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Effects of Biochar and organic fertilizer on the Kenaf (*Hibiscus cannabinus*) growth and control of root knot Nematode

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Kenaf (*Hibiscus cannabinus*) is cultivated for its fiber. In Malaysia, kenaf was grown at the Beach Ridges Interspersed with Swales (BRIS) soil area; which was previously cultivated with tobacco plant. BRIS soil is known as unfertile and need to be treated to improve the growth of plant. In this study, the effect of BRIS soil treated with biochar and its combination with other organic fertilizers on the growth of kenaf and its consequence effect on plant resistance against root knot nematode disease were investigated. The design for the experiment was Randomized Complete Block Design (RCBD) with ten replicates. The plot size for each treatment was 5 meter x 5 meter each. Treatments are (1) chemical fertilizer (NPK), (2) biochar, (3) biochar: cow dung, (4) biochar: organic compost and (5) biochar: cow dung: organic compost with the ratio 1 to 1. The plant growth parameters such as plant height, chlorophyll content, plant dry weight and fiber dry weight were measured at the end of the study. The roots of plant were collected to investigate the indirect effect of these treatments on the plant resistance against root knot nematode disease. Despite of having the significantly higher number of root galls, results obtained shows that the application of chemical fertilizer was significantly improved the growth of kenaf compared to other treatments. The plant height, chlorophyll content, stalk diameter, stalk length, fiber content, plant dry biomass and time of flowering is best when the soil was treated with chemical fertilizer. On the other hand, even though the number of root galls at the plot treated with biochar is significantly lower compared to other treatments; the application of biochar alone does not improve the plant growth performance.

Keywords: Kenaf, biochar, organic fertilizer, root knot nematode

INTRODUCTION

Kenaf (*Hibiscus cannabinus* L.) is an industrial crop that has been introduced in Malaysia to replace tobacco crop under the East Coast Economic Region (ECER) program. This crop has been cultivated for animal food, fiber, protein, oil and varieties of allelopathic chemical products for the industries (Malisa et al. 2011). Kenaf has many advantage in term of fiber source compared to hemp, jute and flax as they are differed in production, anatomical properties, stem processing, fiber quality, yield and prices (Karimi

et al. 2014).

According to Paridah et al. (2017), kenaf is suitable to grown at BRIS soil area as it can adapt to a temperate and tropical climate, not environmentally harmful, biodegradable and low-cost. However, kenaf farmers in Malaysia have complained that they need to invest much on fertilizers and pesticides for kenaf cultivation to get the good yield. BRIS soil is poor in soil chemical, physical and microbiological properties characteristics (Malisa et al. 2011). BRIS soil also suffered from nutrient leaching, low organic matter content and high surface soil temperature. BRIS soil consists of 82-99% sand particles with low water holding capacity, low Cation Exchange Capacity (CEC) and low nutrient retention capacity, thus affecting the growth of kenaf (Abdul Hamid et al. 2009). However, the BRIS soil fertility can be improved with the utilization of organic matter amendments (Malisa et al. 2011).

Besides, the infection of root-knot nematode is another challenge in kenaf cultivation. According to Tahery et al. (2011), nematode can grow well in BRIS soil as this type of soil provide appropriate condition for this plant pest. Nematode infection on kenaf plant can cause the great loss in the crop yield production. As an example, the incident of nematode infestation on kenaf plantation at Telaga Papan, Terengganu Malaysia shows that stem yield was decreased for about 3 tons per hectare (Tahery et al. 2011).

The production of kenaf is depend upon soil fertility and nutrient contents. Previous research shows that the yield of kenaf can be improved by adding the organic or inorganic fertilizers into the soil (Syuhada et al. 2016). The application of organic fertilizer such as compost, mulch, or manure is suitable for nutrient retention compared to mineral fertilizer due to its gradual release of nutrient. In addition, the application of biochar into BRIS soil become as new alternative method to improve the growth of kenaf.

