Fundamental and Applied Agriculture
Vol. 6(2), pp. 119–133: 2021
doi: 10.5455/faa.77702

CROP SCIENCE | ORIGINAL ARTICLE

Combined effects of pyroligneous acid dilutions and *Bacillus subtilis* UPMB10 on growth and yield of dwarf long bean (*Vigna sesquipedalis*)

Muhamad Noor Hazwan Abd Manaf [1*], Halimi Mohd Saud [1], Siti Zaharah Sakimin [2], Samsuri Abd Wahid [3]

[1]Department of Agriculture Technology, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[2]Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[3]Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ARTICLE INFORMATION

Article History
Submitted: 30 Apr 2021
Accepted: 26 May 2021
First online: 30 Jun 2021

Academic Editor
Md. Parvez Anwar
parvezanwar@bau.edu.bd

**Corresponding Author*
M N Hazwan Abd Manaf
mnor901@gmail.com

ABSTRACT

The application of pyroligneous acid (PA) and UPMB10 (*Bacillus subtilis*) can improve the growth and yield of dwarf long bean (DLB) without affecting the environment. PA commonly derived from charcoal production as a by-product. Meanwhile, UPMB10 is a plant growth-promoting rhizobacterium (PGPR) isolated from oil palm root. Previous studies investigated the effects of UPMB10 inoculation and PA separately, and no research examined their combined outcome on crops. Therefore, an experiment was conducted to determine the PA's influence on the combined effect of PA and UPMB10 on the growth and yield of the DLB plant. The study investigated the effect of PA dilutions (v/v), control (water), 1:600 (P600), 1:400 (P400), and 1:200 (P200) and UPMB10 inoculation (uninoculated and inoculated) on the growth and yield of DLB. From the study, UPMB10 did not significantly improved the growth and yield of DLB. However, the PA effect was significant toward the yield production, where P400 had significantly promoted the DLB yield by increasing the total yield dry weight, which was positively correlated with the total number of DLB pods. For future research, an investigation on different application timing between PA and UPMB10 could prevent the potential negative impact of PA toward UPMB10 with reduced rates of chemical fertilizer and type of soil used.

Keywords: PGPR, pyroligneous acid, wood vinegar, *Bacillus subtilis*, growth, yield

Cite this article: Abd Manaf MNH, Saud HM, Sakimin SZ, Abd Wahid S. 2021. Combined effects of pyroligneous acid dilutions and *Bacillus subtilis* UPMB10 on growth and yield of dwarf long bean (*Vigna sesquipedalis*). Fundamental and Applied Agriculture 6(2): 119–133. doi: 10.5455/faa.77702

1 Introduction

With just 1% of the world's agricultural area, organic farming is the fastest-growing sector in world agriculture (Seufert et al., 2017). This promising alternative is based on using and enhancing internal natural resources for agriculture productivity improvement to minimise the environment's negative impacts (Deyn et al., 2017). The pyroligneous acid (PA) is one of the biomass pyrolysis products and also known as wood vinegar. It is acidic and has a translucent reddish-brown property (Wang et al., 2019). Most biomaterials, such as tree branches, paddy straw, bamboo, wood residue, can be used as a feedstock for PA production. It comprises many complex organic components and compounds that can benefit many areas, including agriculture, food and medicine (Lu et al., 2019). The utilization of a by-product from any industries helps reduce the impact of carbon footprint and generate income and job opportunities for society. One of PA's benefits in agriculture is the promotion effect on crop plants' growth and yield. The previous researcher reported that the products of rice, rock melon, soybean, sweet potato, tomato, and several other crops had been significantly promoted by the application of PA (Grewal et al., 2018). The PA may be used as foliar sprays or as a soil drench, and there is no significant difference in the effects on any traits between the two types (Mungkunkamchao et al., 2013). At a suitable dilution, PA can promote the soil microbe and the plant growth and yield. But the dilution rate also depends on the crop and environmental condition as well. If the dilution is to dilute, then the effect will not be seen. And for the concentration which is too high, PA's effect will cause stunted plant growth and soil microbes. Use of a proper dilution can also saving cost by preventing the excess PA from wasting.

