Effects of Aqueous Neem Leaves Extract in Controlling *Fusarium* Wilt and Growth Performance of *Musa* spp.

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Abstract- *Fusarium*wilt diseases of the banana caused by *Fusariumoxysporum*f. sp. *cubense*(Foc), continue to present major challenges for the growth of this important crop worldwide. Intensive research has led to an increased interested in determining the effect of aqueous neem leaves extract (NLE) on controlling *Fusarium* wilt and growth performance of Cavendish banana (*Musa* spp. AAA group cv. 'Grand Nain'). The 40 ml *Fusarium* fungus solution with a population of 1.25 x 10^8spores ml^{-1} was poured onto the soil media. Next, the solution of 800 ml NLE treatment per 25 kg soil media (4:125) treatment was applied after one month of *Fusarium* fungus application. After 10 weeks of the experimental period, banana plants associated fungus showed significant in all disease assessment, leaves number, root size and distribution as well as dry weight of shoot and root. Moreover, it was observed that the application of NL extract improves significantly in vegetative growth performance (plant height and pseudo-stem diameter), root size and distribution (root diameter, root surface area, and root volume), plant biomass production (root shoot ratio, fresh and dry weight of root) and tend to enhance total leaves number, root length, fresh and dry weight of shoot as well as in decreasing diseases assessment of the Cavendish banana under the present of the fungus in the soil media. Thus, the study shows that the application of NLE solution tended to be beneficial to Cavendish banana plants to withstand *Fusarium* wilt infection and significantly promote better growth of the crops.

Keywords – nee1m leaves extract, *Fusarium* wilt, Cavendish banana, in-vivo

I. INTRODUCTION

Neem leaves are a natural material possess a high content of antifungal compounds such as azadirachtin, desactylimbin, quercetin, and sitosterol[1] [2] and have been reported to be active against *Aspergillusniger*[3], *Candida albicans*[3], *Fusarium*verticilloides[4] and *Fusariumoxysporum*f. sp. lycopersici[5], various pathogen that resulting in serious diseases on different agriculture crops. Besides that, neem leaves also contain several micronutrients, thus it might serve as a good source of nutrition as beneficial crop growth promoter in different agriculture cultivation including banana [6]. To test the efficiency of neem leaves on different agriculture crops growth and development [7][8] [9] [10] [11], influence on the disease's severity [5] [11] [12] as well as effects on soil fertility [7] [11][13], varying extraction methods and extraction solvent of neem leaves were examined by a different researcher. However, information is not available on the antifungal properties of Malaysia's neem leaves extract against *Fusariumoxysporum*f. sp. *cubense* (Foc) which causing *Fusarium* wilt disease on local Cavendish banana plantation. Eventually, the widely dispersed of lethal *Fusarium* diseases resulting in a devastating impact on

crops, resulting in significant wilting problems, economic losses, and threatening 80% of the world's banana agriculture production [14]. In Malaysia, those local areas with Cavendish growth-region such as at Peninsular Malaysia and Sarawak are recorded high occurrence of this disease [15][16].

Keeping in view, the above-mentioned facts, aqueous neem leaves extract (NLE) may have the potential to control agriculture diseases at the same time enhances agriculture productivity. Therefore, the primary hypotheses of this study were aqueous neem leaves extract do not shows any adverse effects in plant growth and morphology, root size and distribution, plant biomass production, and disease assessment of cavendish banana while secondary hypotheses show adverse effects. The objective of this study was to determine the effect of aqueous NLE on controlling *Fusarium* wilt, plant growth and morphology, root size and distribution, and plant biomass production of Cavendish banana.

II. MATERIALS AND METHODS

2.1 Experimental site

The experiment was conducted under a rain shelter house at Field 10, Faculty of Agriculture, Universiti Putra Malaysia (UPM), Serdang, Selangor.

2.2 Preparation of soil as growing materials

The soil mixture was prepared with a combination of topsoil, organic matter, and fine sandy loam at a ratio of 3:2:1. Topsoil and fine sandy loam were obtained from Field 2, Universiti Putra Malaysia (UPM) while the organic matter was supplied by HinFatt Fertilizer Sdn. Bhd. company. An adequate amount of soil media with approximate 25 kg was placed into 50.8 cm × 50.8 cm (20" × 20") polybag for growing Cavendish plantlets under field study.

2.3 Treatment and method of application

The neem (*Azadirachtaindica*) leaves (NL) were obtained from Puchong Field, UPM, Selangor. Aqueous neem leaves extract (NLE) was prepared following the modified method described by [17]as well as [11]. Fresh whole NL was surface-sterilized with 5% sodium hypochlorite (NaOCl) for 10 minutes and then rinsed with sterile water. The leaves were then boiled for 80 min with sterile water to achieve the desired 1:2 ratio concentration. Then, the boiled NLE was filtered through layers of gauze and cooled for 24 hours before use for field experiments.

Meanwhile, *Fusariumoxysporum*f. sp. *cubense* (Foc) fungus Race 4 was isolated in pure culture on Potato Dextrose Agar (PDA) media (Difco^{TM}) from culture collection supplied from Microbiology Laboratory 2, Department of Plant Protection, Faculty of Agriculture, UPM. Moreover, 7-day-old culture *Fusarium* was used to prepare fungus suspension and applied in in-vivo experiments. A 5 mm diameter disc was cut from the petri dish, suspended in distilled water, and mixed well. The suspension was then quantified using a haemocytometer and Foc Race 4 spores were adjusted to concentrations of 10^8spores ml^{-1}.