The effect of organic fertilizer that stand alone compared to combined organic fertilizer on kenaf growth would be differ from one another. The study conducted by Akbar Basri et al. (2013) shows that the treatments of mixed organic and inorganic fertilizer resulted in higher stem diameter compared to single fertilizer. These supported by the study of Srivastava et al. (2012) where they reported that the combination between chemical fertilizer and organic materials significantly increased the number of leaves, diameter, fresh weight and leaf length of plant.

Therefore, the objective for this research is to determine the best soil treatment through the evaluation of plant growth performance. The effect of these soil treatments on the incidence of nematode infestation on kenaf was also investigated. Findings obtained from this research will help Lembaga Kenaf dan Tembakau Negara (LKTN) to develop suitable Standard of Procedure (SOP) for kenaf cultivation in Malaysia. Thus, it will help farmers to produce healthy plant, increase the yield of production, and gain more profit.

MATERIALS AND METHODS

Plant Materials

The seeds of kenaf were collected from Lembaga Kenaf dan Tembakau Negara (LKTN), Merang Station, Terengganu. Seeds were sown in the tray contained coco peat (Agropolis, UniSZA) as the medium for seed germination. The tray was covered with black plastic for about 2 to 3 days for seeds germination process. After that, the plastic was removed and the seedlings were watered daily. Seventh day old kenaf seedlings were transplanted into the field plot. Plants were watered daily until harvested at day 103.

Field and Experimental Design

The experiment was carried out at the Faculty of Bioresources, Universiti Sultan Zainal Abidin, Besut Campus, Terengganu Malaysia (5.753454, 102.627249). The experimental design employed was Randomized Complete Block Design (RCBD). The size of a plot is 5 meter x 5 meter each. Plot was divided into 5 rows with four plants were planted per row. Experiment was conducted with five treatments with ten replicates. The treatments were T1 (100 g of NPK), T2 (35 g of biochar), T3 (35 g of biochar and 230 g of cow dung), T4 (35 g of biochar and 235 g of organic compost), T5 (35 g of biochar, 230 g of cow dung and 235 g of organic compost).

Measurement of Plant Parameters

The parameters observed are plant height, chlorophyll content, stalk diameter, stalk length, fibre content, plant dry biomass and flowering time. Plant height was measured once a week starting from the day of transplanted until harvested. Meanwhile, chlorophyll content and stalk diameter were observed a week before harvested. The plant dry biomass, fibre content and nematode infestation were measured after the plant harvested.

The plant height was measured from the ground level to the top of the plants. The stalk

diameter was measured by a slide calliper at 1 cm above the base of the plants. The chlorophyll content of the fully expended leaf from the upper part of the plant was measured by using hand held chlorophyll meter (SPAD 502 Plus Chlorophyll Meter, Japan).

Once harvested, the plant stalks were dried in the oven until the weight of the stalk is constant. The plant dry biomass was quantified by using scientific balance (RADWAG AS 220 R2, Poland). The fibre content of kenaf was measured by soaking the bark 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% NaOH and 0.5% sodium bisulfite (w / v) (NaHS03), and the fiber-to-liquid ratio will be increased to 1:20. The solution was boiled for one hour, with fibres were submerged in it. The filers were removed and washed in hot water until the water ran clear, then submerged quickly in a 0.2% acetic acid (v / v) solution for two minutes and again washed thoroughly in hot water to ensure fibre quality. Harvested fibres were air dried in the oven for 3 days to ensure proper dryness. The fibre was combed and weighted by using scientific balance (RADWAG AS 220 R2, Poland).

Nematode Infestation

The quantification of nematode infestation was modified according to Tahery et al. (2011). The plant root was removed from the soil and observed for the present of root gall. 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 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

Statistical Analysis

Statistical analysis was performed by using R software. The data obtained were analysed by using one-way ANOVA and the significance difference between treatments were compared by using LSMEANS and separation by post-hoc Tukey test.

RESULTS AND DISCUSSION

Plant Height

Overall, the result obtained showed that the height of plants are significantly differ between treatments ($F=89.227$, $P< 0.001$). However, no significant difference was observed between plants in T4 (biochar + organic compost) and T5 (biochar + cow dung + organic compost). Figure 1 shows that the plant height is increased by weeks. At the end of experiment, the height of kenaf recorded in this study was in the range of 99.16 to 206.3 cm. The height of kenaf due to treatment is ranked in the following orders: T1 (206.3 cm) > T5 (180.3 cm) > T4 (169.85 cm) > T3 (121.25 cm) > T2 (99.16 cm).