Generally, the optimal dilution of PA, which promotes plants growth, is in a range of 250-800 times dilution (Shuang et al., 2020). In study, PA application diluted 300 times increased the number of panicles and grain number per panicle of organic rainfed rice (Polthanee et al., 2015). A 0.2% PA (nearly 500 times dilution) also enhanced the growth and yield of MR219 rice (Berahim et al., 2014). Besides, an application of 800 dilutions of PA also significantly promoted the total soluble solutes of tomato fruit (Mungkunkamchao et al., 2013). The total phenolic content of tomato also increased with 250 times dilution of PA (Benzon and Lee, 2016). As compared to the control, lettuce, cole, and cucumber yields also increased by 18.9 – 20.2% with the 500 times dilution of PA (Jun et al., 2006). The PA dilution used some time is beyond the 250-800 times dilution. The PA at five times dilution combined with biochar significantly increase the maize grain yield after a year at abandoned salinized cropland (Lashari et al., 2015). The PA of pineapple biomass, 2 – 4% (approximately 50 – 25 times dilution) affected the okra growth in terms of plant height, leaf diameter, root length, fruits weight, leaves number and fruits number (Mahmud et al., 2016). Even the 4000 times dilution also had found to promote the germination and root growth of wheat seeds (Lu et al., 2019). While some studies found that applying PA at dilutions of the rate of 10% increased crop yield, at higher concentrations, PA has an inhibitory effect on plant development (Mmojieje and Hornung, 2015; Zulkarami et al., 2011). The application of 30% PA was toxic, as most rock melon plants died when exposed to this level of PA (Zulkarami et al., 2011). Although the 10% PA dilution does not limit the plant growth when applied as a post-germination, the dilution rate inhibits the pepper seeds when used in the initial germination stage (Mmojieje, 2016).

The implementation of beneficial bacteria in sustainable agriculture is an initiative that promotes plant health and growth (Robledo-Buriticá et al., 2017). Plant growth-promoting rhizobacteria (PGPR) is a group of bacteria living in the rhizosphere, consisting of *Agrobacterium*, *Bacillus*, *Pseudomonas*, and several other genera (Goswami et al., 2016). Besides acting as a bio fertilizer, PGPR also can be used as biocontrol for phytopathogens (Ahmad et al., 2019). There are plenty of commercialized PGPRs in the markets, and one of them is UPMB10. UPMB10 (*Bacillus subtilis*) is a rod-shaped, Gram-positive, and spore-forming bacteria (Kamaruddin, 2016). The strain was isolated from the root of oil palms and used in the Bacto-10TM commercial product (Kuan et al., 2016). There are several abilities of this PGPR, which include IAA (indole-3-acetic acid) production and atmospheric nitrogen fixation, that can promote growth and yield of crops production (Razak et al., 2019). Other biochemical properties of UPMB10 are phosphate solubilisation, potassium solubilisation, iron siderophore production, cellulase production, and pectinase production (Ali-Tan et al., 2017).

The dwarf long bean (DLB) is the hybrid between cowpea (*Vigna unguiculata* subsp. *unguiculata*) and long bean (*Vigna unguiculata* subsp. *sesquapedalis*) through selective breeding and backcrossing processes (Tantasawat et al., 2010; Ujang, 2011). This DLB plant has similar pod morphology to a long bean plant but with a shrub or bush characteristic (Brathwaite, 1978). This new horticultural crop was first developed in the Philippines by Acosta and Petrache (1960). The areas of cultivation for the long bean are Central and East Africa, tropical America, tropical Asia and West Africa (Tindall, 1983). As with other legumes, DLB is a nutritional component of both the human diet and livestock (Olawuni et al., 2013). Fresh pods are harvested as green vegetables (Ofori and Klogo, 2005) and served as a light meal (Razali et al., 2006). The legume is also suitable for conservation agriculture due to its ability to fix atmospheric nitrogen and facilitate soil nutrients' circulation and water retention (Stagnari et al., 2017). Unlike long bean, DLB does not require trellises and can save about 50% for the site preparation cost (MARDI, 2008). This crop also has a short life span, making it less risk of exposure to pests, diseases, and natural disaster (Tantasawat et al., 2010). The slightly shorter pods of DLB makes the pods are more manageable for storage and transportation compare to the typical long bean (MARDI, 2008). Due to the desirable features, the DLB plant was selected as a test crop for this study for more manageable yield storage and transportation and fast growth.

Most of the previous study had evaluated the effect of PGPR inoculation and PA on crop singly (Rui et al., 2014). There was also minimal research done on growth and yield improvement by PA, especially in co-application with PGPR with the optimal PA dilution. PA alone cannot afford the nutrition required by crops as the amount of NPK of the substance is very low (Mungkunkamchao et al., 2013). Therefore, the role of PGPR, including UPMB10, is beneficial to promote nutrient availability through their plant growth promoting traits. With the help of the UPMB10 combination, the nutrients provided by PA can be fortified to fulfil the plant needs, including leguminous plants. The combination of PA and UPMB10 could contribute to the environment-friendly approach and food security through a sustainable method without causing pollution. Moreover, the utilisation of PA derived from the by-product of charcoal factories in farming can also promote the socioeconomic of the society. At the suitable PA dilution, the combined application of PA and UPMB10 is hypothesised to have a synergistic effect of DLB without affecting the natural environment. Hence, an experiment was conducted to study the PA dilutions effect combined with UPMB10 (as a factorial experiment) on the growth and yield of DLB.