For the in-vivo experiment, four-month-old of tissue culture Cavendish banana (*Musa* spp. AAA group cv. 'Grand Nain') as planting materials which were supplied by Field Puchong UPM, Selangor. A total of thirty-two Cavendish banana cv. 'Grand Nain' was used for the in-vivo study. Upon received from the supplier, the plants were acclimatized under rain shelter for one week before homogenous selected plantlets were transplanted into polybag containing an adequate amount of soil mixture (25 kg). Transplantation was done early in the morning to minimize high-temperature shock. At the time of transplanting, a 40 ml of *Fusarium* fungus suspension (Foc) with 2.50 x 10^8 spores ml^{-1} was poured into the soil medium around the roots of the banana plants using a method explained by [18], the diseased plant was then considered as negative control (Control -ve) treatment. A healthy plant without any *Fusarium* fungus inoculation was considered positive control (Control +ve) was also prepared and used as a comparison. After 30 days grown in polybag an amount of 800 ml of aqueous NLE treatment was applied per 25 kg of soil media (4:125) under both diseases and healthy together. Plants without NLE treatment (0 ml) was also prepared. Thus, there were four treatments in this experiment such as healthy positive control plants (without NLE), healthy positive control plants (with NLE), diseases negative control plants (without NLE), and disease negative control plants (with NLE). The treatments in this study were arranged by randomized complete block design (RCBD) with the factorial design under four replications (2 plants/replication). The experimental layout was showed in Appendix 3. The banana plants were then be watered manually, protected from weeds, sunlight, and excessive rainfall during the experimental period. Similar culture practices with the same agronomic practices and irrigation

were applied to all experimental plants. The study was carried out for 10 weeks. Throughout the experimental period, related parameter data was collected including disease assessments, plant growth, and morphology parameter as well as soil physicochemical properties analysis.

2.4 Data collections

2.4.1 Plant growth and morphology

2.4.1.1 Plant height, pseudo-stem diameter, and total leaf number

In plant growth parameters, the banana crop growth including plant height, pseudo-stem diameter, and total leaf number, for every one-week interval were evaluated. The height of plants was measured at distances 1 cm above the soil level adjacent to the stem, to the shoot apical meristem of the plants. Moreover, measuring the pseudo-stem diameter of the banana plant was done by using a digital vernier caliper (Model: Vernier caliper series 530) at 5cm above the highest ground point. The number of leaves was recorded at weekly intervals from week 1 until week 10 after transplanting by counting manually.

2.4.1.2 Root size and distribution (root length, root surface area, root diameter, and root volume)

Meanwhile, the banana plants were uprooted at the end of the experimental period. Root size and distribution, including root length (RL), root surface area (RA), total root diameter (RD), and root volume (RV) were measured using a root scanner (Model: EPSON Flatbed Scanner 1680).

2.4.1.3 Plant biomass production (shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, and root shoot ratio)

The whole banana plant such as shoot fresh weight (SFW), shoot dry weight (SDW), root fresh weight (RFW), root dry weight (RDW), and root to shoot ratio (R:S) was also collected for weight assessment. The plant material was then separated into the shoot and root part and rinsed by water to remove the soil while tissue paper was used to remove the water droplet. Immediately, the fresh weight of the plant part was determined by using a measuring balance (Sartorious A and D FX200Iwp, Germany). The plant sample was then placed into the oven before the dry matter content was determined at 70℃. After 3 days, the shoot and root biomass were weighted again by using measuring balanced under low humid conditions to prevent plant samples to take up water from the surrounding environment. Besides that, R:S which represents the vigor and health of the plant is also an important factor. The result for R:S was calculated using the following formula [19]:

$$R:S = (\text{Total root dry weight}) / (\text{Total stem and leaf dry weight}) \quad (1)$$

2.4.2 Diseases assessments

2.4.2.1 Leaf symptom index, leaf disease severity index, leaf damage percentage, rhizome discoloration index, rhizome diseases severity index, rhizome wilting percentage, and reduce in *Fusarium* wilt

The diseases assessments data of banana crops were collected and recorded at the end of the experiment for leaf symptom index (LSI), leaf disease severity index (LDSI), leaf damage percentage (LDP), rhizome discoloration index (RDI), rhizome diseases severity index (DSI), rhizome wilting percentage (RWP) and reduce in *Fusarium* wilt (RFW). LSI represents external wilting symptom on the leaf of banana crops while RDI represents internal *Fusarium* disease symptom that indicated the discoloration scale of the rhizome stellar region was recorded based on a scale presented in Figure1 and Table 1 respectively.