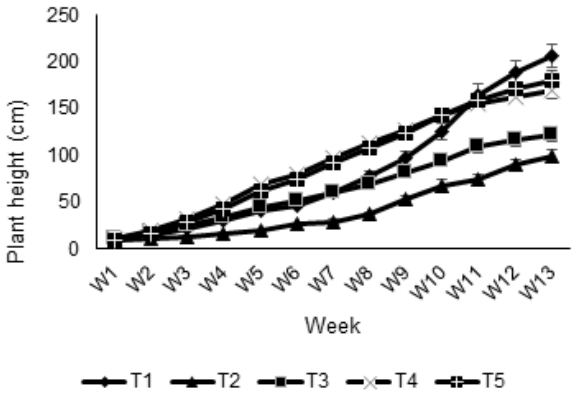

Figure 1: Kenaf height (±SE) in different soil treatment. T1 (NPK), T2 (biochar), T3 (biochar + cow dung), T4 (biochar + organic compost), T5 (biochar + cow dung + organic compost).

Plants treated with NPK fertilizer (T1) is the highest and the shortest is plants treated with biochar (T2). Akbar Basri et al. (2013) reported that biochar is able to retain nutrient, reduce nutrient leaching, improve soil quality and carbon sequestration, thus supply nutrients to the plants. Thus, the application of biochar into BRIS soil may increase plant growth and crop yield (Graber et al. 2010). However, our result indicates that the application of biochar alone gave lower height increment as compared with the NPK fertilizer application.

Organic fertilizer is the slow-release nutrient fertilizer (Gendy et al. 2012); meanwhile, kenaf plant is a fast-growing crop (Hossain et al. 2014). Thus, the application of organic fertilizer at inappropriate time of planting is meaningless; as it cannot be fully exploited by the plant. Meanwhile, nitrogen, phosphorus and potassium contained in the inorganic fertilizer give best effect on plant growth and development. As an example, Brown et al. (1995) reported that the application of NPK increase the okra plant height and the number of leaves. This is because the application of NPK in soil plays an important role in forming molecules of phospholipid, nucleotides, nucleic acid and certain coenzymes that help in plant metabolism; thus, increase plant growth (Hassan et al. 2015).

The commercial organic compost used in this experiment is made from the mushroom based. Seehausen et al. (2017) reported that the organic compost made from mushroom based could be used to treat unfertile soil; where it is proven give positive effects on the plant growth and soil function. The studies also reported that fruit yield was increase when the soil is fertilized with mushroom compost at high dose. In other hand, the application of cow dung could improve the soil physical properties as it is rich in organic matter content; which results in adequate moisture retention in the soil. This confirmed by Dalorima et al. (2018), who found out that the increment of organic matter content, water retention capacity and crop nutrient was achieved by applying cow dung into the soil. Cow dung also could enhance the physical properties of the soil by improving the mineral fertilizer if they were combined together.

Time of flowering

The time of flowering was significantly influenced by the soil treatments. It is took 74 days for the plant treated with NPK fertilizer (T1) to produce its first flower. Plants in treatment T3 took 99 days to produce flower, while plants in treatment T2, T4 and T5 did not produce flowers when harvested at 103 days old. The supply of nitrogen, phosphorus, and potassium fertilization into the soil affects the flowering time of the plants (Ye et al. 2019). This explained our findings as the application of NPK has supplied instant nutrient to the plant compared to the organic fertilizer which are slower release. Early flowering is important for kenaf plant as it enhances the early picking, better returns and also widens fruiting period of the plants (Aseffa, 2019).

Chlorophyll Content

The result obtained showed that types of soil treatment influenced the chlorophyll content of leaves (F= 16.36, P< 0.001). However, no significant difference was observed between plant in T2, T3, T4, and T5 (Figure 2). Chlorophyll content of kenaf was observed higher in treatment T1 (NPK fertilizer) compared to other treatments.