2 Materials and Methods

2.1 Experimental design and treatment

The study was a two-factorial experiment. The first factor was the different PA dilution treatments: 0:1 (control), 1:600 (P600), 1:400 (P400), and 1:200 (P200). And the second factor was the inoculation of the UPMB10: inoculated and uninoculated. The investigation was conducted at the Field 16 Unit, Faculty of Agriculture, Universiti Putra Malaysia, for nine weeks in November 2016 and was replicated four times under a completely randomized design. The agro-climatic condition is classified as warm, humid tropics with a regular dry season (FAO, 2004). The temperature is relatively uniform with high relative humidity (85-95%) and typical rainfall (200-250 cm a year) (FAO, 1996). During the experiment, the temperatures range from 34 °C during the day to 22 °C at night. The maximum daily precipitation is 40 mm and with a total of 452 mm and 49 days of rain from 16th November 2016 to 21st January 2017. The cocopeat used had an acceptable pH (6.6), electrical conductivity (0.16 mS cm^{-1}), and bulk density (0.07 g cm^{-3}) (Awang et al., 2009). The chemical properties (%) of the cocopeat were N (2.08), P (1.80), K (2.72), Mg (0.87), and Na (0.49) (Ismail et al., 2020).

2.2 Preparation and dilution of PA

The PA was obtained from a local agriculture store at Seri Kembangan, Selangor, Malaysia. It was produced from a liquid condensate extracted from the carbonisation process during charcoal making at a temperature of 240 – 500 °C of mangrove tree billets (*Rhizophora apiculata*). The condensate was allowed at room temperature for three months to separate refined PA (brown liquid, located at middle layer) from clear oil (at top layer) and dark tar (at bottom layer). The thick brown liquid of the intermediate layer was collected and stored in a new container before used as commercial PA. Depending on the rate of application, PA was diluted with distilled water (v/v) into 0:1 (control), 1:600 (P600), 1:400 (P400) and 1:200 (P200). Each PA treatments was watered into the planting media with a similar volume as the scheduled fertigation volume. Both PA and UPMB10 treatments were applied twice during four and six weeks after sowing (WAS) in the afternoon (around 5.0 pm).

2.3 Preparation of UPMB10 culture

The pure culture of UPMB10 was obtained from the Department of Land Management, University Putra Malaysia, Serdang. The culture was prepared by growing in tryptic soy broth (TSB) (Merck, USA) for 24 hours and was centrifuged (5810 R, Eppendorf, Germany) at 9000 rpm for 10 minutes in a 50 mL Eppendorf tube (Tan et al., 2015). The supernatant was removed aseptically and replaced by 0.85% of sterilised phosphate buffer saline (PBS). The solution was then mixed thoroughly by using a vortex mixer (FINEVORTEX, FINEPCR, Korea) for 30 seconds. The colony-forming unit (CFU) of the bacteria culture was observed using the total plate count technique (Tan et al., 2015). One millilitre of the solution was placed into a series of test tubes at 10-fold dilution and streaked on tryptic soy agar (TSA) (Merck, USA). The colony count of the 24 h culture was approximately 1×10^9 CFU mL^{-1}. These microbial cultures were directly inoculated on the basal of plant stems at the rate of two millilitres per plant.

2.4 Preparation of planting media

The cocopeat used in the experiment was collected from a local agriculture store at Seri Kembangan, Selangor, Malaysia. All polybag (32 polybags equivalent to 32 experimental units) were filled with cocopeat until 1 inch left from the top using 12 inches × 16 inches garden polybags (black). The cocopeat was rinsed with water before sowing. The seeds of dwarf long bean were sowed into the polybag at the rate of two seeds per polybag. The polybags were watered twice daily to ensure healthy seedling germination and plant growth. The electrical conductivity (EC) of the solutions were maintained according to local agricultural practices (Table 1). The rate was 1.5 dS m^{-1} for the vegetative state (1-4 WAS), 2.0 dS mm^{-1} for the flowering stage (5 WAS), and 2.5 dS mm^{-1} for the fruiting stage (5-9 WAS) in all fertilizer storage tanks. A drip fertigation system was used to irrigated

Table 1. Irrigation volume, electrical conductivity (EC), and plant growth stage for each week

Week after sowing (WAS)	Irrigation volume (L bag^{-1} d^{-1})	EC of liquid fertilizer (dS/m)	Plant growth stage
0-1	0.25	1.5	Vegetative
1-2	0.5	1.5	Vegetative
2-3	0.75	1.5	Vegetative
3-4	1	1.5	Vegetative
4-5	1.25	2	Flowering
5-6	1.5	2.5	Fruiting
6-7	1.75	2.5	Fruiting
7-8	2	2.5	Fruiting
8-9	2	2.5	Fruiting

the nutrient twice a day with a total of 0.25 L per day for each polybag and increased by 0.05 L for each coming week. Once per week, the polybags were weeded out to ensure healthy plant growth without the competition of weed. Local farmers' practice was adopted to control the disease and pest by using fungicides and insecticides when necessary.