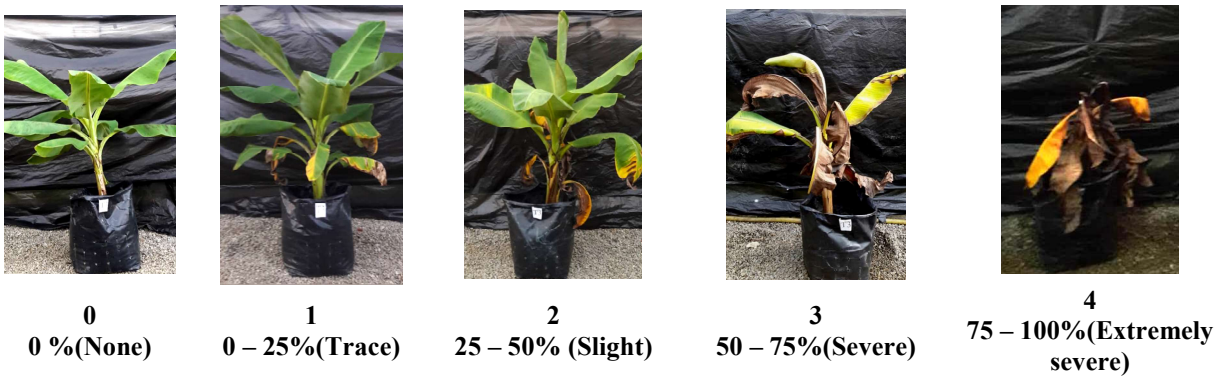

Figure 1- The LSI of Cavendish banana crops [20]. The number represent score and total percentage of wilting symptom on banana leaves.

Table 1- The RDI of Cavendish banana crops [20].

Score	Total percentage of wilting symptom		Photo (Stem- horizontal cut, stem- longitudinal cut and rhizome)
0	0 %	None	
1	0 – 5%	Trace	
1	5 – 35%	Slight	
2	35 – 50%	Moderately severe	
3	50 – 75%	Severe	
4	75 – 100%	Extremely severe	

Both LDSI and RDSI readings were then be taken to calculate the disease severity index (DSI) which represents the overall *Fusarium* disease scale. DSI value was calculated using the formula below:

$$DSI = (ni \times si) / (N \times S) \times 100\ \%(2)$$

Where, ni: number of banana crops with its scale of symptoms, si: the value of the ith scale of symptoms, N: total number of experimental crops, and S: the highest value of the scale of symptoms [21]. The DSI on leaves and rhizome were recorded and classified into six Index based on the severity of Immune (Im) to Very susceptible (Vs) as followed in Table 2.

Table 2- The DSI evaluation of the banana plant [22].

DSI index (%)	Translation
0	Immune (Im)
0 to 5	Resistance (Rs)

5 to 10	**Moderately resistance (Mr)**
10 to 25	**Moderately susceptible (Ms)**
25 to 50	**Susceptible (Sc)**
More than 50	**Very susceptible (Vs)**

Both LDP and RWP indicated *Fusarium* wilt disease incidence for external and internal part respectively by following the formula below:

Wilt(%) = (Number of plants showing wilting symptom)/(Total number of experimental plants) x 100 (3)

RWF shows the percentage of reduction in *Fusarium* wilt disease compare to the diseases-control banana plants which infect by pathogen only without any extra treatment. The reduction percentage (%) was calculated using the following formula:

Reduction (%) = (Index of treated plant) / (Index of diseases-control plant) x 100 % (4)

2.4.2.2 Microbial population

Finally, the soil microbial population was recorded before planting and before the plant to be harvested. The soil microbial population was counted by using the serial dilution plate method which spread, plate, and count of live microorganisms to determine the number of microorganisms in a given soil sample [23]. Firstly, the soil sample was collected from the experimental media at each sampling period (after Foc treatment application period and at the end of the experimental period) and weighed for 10 g. The soil sample was then added into a conical flask containing 95 ml sterile distilled water. Flasks were shaken for 30 minutes using a mechanical shaker to prepare the soil suspension. In the same time, five sterile test tube containing 9 ml sterile distilled water was prepared and label from 10^{-2} to 10^{-6}. 1 ml of the original solution was then pipetted into the first test tube. Next, 1 ml of the diluted suspension from the first test tube was pipette into the second test tube, and so on. The suspension was mixed thoroughly before 1 ml of dilution from serial dilution 10^{-4} to 10^{-6} were collected and spread in a petri dish using a sterilized bent glass rod. All petri dishes contained PDA media that supports the growth of the micro-organisms was incubated at 27 ± 2ºC to undergo colonies count after 7 days of incubation. The data were expressed in the colony-forming unit (CFU) per gram soil sample.

2.5 Statistical Analysis

The data were subjected to the two-way-ANOVA (Analysis of variance) using GLM (General Linear Models) procedures followed by LSD at 5% probability with SAS 9.4 software package, SAS Institute Inc., Cary, NC, USA [24].

III. RESULT

3.1 Plant growth and morphology

3.1.1 Plant height, pseudo-stem diameter, and total leaf number

Application of different NLE significantly alters plant height and pseudo-stem diameter but no significant difference was shown among sick and healthy plants on week 10. In contrast, when comparing the plants under the attack of Race 4 Foc, significant differences were observed in total leaf number but no signature was showed between NLE treatments at the end of the experiment.

From week 1 to week 10 there was a rise in plant height of the Cavendish after treatment application (Figure 2A). Application of aqueous NLE resulted in significantly taller plants for uninoculated plants (Control +ve) and inoculated plant (Control -ve) with value 66.58 cm and 59.22 cm respectively when compared to the absence of the NL treatment on both uninoculated plants (57.82 cm) and inoculated plant (52.63 cm) on week 10. This is consistent with results by previous studies that, aqueous NLE significantly increases the height of the carrot [25], plantain [26], brinjal[27], citrus [10] and maize [7] [11].