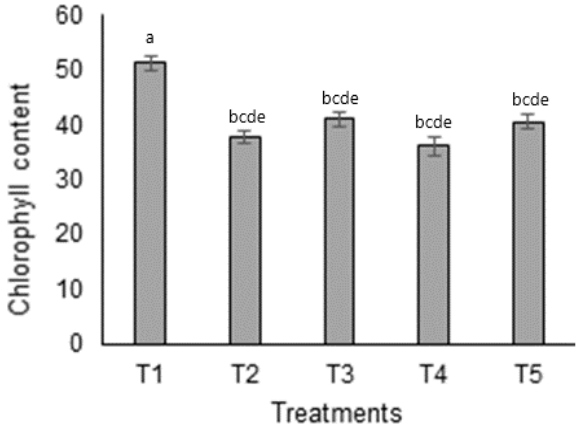

Figure 2: Chlorophyll content (±SE) of kenaf in different soil treatment. T1 (NPK), T2 (biochar), T3 (biochar + cow dung), T4 (biochar + organic compost), T5 (biochar + cow dung + organic compost).

The physiological performance of the plants is the translation of the chlorophyll content which is closely related to the amount of nutrient absorbed by the plant from the soil (Dalorima et al. 2018). Karanatsidis and Berova (2009) stated that the chlorophyll content in plant was positively affected by the type of organic fertilizers. The humic substances in organic manure were responsible for the increment of chlorophyll content observed in plants which may increase the photosynthetic rate in plants (Sayed et al. 2019). The organic matters in the soil affect the opening and closure of stomata which could affect photosynthetic CO^2 fixation in mesophyll tissues (Dalorima et al. 2018). The chlorophyll content in plant is strongly related to the nitrogen concentration in the soil (Sumeet et al. 2009). Nitrogen supply in the soil through the application of NPK fertilizer affect the net photosynthesis by increased light interception. In addition, nitrogen is the important component of rubisco and other protein; thus, it affects the morphology and size of chloroplast (Khandaker et al. 2017).

Stalk Diameter

The result obtained showed that the stalk diameter is significantly differed between treatments (F=16.82, P< 0.001). However, no

significant different on the stalk diameter was observed between T1 and T5; T2 and T3; T3 and T4; T3 and T5; T4 and T5 (Figure 3).

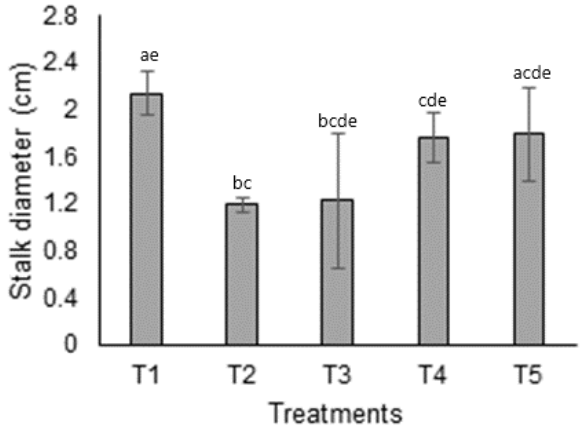

Figure 3: Stalk diameter (±SE) of kenaf in different soil treatment. T1 (NPK), T2 (biochar), T3 (biochar + cow dung), T4 (biochar + organic compost), T5 (biochar + cow dung + organic compost).

The diameter of kenaf stem is influenced by the soil treatments. Plant grown at the plot treated with chemical fertilizer (NPK) showed the biggest stem diameter. The NPK fertilizer is in the form of readily available nutrient; make it easier for plants to absorb the nutrient (Akbar Basri et al. 2013). Mahapatra et al. (2009) reported that the deficiency in nitrogen reduce the plant height as well as basal diameter of jute plants. In addition, Khandaker et al. (2017) stated that phosphorus help stem of okra to be widen or lengthen by increasing the cell division. This led to the increment of stem width and length. The carbon level also affects stem diameter and plant height of different kenaf varieties at different level (Hossain et al. 2014).