2.5 Parameters measurement

Growth characters (plant height and chlorophyll content) were observed weekly for nine weeks. Yield components (flowering date, pods number, dry weight and relative water content) were recorded every two days started from emerged of the first flower. Plant height was recorded by measuring the length of the shoot system (from the top base of planting media to apical bud) by using a meter ruler. The date of flowering was observed based on the number of days taken by a plant to emerge the first flower. The number of pods was the total pod number harvested during the reproductive stage from a plant. Total yield was presented in terms of total dry weight (in gram) produced by a plant. Total yield per pod was the average weight of all pods produced by a plant. The chlorophyll content was taken using a portable SPAD meter (SPAD-502, Konica-Minolta, Japan) at the fourth trifoliolate leaves from the top of the plant (Hgaza et al., 2009). The readings were taken at two-thirds of the distance from the leaf tip towards the stem (Argenta et al., 2004). Three measurements of SPAD value were taken per plant and were averaged (Rodriguez and Miller, 2000). Relative water content (RWC) of harvested pods was calculated based on the following formula given by Sumithra et al. (2006):

$$RWC\ (\%) = \frac{(FW - DW)}{FW} \times 100 \qquad (1)$$

where FW and DW are the fresh and dry weight, respectively. DW was measured after oven-drying the pods for 48 h at 90 °C (Egashira et al., 2016).

2.6 Statistical analysis

All data were analysed by one-way analysis of variance (ANOVA). Mean differences among tested treatments were compared using the least significant difference (LSD) test at 5 % significance ($P < 0.05$). ANOVA and LSD test were performed through the Statistical Analysis System (SAS) version 9.4.

3 Results and Discussion

There was no significant interaction between PA and UPMB10 on all of the parameters. The effect of PA treatments on the parameters was not influenced by UPMB10 treatments or vice versa. The results are presented and discussed on the main effect of PA and UPMB10 individually for each treatment. However, the average plant height of DLB, which were taken weekly, was the only parameter that had shown a significant effect of interaction, which was not demonstrated within each week's comparison.

3.1 Chlorophyll content

In the study, the leaf chlorophyll content of DLB was observed in eight treatment combinations. For the main effect of PA on chlorophyll content, there were no significant differences within each week, except in 8 WAS (Table 2). Although the result was not significant, the highest average SPAD value was presented by DLB treated with P400 with an average value of 53.2. It was 10.1% higher than control. The lowest average SPAD value among PA treatments was found in DLB plants treated with P600 with an average value of 50.4. The value was only 4.3% over the control. The insignificant result of PA treatments in this study, except for 8 WAS, might cause by the high availability of nutrients provided by liquid fertilizer irrigated into the cocopeat media, as cocopeat media only had a low concentration of available nitrogen, calcium, magnesium and micro-elements. In contrast, phosphorus and potassium concentrations were high (0.28

Table 2. Effects of PA (pyroligneous acid) dilution treatments and UPMB10 inoculatation on leaf chlorophyll content (SPAD value) of yeardlong bean at 5, 7, and 9 WAS (weeks after sowing)

Treatment	SPAD value				
	5 WAS	6 WAS	7 WAS	8 WAS	9 WAS
PA dilution					
Control	42.6	46.3	68.8	37.6b	46.1
P600	42.9	46.3	63	50.3a	49.4
P400	42.3	46.8	71.7	51.8a	53.2
P200	41.3	48.6	65.9	50.3a	47.5
Sig. level	NS	NS	NS	*	NS
UPMB10 inoculatation					
Uninoculated	42.2	47.6	68.4	44.5	48
Inoculated	42.3	46.4	66.4	50.6	50.1
Sig. level	NS	NS	NS	NS	NS

Values with different letters in a column designate significant difference among the treatment means. NS = not significant.

– 2.81 mol m^{-3} and 2.97 – 52.66 mol m^{-3}, respectively) (Abad et al., 2002). A study on rice plant also reported no significant difference between control, 500-times dilution, and 1000-times dilution of PA (Jeong et al., 2015). The study also stated that the application of fertilizer dominated plant growth improvement that PA could derive. The application of reduced chemical fertilizer rate might help understand how PA affects chlorophyll content in future study. Besides that, an experiment was done on tomato plants with a 1:800 PA dilution ratio and found no change in chlorophyll leaf content at any growth stage (Mungkunkamchao et al., 2013). The insignificant difference might be related to the small amounts of N, P, and K contained in PA, as shown in their research. They had concluded that PA might not have the same effects as synthetic fertilizer.