In the period between week 1 and week 10, an upward trend was recorded in the pseudo-stem diameter of the treated-fruit crops (Figure 2B). At the end of the experiment, the maximum value of pseudo-stem diameter was observed under *Azadirachtaindica* extract application on both healthy (71.21 cm) and sick (66.73 cm) plants. As a comparison, the plants without NL treatment shows narrower in pseudo-stem diameter with a value 62.52 cm for

healthy plants while 60.13 cm for sick plants. In [7]study, modified neem leaf extracts significantly encouraged growth development with 27.43% and 24.48% increases in stem girth of maize while also in maize and watermelon intercropping. Furthermore, the plantain stem diameter also increased significantly under the aqueous extract of neem leaf with more 58.76% than the control plantain under [26]research.

The fluctuation in total leaf number in inoculated-banana plants applied with NLE throughout 10 weeks experimental period was shown with ranging between 7.38 and 11.83. For leaves number on week 10, crops with NLE treatment had more leaves on both uninoculated plants (11.17) and inoculated plants (10.75) compare to both planting material without NLE treatment on uninoculated plants (8.43) with inoculated plants (7.38) suffered more defoliation among all treatments (Figure 2C). Egunjobi and Afolami[11] also observed that the total number of leaves were minimum in inoculated treated plant and maximum in NLE treated plants althoughthese differences were not significant. Likewise, the results in previous studies showed that there were significant increases in the carrot [26], plantain [26], and brinjal[27]leaf population under NLE treatments respectively.

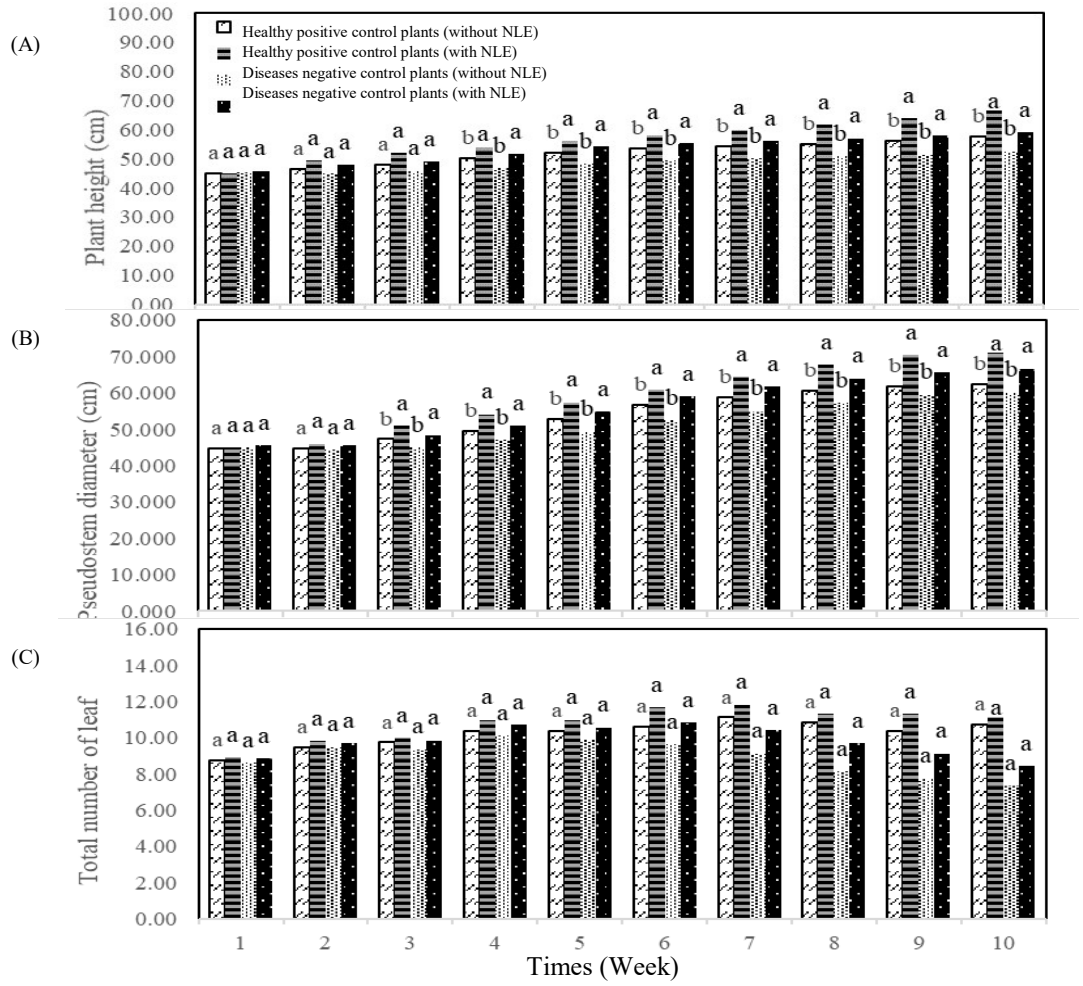

Figure 2. Effect of aqueous NLE on plant height (a), pseudo-stem diameter (b), and total leaf number (c) of Focuninoculated (Control +ve) and inoculated (Control –ve) Cavendish banana plants throughout 10 weeks after planting. Mean values with the same letter are not significantly different using LSD at P>0.05.