Plants in treatment T2 showed smallest basal diameter compared to T5. This shows that the application of biochar alone does not help in kenaf growth. However, the combination of biochar with other organic fertilizers enhances the plant growth. Ahmed et al. (2010) reported that the combination of chicken dung with biochar produced better result compared to the single fertilizer application. In our experiment, there is no significant different on stem diameter was observed on the plant grown at plot treated with T2 and T3. However, we found that the application of organic compost fertilizer were influence the size of stalk diameter. This finding shows that organic compost fertilizer gives better result compared to biochar alone or when it is combined with cow dung.

Stalk Length

The result showed that stalk length are significantly differed between treatments ($F=18.56$, $P<0.001$). However, the stalk length of T2 and T3 and; T4 and T5 are not significantly differs (Figure 4).

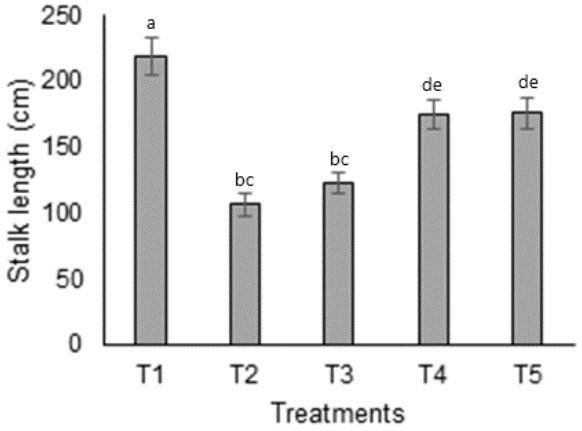

Figure 4: Stalk length (±SE) of kenaf in different soil treatment. T1 (NPK), T2 (biochar), T3 (biochar + cow dung), T4 (biochar + organic compost), T5 (biochar + cow dung + organic compost).

Result showed that plant treated with NPK fertilizer has the longest plant stalk. The application of nitrogen, phosphorus and potassium give positive effects in plant growth and development (Khandaker et al. 2017). It is shows that, under certain circumstances, biochar which has a high absorption ability can lead to reduced availability of nutrients in particular mineralized N or available P for plant (Atkinson et al. 2010). Thus, the combination of biochar and mushroom compost may influence the availability of nutrient; which then influence the plant growth (Seehausen et al. 2017). This explain that the biochar in combination with other organic fertilizer give lower result compared to chemical fertilizer. However, biochar that combined with organic compost gives better growth performance compared to the application of biochar alone.

Plant Dry Weight

We found that the plant dry weight are significantly differs between treatments ($F=23.18$, $P<0.001$). The yield of plant dry matter was increased when BRIS soil is treated with chemical fertilizer (55.89 g); while the lowest yield was in biochar treatment (9.32 g) (Figure 5). As the plant height is increase, the biomass yield produced

also increase. The yield of biomass is directly related with the plant height and leaf area (Aseffa, 2019). The biomass of plant stalk is an important factor in selecting cultivars for kenaf fiber production.

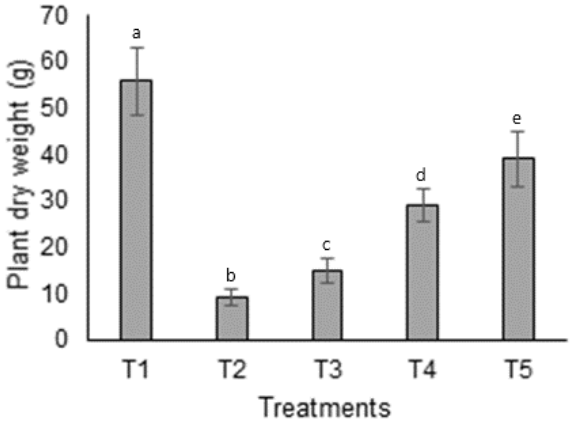

Figure 5: Dry weight (±SE) of kenaf in different soil treatment. T1 (NPK), T2 (biochar), T3 (biochar + cow dung), T4 (biochar + organic compost), T5 (biochar + cow dung + organic compost).