The significant reduction of SPAD value for the control during 8 WAS might cause by the effect of remobilization of nutrients during seed filling. The nitrogen accumulated earlier in the vegetative tissues was redistributed into the pods (yields) to provide enough nitrogen for the seed filling and subsequently reduce SPAD value from the leaves. A study done on several legumes found out that the nitrogen requirements during seed filling are greater than biological nitrogen fixation (BNF) and nitrogen uptake (Pampana et al., 2016). But, the application of PA was significantly preventing the SPAD reduction. Other research studied the combined effect of PA and herbicides on leaf chlorophyll content of rice plants (Seo et al., 2015). They also had found a small increment of chlorophyll content on the plants treated with the combination of PA and several types of herbicides compared with the plants treated with herbicides only. They stated that the improvement might be due to the additional nutrients and organic substances in PA. Besides, the increment also might be due to the ability of PA that can reduce the toxic effect of herbicides on rice plant. Furthermore, other study had found that PA is able to increase leaf chlorophyll content of rice, but it was not the effect of PA alone (Kang et al., 2012). In their study, the PA was mixed with charcoal. During the maximum tillering stage of rice, the SPAD value of rice plant treated with a mixture of charcoal, PA and half of the recommended rate of chemical fertilizer had a similar result with the one treated with the recommended rate of chemical fertilizer only. However, during the heading stage of plant rice, the treatment of the mixture was increased significantly at 13.8% compared to the recommended rate of chemical fertilizer treatment.

The main effect of UPMB10 treatments on leaf chlorophyll content was also no significant within each week (Table 2). However, DLB plants inoculated with UPMB10 had higher leaf chlorophyll content than control at 2.0% of increment. UPMB10 had been proved to have several beneficial effects on plants, such as increasing N, P, and K in plant tissues and promoting overall plant growth (Tan et al., 2014). With these abilities, UPMB10 supposed to increase leaf chlorophyll content as the leaf chlorophyll content is also correlated with the leaf nitrogen content (Ling et al., 2011). However, the insignificance increments by the UPMB10 inoculation for the chlorophyll content might be due to the high availability of nutrient inside the planting medium—the same reason as insignificant treatments of PA. A study done with inoculation of *Rhizobium phaseoli* found that the plant growth enhancement was overridden by high fertilizer application during maturity stage and grain yield of rice plant (Jeong et al., 2015).

3.2 Plant height

There was no interaction between PA dilution and UPMB10 inoculation treatments on plant height at each specific week (5, 6, 7, 8, and 9 WAS). Therefore, the results on plant height for each week (5, 6, 7, 8, and 9 WAS) were compared and discussed separately between the main effect of PA dilution treatments and the main effect of UPMB10 inoculation treatments (Table 3). For the main effect of PA (Table 3), no significant differences among PA treatments were observed at 5, 6, 7, 8, and 9 WAS, except for the overall (average) height (Fig. 1). P400 had shown the highest DLB height for each week, and it was significantly higher than control at 7.0% on average. The average height of P400 was also considerably higher than P600 and P200 at 15.4 and 8.4%, respectively. The lowest plant height from 5 to 9 WAS was DLB treated with P600, and it was 7.2% less than control on average. Above all, no PA treatments reduced DLB height significantly for each week (Table 3). Thus, PA was not adversely affecting the growth of DLB, especially in terms of tallness. The PA was proved to promote plant growth in previous studies due to its biological-active components (Wang et al., 2019). Phenols, esters and acetic acids in PA are correlated with the growth promotion properties (Grewal et al., 2018). Researchers also have shown that PA performs similar to a plant growth regulator. A hormone in PA, called karrikinolide, promotes plant growth, especially at the early stages of plant growth (Mungkunkamchao et al., 2013).

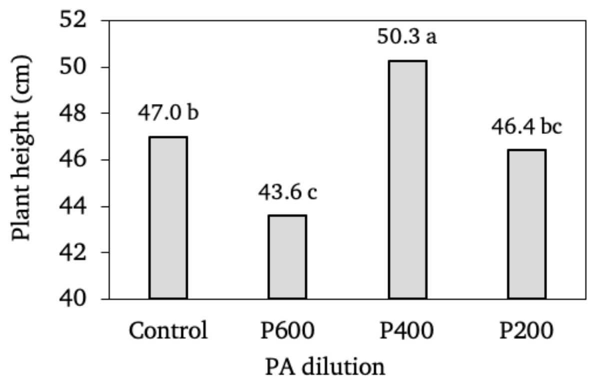

Figure 1. Mean comparison between PA (pyroligneous acid) dilution treatments in terms of overall (average) DLB (dwarf long bean) height. Different lowercase letters indicate significant differences ($p<0.05$) between treatment means.

The indirect effects of PA on DLB, such as controlling pests and diseases, and improving soil biology and chemical, also influence plant growth. A yellow gum disease caused by bacteria named *Corynebacterium agropyri* also is inhibited by PA's application (Grewal et al., 2018). The PA at 1% also had shown high pest repellent characteristics (Siriwardena et al., 2019). Besides, weed also can be controlled by the PA application (Grewal et al., 2018). The presence of phenols, formaldehyde, organic acids, carbonyls, and alcohol in PA enable it to have biocide effects at high application concentration (Grewal et al., 2018; Steiner et al., 2008). PA can act as a catalyst for soil microbes and enzyme activation (Grewal et al., 2018). It was also proved that 1% of PA dilution can improve soil biology by increasing biological biomass (Cardelli et al., 2020). The influence of PA on soil exchange complex through ion displacement had enhanced nutrient availability. PA increases the soil pH by increasing the availability of basic cations (Grewal et al., 2018). With the application of PA, the 1:100 dilution enhances the uptake of N and K (Benzon and Lee, 2017). PA also promotes P uptake by increasing useable phosphoric acid (Grewal et al., 2018).

