weight of kenaf

The dry weight of kenaf was influenced by the different types of soil treatment (F= 56.58, P<0.001). However, there were no significant difference was observed between the plant in T2-T4; and T3-T5 (Fig. 5).

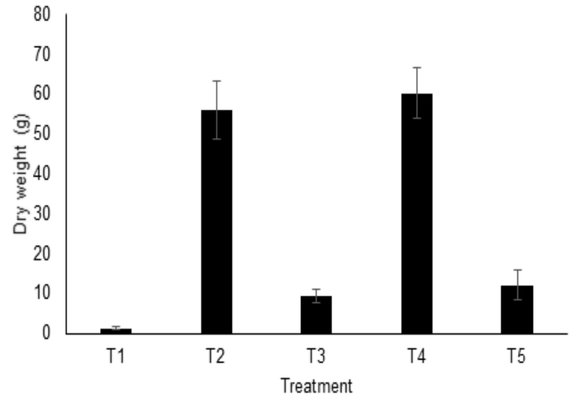

Figure 5: The dry weight (±SE) of Kenaf was treated with different waste materials. (T1) untreated soil; (T2) NPK fertilizer; (T3) Biochar; (T4) Cow dung; (T5) Organic compost.

The plant grown at the plot treated with cow dung has the highest dry weight (60.28g) followed by NPK (55.89g), Organic compost (12.13g) and Biochar (9.23g). Plants grown at untreated plots have the lowest dry weight (1.31g) (Fig. 5). This finding is supported by Njoku et al. (2008) that the increase of dry matter content of *Glycine max* was affected by the application of manure. The result shows that plants grown at the plot treated with biochar produced the lowest dry weight compared to the plant grown at the plot treated with other waste material as the positive effect of biochar may depend on the characteristics of biochar and soil type. The size of biochar is too small and leached since the BRIS soil is larger in size (Liu, 2016). Since biochar itself cannot hold the BRIS soil particle, there are other organic materials needed in the application of biochar. In addition, the organic pyrolytic product may present from biochar such as phenolic and polyphenolics that can give a harmful effect on soil microorganisms (Warnock et al. 2007).

Plant maturity

Based on the observation of phenology, plant grown at the plot treated with NPK treatment took 74 days while cow dung treatment takes 89 days to produce its first flower. However, the other treatment did not produce flowers until day or harvest on day of 103. This support by Gray et al. (2006) that at the best condition, kenaf variety V36 took 72 days to produce a flower. The result shows that kenaf plant grows best at the plot treated with NPK.

Determination of fiber content

The application of waste materials influenced the fiber content of Kenaf. Based on the result, plant grown at the plot treated with cow dung produce (124.14g) higher fiber content followed by the plants grown at the plot treated with NPK (113.3g), organic compost (61.08g) and biochar (19.48g). The plant grown at the untreated plot produced the lowest fiber content (2.8g). The result shows that Kenaf plant grown on BRIS soil will grow best when treated with cow dung.

Effect of different soil treatments on nematode infestation

The result obtained showed that the soil treatment significantly affects the root-knot nematode infestation on kenaf (F= 70.200, P<0.001). However, no significant difference was observed between the plant in T1 and T3, T1 and T4; and T3 and T4 (Fig. 6).

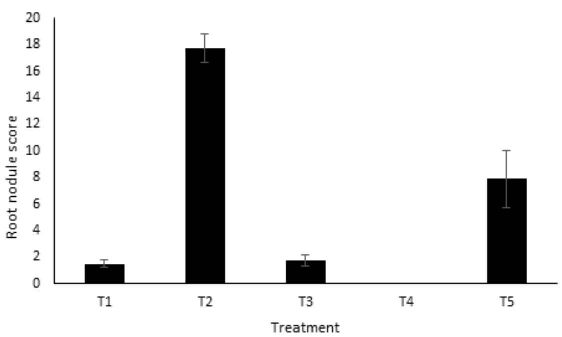

Figure 6: Root-knot nematode infestation (±SE) on Kenaf. (T1) untreated soil; (T2) NPK fertilizer; (T3) Biochar; (T4) Cow dung; (T5) Organic compost