The combination of biochar, cow dung and organic compost gives the high yield compared to the application of biochar alone; or when it is combined with cow dung or compost. Malisa et al. (2011) reported that biochar could not function as fertilizer by itself and it has to be applied together with additional nutrients to maximize its function.

Fiber content

Plants in T1 that treated with chemical fertilizer produced higher amount of total fiber (113.3 g) followed by T5 (93.6 g), T4 (63.7 g), T3 (34.1 g) and T2 (19.84 g). Thus, it can be conclude that the application of inorganic fertilizer gives the highest amount of fiber compared to the plant treated with the organic fertilizers. According to Mahapatra et al. (2009), the quality of fiber can be improved with the application of K nutrient. However, we also found that the combination of organic fertilizers positively effects the fiber content as the combination of biochar, compost and cow dung was performing better than other combination or single biochar.

Nematode infestation on kenaf

The result obtained showed that the soil treatments are significantly influence the infestation of nematodes on kenaf root ($F= 19.33$, $P< 0.001$). However, no significant difference on the attack of nematodes was observed between T3, T4 and T5 (Figure 6).

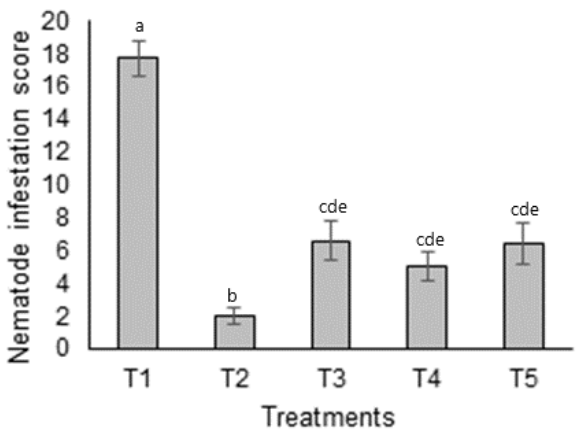

Figure 6: Nematode infestation score (±SE) on kenaf in different soil treatment. T1 (NPK), T2 (biochar), T3 (biochar + cow dung), T4 (biochar + organic compost), T5 (biochar + cow dung + organic compost).

Nematode infestation greatly influences the growth of the plants, quantity and quality of kenaf yield. However, our result shows that the plants in T1 have better growth performance with high quality kenaf yield even though it were greatly infected by root knot nematode. This result is contradicted with the findings reported by Hafiza et al. (2016).

Our result shows that the application of organic fertilizers was more effective in suppressing the gall formation caused by nematode infestation. Studies by Abolusoro et al. (2013) reported that the different application of organic manures significantly reduce root knot nematode population in the soil and improved growth of eggplant. This supported by Summers (2011) that organic manure amendment encourages the multiplication of microorganism such as fungi and bacteria which are able to suppress the nematode in soil and subsequently promoting growth and development of the plants. These good microorganisms would produce toxins that give adverse effects on root knot nematode; thus, reducing the speed of nematode activities, nematode survival and population (Abolusoro et al. 2013).

The least nematode infestation was observed in T2; which is the application of biochar that stand alone. The lower number of nematodes galls may be due to the lower plant growth performance and the root system that do not develop well. In addition, Ibrahim et al. (2018) reported biochar reduce the harmful effect of nematodes which also resulting the decline in galling and improve the growth and yield of tomato.

CONCLUSION

The application of inorganic fertilizer (NPK) had positively affected the growth of kenaf compared to organic fertilizer as kenaf is categorized as fast-growth plant. The plant growth performance showed by the increment in plant height, stem diameter, plant dry weight, chlorophyll content and stalk length. Meanwhile, the application of biochar alone or biochar combined with other organic fertilizer are able to reduce the nematode infestation. Theoretically, the reduction of nematode would improve the plant growth performance, but in this study the infestation of nematode seems not affect the plant growth. Thus, further research should be done to assess of the fertilizer's capacity especially on nutrients in the soil following repeated application. The further research also necessary to improve the understanding on the effect of combination of organic fertilizers.

CONFLICT OF INTEREST

The authors declared that 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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