For the main effect of UPMB10 inoculation on plant height, there was no significant difference between control and inoculated treatment from 5 to 9 WAS (Table 3). The highest promotion effect was at 7 WAS by 4.7% increment, and the lowest reduction was at 6 WAS by 2.4% reduction. On average, the UPMB10 treatment was only 0.2% higher than the control. The little influence of UPMB10 on plant height might be due to several factors such as the overlapped effect caused by the fertilizer application or the small inoculation volume given into the planting media. Previous studies had reported that UPMB10 significantly promoted seedling growth (Mia et al., 2012). A study done on rice had found that UPMB10 significantly increased seedling height (Tan et al., 2014). The UPMB10 also increased plant height up to 30% on banana growth (Ibrahim, 2008). The ability of UPMB10 to produce IAA helps in promoting plant height (Kuan et al., 2016). The UPMB10 also fix atmospheric nitrogen, which increases the accumulation of N and subsequently promotes plant growth (Mia et al., 2010). The possible factors which might influence the insignificant result of UPMB10 on DLB height are the late application timing, the low application frequency, and the small inoculant volume. In our experiment, the application of UPMB10 (which was a similar date as PA application) was conducted only twice at 4 and 6 WAS (flowering stage started at 5 WAS) with 2 mL of UPMB10 culture (without diluted). These conditions might reduce the success rate of UPMB10 inoculation into planting media. Earlier application of UPMB10 might help the effectiveness of the inoculation process in plant media. It has been reported that the timing of application of PGPR influences plant growth (Kurokura et al., 2017). Plants inoculated with PGPR at an early stage might respond more, where younger seedlings are more responsive. Seeds inoculated with PGPR also increase the plant growth rate, especially the microbial seed coating approach (Rocha et al., 2020).

Table 3. Effects PA (pyroligneous acid) dilution treatments and UPMB10 inoculation on plant height of yardlong bean at 5, 7, and 9 WAS (weeks after sowing)

Treatment	Plant height (cm)				
	5 WAS	6 WAS	7 WAS	8 WAS	9 WAS
PA dilution					
Control	39.9	43.5	45.3	51.9	56
P600	40.1	38.8	43.3	47.1	48.8
P400	42	45.1	48.6	55.4	60.3
P200	40.9	41	44.3	51.4	54.6
Sig. level	NS	NS	NS	NS	NS
UPMB10 Inoculation					
Uninoculated	40.5	42.6	44.3	51.4	55.4
Inoculated	40.9	41.6	46.4	51.4	54.4
Sig. level	NS	NS	NS	NS	NS

Dosage and frequency of PGPR application also affect plant growth (Naihati et al., 2018). More frequent application of UPMB10 might help in increasing the significant rate of plant growth. The high volume of inoculant also might increase the success rate of UPMB10 inoculation into the rhizosphere by increasing the spread area. Moreover, agronomic practice dilutes the microbial culture first before use. This approach increases spread area without reducing PGPR population per plant, especially toward old or grown crops which have larger rhizosphere volume compared to seedlings rhizosphere. Another possible factor was the usage of the standard amount of fertilizer rate. The use of 100% amount of fertilizer can results in high nutrient in soil media, which result in a high rate of plant growth (Jeong et al., 2015). A comparison between the control and inoculated plant might be less significant in terms of plant height when a standard amount of fertilizer is applied. The below-recommended rate of fertilizer might help in differentiate the plant height between the control and treated plant.

3.3 Days to flowering

For the main effect of PA (Table 4), there was no significant difference between the treatments in terms of days to flowering. The earliest flowering was obtained from P400 with a mean value of 35.29 days, but it was only 0.4% faster than the control. Meanwhile, the latest flowering was recorded when treated with P600 or P200, with both have a mean value of 36 days, and it was only 0.2% faster than the control. Studies indicate that PA stimulates and accelerates flowering (Kulkarni et al., 2006; Wang et al., 2019). A mixture of PA and charcoal also shorten the days to flowering of zinnia, Melampodium and scarlet sage (Kadota and Niimi, 2004). However, other studies also have proved that there was no effect of PA application on the flowering date of tomato (Mungkunkamchao et al., 2013). The insignificant result on the days to flowering of DLB may be due to different species of test crop or late timing application of PA. Some plants are more responsive compared to others. Less sensitive plants may need a more frequent application of PA during their growing stage. Also, the known compounds in PA may be more responsive toward younger seedlings (Mungkunkamchao et al., 2013). The application of PA at the early stages of DLB growth might accelerate the flowering process. Future studies are required to understand the influence of the initial application of PA toward the days to flowering.