3.1.2Root size and distribution

Data on root size and distribution of Cavendish sampling affected by the treatment is presented in Table 3. All NLE treatment applied had a strongly significant effect on root length (RL), root area (RA), root diameter (RD), and root

volume (RV) of the inoculated plant. In contrast, only RL of banana seedlings does not show any adverse effect by NL treatment.

Plantlets tended to have higher root measurements of RL (5174.36 cm), RA (3731.44 cm^2), RD (223.15 mm), and RV (14.574 cm^3) after NLE application. The root assessment data then decreases to plants without any treatment application (positive control) and next to diseases plant with NLE treatment. The lowest root data was recorded on diseases under stress plants (negative control) without NLE treatment with 3294.58 cm, 2213.87 cm^2, 121.54 mm, and 7.08 cm^3 on RL, RA, RD, and RV assessment respectively (Table 4). These results were similar to[11]study that the root system of grain improved significantly underwater extract of NLE treatment. Significant variation was also recorded in [25] experiment that the application of neem leaf powder products gave the longer RL (14.00 cm), as well as higher RD (4.18 cm) of carrot, compare to control RL (10.00 cm) and RD (3.24 cm). The NLE causes a promotive growth on RL of infected *Fusarium* tomato seedlings as claimed by [28]. In the same experiment, tomato seedlings grown in soil media with *F. oxysporum*pathogenic are markedly less in root lengths data than that of the infected seedling. A previous study of the pathogenicity of *Fusarium* pathogen in [29]has reported a reduced root system of soybean crops. While the banana plant exposed to Foc in our experiment also exhibited the same significant observation on all root measurements than the uninoculated plant.

Table 3- Effect of aqueous NLE on RL, RA, RD, and RV of Focuninoculated (Control +ve) and inoculated (Control –ve) Cavendish banana plants

Means followed by the same small and capital letters within a column are not significantly different at (P<0.05) by Least Significant Difference (LSD) with n=32*, **, *** significantly different at P<0.05, 0.01, 0.001, respectively, and NS=not significant.

Table 5- Effect of aqueous NLE on LSI, LDSI, and LDP of Focuninoculated (Control +ve) and inoculated (Control –ve) Cavendish banana plants on week 10

Treatment	Parameter			
	RL (cm)	RA (cm^2)	RD (mm)	RV (cm^3)
Control +ve				
0 ml NLE	4507.99a	3175.34b	189.88b	14.02b
800 ml NLE	5174.36a	3731.44a	223.15a	14.57a
Means	4841.17A	3453.39A	206.51A	14.30A
Control -ve				
0 ml NLE	0.25a	5.00a	Mr	25.00a
0 ml NLE	3294.58a	2213.87b	121.54b	7.08b
800 ml NLE	3873.26a	2995.98a	192.39a	11.71a
Means	3583.92B	2604.92B	156.96B	9.39B
LSD of means at P<0.05n and levels of significance for a two-factor ANOVA				
NLE	ns	**	*	*
Foc	**	**	*	**
NLE × Foc	ns	ns	ns	ns

3.1.3 Plant biomass production

Results of plant biomass production including shoot fresh weight (SFW), shoot dry weight (SDW), root fresh weight (RFW), root dry weight (RDW), and root to shoot ratio (R:S) of the banana crops under 10 weeks experimental period from the treatment can be observed in Table 4. None of the treatments applied had a significant effect on SFW in Cavendish plants. However, NL treatment is applied to differ significantly RFW, RDW, and R:S while Foc treatment shows significant dry shoot data.

The highest SFW, SDW, RFW, RDW, and R:S was recorded in banana seedlings with NL treatment only with value 1335.93 g, 342.56 g, 284.39 g, 55.95 g, and 0.19 respectively whereas the lowest was observed in negative control treatment with value 891.49 g, 194.12 g, 144.16 g, 18.01 g and 0.09 respectively (Table 4). Consequently, positive control has lower plant biomass production but higher SDW when compare to plant under the stress of disease pathogen with NL treatment. A similar observation was made by previous studies on root weight of grain [11], citrus[10], and carrot [25] that increases significantly when exposed to the plot that receiving the water extracts of NL as compare to the control. The respond of *Fusarium* on plant biomass production was also reported by [30] that, eventually both fresh weight (22.890 g) and dry weight (1.815 g) was poor in the Foc-inoculated banana seedlings as compared to the control fresh (36.220 g) and dry weight (2.891 g).

800 ml NLE	**0.17a**	**2.50a**	**Rs**	**16.67a**
Means	**0.21A**	**3.75A**		**20.84A**
Control -ve				
0 ml NLE	**3.25a**	**65.00a**	**Vs**	**100.00a**
800 ml NLE	**2.14a**	**40.00a**	**Sc**	**75.00a**
Means	**2.70B**	**52.50B**		**87.50B**
LSD of means at P<0.05n and levels of significance for a two-factor ANOVA				
NLE	**ns**	**ns**		**ns**
Foc	***	**		**
NLE × Foc	**ns**	**ns**		**ns**

Means followed by the same small and capital letters within a column are not significantly difference at (P<0.05) by Least Significant Difference (LSD) with n=32*, **, *** significantly different at P<0.05, 0.01, 0.001, respectively and NS=not significant. Im = Immune, Rs = Resistance, Mr = Moderately resistance, Ms = Moderately susceptible, Sc = Susceptible and Vs = Very susceptible.