In this experiment, the plants treated with NPK has the highest root knot nematode infection followed with organic compost while untreated soil and biochar have almost the same number of root nodules. Meanwhile there are no root knot nematode galls found at the plant roots in cow dung treatment. This shown that waste material significantly reduces root knot nematode infestation in kenaf. This finding is consistent with Amulu and Adekunle (2015) that the application of poultry manure or cow dung potentially suppresses nematode population density and reduces damage to the plant.

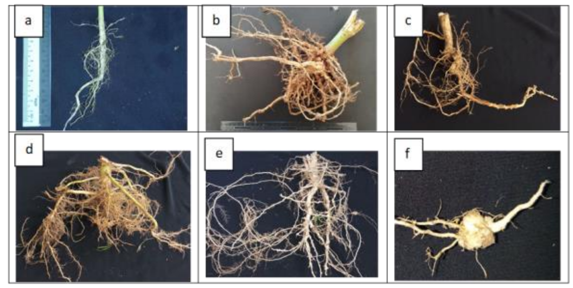

Figure 7: The root of kenaf infected with root-knot nematode at (a) untreated soil; (b) treated with NPK; (c) treated with biochar; (d) treated with cow dung; (e) treated with organic compost and (f) root nodule.

Our findings could be influenced by the activity of soil microbes that facilitated with the organic amendment. This is consistent with the previous study by Orisajo et al. (2008) and Iqbal et al. (2011). Blanchet et al. (2016) found that the addition of manure will increase the soil microbial activity. Figure 7 shows that root from the plant grown at the plot treated with cow dung treatment was healthier with a high density of root hair and many fleshy white tips compare to other treatment. The addition of manure has been proved to increase the population of ammonifying and nitrifying bacteria in soil (Shukla et al. 2008). However, the higher number of infestations of root knot nematode in NPK treatment does not influence its growth.

CONCLUSION

Our finding shows that the application of agricultural waste materials gives a positive impact on the growth of kenaf and at the same time enhances its resistancy against nematode infestation. Among the agriculture waste materials tested in this study, the addition of cow dung into the BRIS soil significantly enhances the growth of Kenaf and reduces the infestation of root-knot nematode to the lowest level compared to other organic compost. Even though the performance of kenaf plants in the plot treated with cow dung is nearly similar to the plant at the plot treated with chemical fertilizer (NPK), the usage of cow dung is much better because it manages to reduce the nematode infestation compared to NPK which is not. Thus, the usage of waste materials as soil treatment in enhancing plant growth and controlling the root-knot nematode is recommended in an aspect of economic, health and environment. Further study should be done to investigate the nutrient content that available in that agriculture waste materials and how it influences plant physiology in order to explain the importance of that nutrient for plant growth. Findings from that study will help researchers and farmers to understand how the plant nutrient will help plant build their resistancy against pests and disease. This research will help NKTB to develop a suitable Standard of Procedure (SOP) for Kenaf cultivation that will help the farmers to grow a healthier plant, thus gain more profit. It also will give a value-added to some waste materials such as cow dung, biochar and organic compost where it can be recycled as an agriculture product. Owners of animal farms and paddy growers can generate more income if they process their agriculture waste properly; ie as a fertilizer for plant growth. The management of those agricultural wastes will indirectly reduce pollution caused by agriculture sectors.

CONFLICT OF INTEREST

The authors declared that the present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

NN conceived the idea, designed the research methodology, planned the study, supervised the project, performed and interpreted the data analysis, and write the manuscript. SNF, AR planned and performed the experiment, collecting the data and write the manuscript. MHS, TAA, AJZ, SM conceived the idea, interpreted the data analysis, and write the manuscript. MNY, KM conceived the idea and designed the research methodology. All authors read and approved the final version.

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