For the main effect of UPMB10 (Table 4), there was also no significant difference between the treatments in terms of days to flowering. Inoculated UPMB10 treatment, which had a mean value of 36.25 of days to flowering, was only 1.9% slower than uninoculated control with no significant difference. Thus, the application of UPMB10 was not adversely affected the date of flowering. In another study, UPMB10 significantly induced the early flowering of banana plants (Mia et al., 2005). The experiment reported that the combined application of UPMB10 with 33% of N fertilizer showed three weeks early flowering compared with uninoculated control combined with the same fertilizer rate. However, the combination of UPMB10 and 100% of N fertilizer showed only five days earlier compared with uninoculated control and 100% N rate. In our study, 100% of the standard fertilizer rate was given through the fertigation system, which might be responsible for the slight influence of UPMB10 on the days to flowering of DLB. Reduced fertilizer rate might help in increasing the significance, as the high nutrient effect might interfere with the promotion effect of UPMB10. Other possible reasons were small inoculation volume and no early inoculation of

UPMB10 during sowing. The PGPR application favors the early stage of plant life. Frequently repeated inoculation can also help increase the promoting effect of UPMB10, as there was also interaction with other millions of indigenous microbes that may compete with each other.

3.4 Pod number

For the main effect of PA (Table 4), the highest pod number of DLB was obtained from the plant treated with P400 with a mean value of 19.50 pods. The P400 was significantly higher than P600 and P200 at 173.5 and 62.5 %, respectively. The P400 was also higher than control at 36.8%, but the difference was not significant. Meanwhile, the lowest pod number of DLB was obtained from P600 with a mean value of 7.13 pods. P600 was significantly lower than control and P400 at 50 and 63.4%, respectively. The P600 was also lower than P200 at 40.6%, but the difference was not significant. The increment of the pod number of DLB, when treated with P400, was might be due to the promotion effect of PA on the number of produced flowers. It was suggested that the impact of PA vary depending on the plant species (Kadota and Niimi, 2004). The difference might be due to the different pH tolerance of plants toward the soil pH fluctuation caused by the PA application. Some plants prefer acidic condition like chilli, while others favor neutral pH like tomato (Luo et al., 2019). Preferable soil pH of plant species is essential for optimum growth. The positive effect of PA on vegetative growth also had influenced the number of flower production of DLB. In a study, the number of the flower bud of Melampodium was increased significantly with the mixture of PA and charcoal (Kadota and Niimi, 2004). However, there was no significant difference in the number of the flower bud of French marigold and zinnia in the same study. The small (insignificant) promotion of fruit number by PA application was also reported by other researchers (Mungkunkamchao et al., 2013). Besides, an experiment conducted on soybean revealed that the 1.5, 3.0, and 6.0% of PA dilution (v/v) did not significantly affect the number of pods per plant (Petter et al., 2013). Our study found a significant positive correlation (r = 0.70, p>0.05) between plant height and pod number. In a study on rock melon, the root system and plant growth were promoted by the PA application, which subsequently promoted the flowering of rock melon (Zulkarami et al., 2011). The PA contains nutrients essential in the constitution of organic compound that contributes to all plant growth phases such as growth and flowering when given at a low dosage of application (Silva et al., 2017).

Although P600 was more diluted than P400, P600 had a lower pod number than P400. In theory, if the dilution rate of P600 is located between P400 and control, then the pod number should also be in between around P400 and control, which reflect the concentration of the given PA or at least had a similar pod number with control. However, the mean value of 7.13 pods of P400 was significantly lower than control which has a mean value of 14.56 pods. It was also a possibility that the location of the experimental unit for P600, which exposed more compared with other PA treatments, caused the plants to face external stresses more, such as insect pest and herbicide injury. The examples of insect pests of DLB are aphids, pod borer, and leaf miner. And examples of herbicide injuries are herbicides drift and misapplication. The environmental stresses might interfere with the pod number production, which also correlated with the plant height parameter.