Table 6- Effect of aqueous NLE on RDI, RDSI, RWP, and RWF of Focuninoculated (Control +ve) and inoculated (Control -ve) Cavendish banana plants on week 10

Table 4. Effect of aqueous NLE on SFW, SDW, RFW, RDW, and R:S of Focuninoculated (Control +ve) and inoculated (Control –ve) Cavendish banana plants.

Treatment	**Parameter**				
	SFW (g)	**SDW (g)**	**RFW (g)**	**RDW (g)**	**R:S**
Control +ve					
0 ml NLE	**999.74a**	**312.69a**	**210.66b**	**26.42b**	**0.09b**
800 ml NLE	**1335.93a**	**342.56a**	**284.39a**	**55.95a**	**0.19a**
Means	**1167.84A**	**327.63A**	**247.53A**	**41.19A**	**0.14A**
Control -ve					
0 ml NLE	**891.49a**	**194.12a**	**144.16b**	**18.01b**	**0.09b**
800 ml NLE	**1183.21a**	**254.13a**	**251.94a**	**37.80a**	**0.18a**
Means	**1037.35A**	**224.13B**	**198.05A**	**27.91B**	**0.14A**
LSD of means at P<0.05n and levels of significance for a two-factor ANOVA					
NLE	**ns**	**ns**	*	***	*
Foc	**ns**	*	**ns**	*	**ns**
NLE × Foc	**ns**	**ns**	**ns**	**ns**	**ns**

Means followed by the same small and capital letters within a column are not significantly different at (P<0.05) by Least Significant Difference (LSD) with n=32*, **, *** significantly different at P<0.05, 0.01, 0.001, respectively, and NS=not significant.

3.2 Diseases assessments

3.2.1 LSI, LDSI, LDP, RDI, RDSI, RWP and RWF

*Fusarium*disease severity of banana assesses at week 10 by recording a wilting incident of leaves and discoloration incidence of the rhizome. Table 5 showed leaf disease assessment of *Fusarium* wilt on Cavendish banana as influenced by aqueous NLE. The results of rhizome disease assessment as affected by NLE on Cavendish plants inoculated with Foc were indicated in Table 6. Apart from that, all leaves and rhizome results indicated no significant difference (P<0.05) for NL treatment factors on banana crops inoculated with Foc even-thought significant effect was shown between sick and healthy plants.

On week 10, all leaf symptom index (LSI), leaf disease severity index (LDSI), leaf damage percentage (LDP), rhizome discoloration index (RDI), rhizome diseases severity index (DSI), and rhizome wilting percentage (RWP) assessment was found highest in diseases crops without NL treatment application with value score 3.25, 65.00% (very susceptible resistance), 100.00%, score 3.00, 70.00% (very susceptible resistance) and 100.00%, respectively while the lowest value of score 0.17, 0.00% (immune resistance), 16.67%, score 0.00, 0.00% (immune resistance) and 0.00% was found in healthy crops with NLE treatment. Healthy crops without NLE treatment also shows the lowest rhizome data. In contrast, reduction in *Fusarium* wilt (RFW) shows opposite results with the lowest value of 0.00% in disease plants (without NLE) and the highest value of 100% in both healthy plants (with or without NLE). This is consistent with results by [31] that yellowing and browning in leaves of gladiolus were due to *Fusarium* disease caused by *F. oxysporumSchlecht*. f. sp. *gladioli*. According to [32], the symptom of wilting was clearly shown by a banana plant grown in a field contaminated with *F. oxysporum*f. sp. *cubense*. This is further supported by [33] study that banana clones tested also show high LDSI and RDSI after a few months inoculation of *Fusariumoxysporum*f. sp. *cubense* spore. Ribeiro et al. (2011)[34] also proven that Foc race 1 caused internal necrosis on different banana cultivar such as Silk (susceptible), ThapMaeo (intermediately resistant) as well as Tropical (intermediately resistant) compare to 'Grand Nain' cultivar that was resistant to Foc race 1. Dita et al. (2011)[35] experiment also show similar results at week 1, 2, 3, and 5 after inoculation of Foc race 4 on 'Grand Nain'.

Treatment	Parameter				
	RDI	RDSI (%)	Resistance	RWP (%)	RFW (%)
Control +ve					
0 ml NLE	0.00a	0.00a	Im	0.00a	100.00a
800 ml NLE	0.00a	0.00a	Im	0.00a	100.00a
Means	0.00A	0.00A		0.00A	100.00A
Control -ve					
0 ml NLE	3.00a	70.00a	Vs	100.00a	0.00a
800 ml NLE	2.14a	47.50a	Sc	75.00a	25.00a
Means	2.57B	58.75B		87.50B	12.50B
LSD of means at P<0.05n and levels of significance for a two-factor ANOVA					
NLE	ns	ns		ns	ns
Foc	**	***		***	***
NLE × Foc	ns	ns		ns	ns

Means followed by the same small and capital letters within a column are not significantly difference at (P<0.05) by Least Significant Difference (LSD) with n=32*, **, *** significantly different at P<0.05, 0.01, 0.001, respectively and NS=not significant. Im = Immune, Rs = Resistance, Mr = Moderately resistance, Ms = Moderately susceptible, Sc = Susceptible and Vs = Very susceptible.

However, in the present study, even-though all NLE-treated banana does not show a significant decrease in leave wilting and rhizome discoloration incidence for both healthy and diseased crops, but NLE tends to have a lower value of diseases assessment. In Joseph et al. (2008) [12] study, the researchers still recommended NLE against *Fusariumsolani* f. sp. *melongenae*that causes brinjal wilt in their experiment. As claimed by [27], 40% NLE has significantly reduced the percentage of leaf spot diseases of brinjal plants up to 41.34%. Successful attempts have been made in [5] report that neem treated pots inhibit vigorously the vascular discolouration of tomato. This finding was similar to the result of [36], the southern blight disease of chili with the high dose 4% dry NL amendment in soil was low with disease incidence 30%. The results presented in [37]experiment revealed that maximum mean inhibition on the grayish discoloration of okra stem which was attacked by *Fusarium*solani was obtained in NLE with value of 48.46%.

3.2.3 Microbial population

Significant result was observed for inoculated plant compare to uninoculated plant on both phases (Table 7).Focuninoculated soil media showed colony-forming units (CFU) with the ranged between 0.793 to 0.852 × 10^5 CFU g^{-1} while ranged between 0.929 to 1.036 × 10^5 CFU g^{-1} in the initial and final experimental period respectively. Inoculation of the plants with Race 4 Foc revealed significantly higher numbers of plate count in both starting (ranged between 1.375 to 1.403 × 10^5 CFU g^{-1}) and ending period (ranged between 1.778 to 2.038 ×10^5 CFU g^{-1}). This show that, numbers of CFU was comparatively higher in diseases planted with compared to healthy plant due to the presence of *Fusarium* microorganism in the soil. Yao and Wu (2010) [38] also support that, the microbe populationwas higher in the *Fusarium* wilt susceptible cucumber cultivars.

III. DISCUSSION

The fact that aqueous NLE significantly improved the vegetative growth parameters such as plant height and pseudo-stem diameter of Cavendish banana was proven from the result above while the NLE may also have immense potential in the total leaf number of the crops. The positive effect of NLE could be attributed to the fact that the combined nutrient (N, P, K, Ca, and Mg) in the leaves promoted the vegetative growth of the crops (Figure 2). Moyin-Jesu (2014) [6] also proven that the high amount of combined nutrient such as N (3.56%), P (0.83%), K (1.67%), Ca (0.77%), and Mg (0.75%) can be found in NL Nitrogen in neem leaves may encourage plants to shoot and leaves development during the banana leaf stage [39] [40][41]. Combined nutrients in neem tissue increased available nutrients for crops in soil. Adequate supplies of nutrients will enhance nutrient uptake by crops which thereby stimulated plant vegetative growth and finally may maximizing the inherent resistance of the plant to diseases infection. Stronger plantlets will enhance the immune system of the crops in tolerating infectious pathogens as been mentioned by [42]. Besides that, they further supported that, plant-nutrient interaction also affects the plant's ability in limiting penetration, development, colonization, and reproduction of invading pathogens to minimize the attack of the pathogen. So, nutrition supplied by NLE to the banana plant was an important component in enhancing plant growth and reduced *Fusarium* wilting incidence. Apart from nutrients, plant vegetative growth is also closely related to diseases infection. The general external symptoms on the vegetative part of the infected plants by

Fusarium are usually yellowing, wilting, and drop off of the leaves with the report of [43]. Hence, plant nutrient, vegetative growth, and diseases development proves to be closely related between each other from the experiment.

The data on the maximum root measurement of Musa spp. plants reveal that application with NLE had efficiency on root growth and may protect host plants against races 4 of the Panama wilt pathogen. The promotive effect of aqueous extract of NLs on root growth of the agriculture plants could be due to complex nutrient which acts by providing the effectual nutrient to plants as reported by many authors [6][39] [40] [41]. Akhtar (1999) [44] further documented that the presence of triterpeneazadirachtin in the neem leaves may be delaying and slowing the conversion of ammonium nitrogen into nitrate nitrogen to continuous provide available nitrogen during plant growth development besides acts by delaying diseases development. Besides inhibiting wilting symptoms, the other beneficial effect due to Panama wilt suppression realized in this research is better to root development. A by-product of some toxins by pathogen as well as the accumulation of the harmful phenolic compounds during cell wall degradation of fungal elicitors which affected the root development of the host plant may be avoided by suppression of *Fusarium* wilt had been explained well by [45].

These results highlighted that application of NLE able to improve plant biomass production traits on seedling infected by the Foc pathogen. It could be assumed that enhancing in plant biomass production possibly by complex nutrient provided by *Azadiractin* leaf which increases nutrient availability[44], favor biotic and abiotic soil characteristics for plant growth as well as an increase in diseases resistance in the plant [36]. Henceforth, the protection of the host plant from disease development may also be resulting in better plant biomass development even-though only dry shoot assessment shows the significant result in the present trial. Paul and Sharma (2002) [5] suggest that the treatment of barley plantlets with NLE induced an increase in the activity of host enzymes which led to rendering the crops resistant to diseases and shows better growth development. Equally, *Fusarium* wilt disease suppression could also avoid decreases in morphological expression of hormonal imbalance in crops which in turn lowering abscisic acid (ABA) and increases Indole-3-acetic acid (IAA), gibberellic acid (GA3), and cytokinin accumulation which may affect shoot and root growth and development of the crops as demonstrated by [46]. Plant biomass production could be affected indirectly through the induction of *Fusarium* disease resistance in Cavendish banana by NLE. However, shoot biomass only differed slightly, and not significantly with NL treatments was observed in this study. Kartika et al. (2018) [47] prove that increasing the frequency of fertilizer application will improve nutrient uptake efficiency by plants. Thus, increase the application of NLE to 1-month-interval until the harvesting period may enhance growth characteristics and reduce *Fusarium* wilting effect more significantly compare to the only one-time application during transplanting.

As a result, Cavendish plants with visually noticed in this study shows that *Fusarium* inoculation causes the yellowing and wilting of the leaf might due to the microbe activity which in turn affected LSI, LDSI, and LDP (Table5). *Fusariumoxysporum*f.sp. *cubense* (Foc) is a soil-borne pathogen that infects banana crops via wounds on root tips or around lateral root during the adhesion and penetration stage [48]. The fungal subsequently colonize the root and invades the xylem vessels. Foc can stimulate the plants' immune system, leading to secretion of tylose, clogging of vessels, blocking of water and nutrient transmission, leaf wilting symptoms, and finally plant death. Thus, Foc resulting in higher LSI, LDSI as well as LDP.

At the same time, *Fusarium* wilt controlled using aqueous NLE was possible as it did delay the foliar wilting of the banana plants including in decreasing LSI, DSI, and LDP even-though the result was not significant. Whether these wilting inhibitions of leaves resulted directly from affecting in Foc activity within the soil media or to possible physiological effects on the banana host plant itself or on the media nutrient status is not well understand. But because resulting high LSI, DSI and LDP are known symptoms of *Fusarium* infestation which in turn causing wilting diseases [48], it seems feasible that this could be due to fungicides properties of the NLE used. According to [1] [2] [49] [50], NL antifungal properties was due to the presence of active compounds such as azadirachtin, nimbin, nimbidin, nimbinene and azadirone ingredient in the leaves. Although the details on its compound basis on pathogens are still emerging, the discovery of the antifungal, antibacterial and antiinsecticidal and anti-pesticides properties ushered in a new era of plant pathology. Yet, NLE effect on *Fusarium* disease severity was not always significant may associate with different extract concentrations. Different NLE concentrations could control the disease, but suitable concentration may be more effective and may get more significant results. As reported previously for barley by [5], leaf stripe diseases by *Drechsleragraminea* were minimum in *Azadiractin* leaves with a rate 1:10 extract than 1:100 with LDSI score 11.2% and 30.6 % respectively.

Not only this, but aqueous NLE is also an important treatment in affecting rhizome assessment even-thought the result in this study was not significant. The difference in the effects of the different extract solvent in the severity and incidences of the disease may be due to the solubility of the active ingredient [51] [52]. This finding was demonstrated earlier by [53] that ethanolicneem leaves extracts (3±0.9) exhibited the best control of the rice blast pathogen (*Pyriculariaoryzae*) and subsequent disease symptom followed by cold water (3±0.6) and then hot water extracting solvents (4±0.8). Using a suitable solvent may increase the significant effect of the extract in delaying the rhizome severity.

As depicted in Table 7, none of the NL treatments applied had a significant effect on the microbial population of soil but a slight reduction in the microbe population was found at the end of the experiment. According to [1] [2], NL antifungal properties were due to the presence of azadirachtin compound in the leaves. This is consistence with the research by[17], where supplementation with 2.5 µg mL^{-1}azadirachtin compound in the soil for two months resulting a significant reducing of the microbial population in soil ($4.82\pm0.46 \times 10^5$CFU g^{-1}) compare to control treatment ($9.57\pm0.88 \times 10^5$ CFU g^{-1}). Interestingly, in the same study, 4 g mL^{-1} aqueous neem leaf extract exhibited lower CFU in-vitro but increased the population of microorganisms in soils under in-vivo conditions ($15.95\pm3.23 \times 10^5$CFU g^{-1}). This might probably due to other compounds in the *Azadiractin* extract that enhance or support the growth of the microorganism to some extent.

IV.CONCLUSION

At the end of the experiment, Foc inoculation on Cavendish banana crops resulting significant in all disease assessment leaves number, root size, and distribution as well as dry weight of shoot and root. Meanwhile, it could also be concluded from the research result that NLE gave the significant vegetative growth performance (plant height and pseudo-stem diameter), root size and distribution (root diameter, root surface area, and root volume), plant biomass production (root shoot ratio, fresh and dry weight of root) on both bananas inoculated or uninoculated with Foc. It also plays a possible role in influencing the total leaves number, root length, fresh and dry weight of shoot as well as in decreasing disease assessment although no obvious significant differences existed between treatments.

The resulting hypothesis that *Fusarium* wilt suppression will be maximized by the application of aqueous NLE due to the antifungal active ingredient in it. Furthermore, the complex nutrient in neem leaves will also enhance concentrations of bioavailable mineral nutrient in the soil, altering soil pH level to the optimum value possible with a higher amount of nutrient uptake by crops, impacting positively on plant vegetative growth respond, maximizing plant resistance on pathogen attack, which largely controls the *Fusarium* diseases severity and symptom index.

As a conclusion, NLE had the potential to be an alternative organic fungicide or fertilizer in improving Malaysia banana cultivation and suppression in *Fusarium* wilt diseases. Yet, it is recommended that for better NLE performance, preparing suitable NLE using effective solvent, method, and concentration under favorable field conditions with the more frequent application by paying more attention to plants-nutrient interaction can be taken in noted during future studies to obtain a more significant result.

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