For the main effect of UPMB10 (Table 4), the number of pods that inoculated with UPMB10 was 18.4% lower than control (uninoculated). However, the difference was not significant. Thus, the application of UPMB10 did not significantly reduce the pod's number produced per plant. The positive effect of UPMB10 might be faded by the impact of fertilizer that applied with 100% from the recommended rate. A low fertilizer rate might increase the difference between inoculated and uninoculated treatment in terms of pods number. In a previous study, the number of hands per bunch and the number of fingers per hand for banana plants were reduced by the inoculation of UPMB10 (Mia et al., 2005). Still, the differences were also not significant. A study on blackberry also found no significant difference in the number of flowers for plant treated with *Bacillus subtilis* and *Bacillus pumilus* (Robledo-Buriticá et al., 2017). However, some studies reported that *Bacillus subtilis* promotes the number of flowers and pods. Mixed culture of mutant strains containing *Bacillus subtilis* had encouraged the number of flowers of *Catharanthus roseus* under saline condition (Hingole and Pathak, 2016). A study done on chickpea under sandy soil found that the number of pods per plant was increased significantly over control when inoculated with *Bacillus subtilis* (Khan et al., 2020). The promotion effect of UPMB10 on the pod's number of DLB might also be more significant in our study if the plant undergoes stress such as under saline or sandy soil condition. Main effect of PA and UPMB10 on pods number of DLB were not significant within their treatments. The number of pods might be more significant if the rate of fertilizer was reduced. If the nutrient availability supplied by the irrigated fertilizer (through fertigation system) was limited, then the plant can rely and utilize more on beneficial properties of PA and UPMB10. It was found that the fruit number of a test crop was significantly increased with the high application rate of inorganic fertilizer (Mungkunkamchao et al., 2013).

Table 4. Effects PA (pyroligneous acid) dilution treatments and UPMB10 inoculation on yield and yield characteristics of yardlong bean

PA dilution	Days to flowering	Number of pods	Dry weight (g)		RWC (%)
			Total yield	Per pod	
PA dilution					
Control	36.5	14.25ab	14.81b	1.11	91.9
P600	36	7.13c	7.9c	1.2	91.7
P400	35.29	19.5a	22.03a	1.29	92.4
P200	36	12bc	13.64bc	1.06	91.5
Sig. level	NS	*	*	NS	NS
UPMB10 Inoculation					
Uninoculated	35.58	14.56	15.64	1.15	91.9
Inoculated	36.25	11.88	13.54	1.18	91.9
Sig. level	NS	NS	NS	NS	NS

Values with different letters in a column designate significant difference among the treatment means. NS = not significant.

3.5 Pod yield plant^{-1}

For the main effect of PA (Table 4), the highest total yield (dry weight) produced by a plant was the P400 with a mean value of 22.03 g. The P400 was significantly higher over control, P600 and P200 at 48.8, 178.9 and 61.5%, respectively. The P600 was the lowest in total yield production per plant, and it was significantly lower than control at 46.7%. The P200 was also lower than control at 7.9% less, but it was no significantly different. Moreover, the promotion effect on total yield per plant has a positive correlation with the production of pods number per plant (r = 0.95, p<0.05) and the height of the plant (r = 0.62, p<0.05). Another study also had proved that PA significantly promotes the yield production of soybean (Travero and Mihara, 2016). Grain yield of rice was also increased with PA application alone (Sun et al., 2020) or combined with animal manure (Polthanee et al., 2015). The yield of the rock melon plant was also increased with 20% of PA (Zulkarami et al., 2011). The positive effect of P400 on the yield of DLB might be related to the regulating effect of PA on soil pH and nutrient condition (Sun et al., 2020). The earlier beneficial effect of PA on plant germination and growth and increased leaf area and nutrient uptake might also cause yield promotion (Lashari et al., 2015). Moreover, the improvement of yield biomass might be due to the improved assimilate partitioning and sink strength of carbon through the seeds (Berahim et al., 2014). Although P600 and P200 had lower yield than control, they were not significantly different. A study conducted on soybean had shown no significant difference in the effect of PA on soybean yield (Petter et al., 2013). The yield of the tomato plant also had shown small increases (not significant) when applied with PA derived from eucalyptus (Mungkunkamchao et al., 2013). Another beneficial effect of PA, including insect pests control (such as *Anticarsia gemmatalis* and *Pseudoplusia includens* in soybean crop), can be gained without interfering with the crop yield (Petter et al., 2013). The acids might cause a slight decrease of P600 and P200 on total yield, ketones, and phenols in PA, which are harmful for crop production if at a high level (Sun et al., 2020).

For the main effect of UPMB10 (Table 4), the total dry weight per plant for UPMB10 inoculated treatment was 13.4% lower than uninoculated control, but it was insignificant. Banana plant treated with UPMB10 showed a slight improvement over control in terms of bunch weight per plant with no significant difference (Mia et al., 2005). With only a tiny increment, sweet potato yields also had shown no significant difference over control when inoculated with UPMB10 (Yasmin et al., 2007). Although UPMB10 did not promoted the quantity of crop yield significantly, the inoculant had increased the yield quality. N, P and Mg of sweet potato storage root were significantly increased with the inoculation of UPMB10 (Yasmin et al., 2007). UPMB10 also improved other fruit physical attributes of banana plants, except for the bunch weight per plant, including the fingers weight, length and diameter, and pulp to peel ratio, through the improvement of the nutrient uptake K and Ca (Mia et al., 2005). Another factor that can be considered was not all PGPR were designed to benefit all plant species on all growth or yield parameters. Selected PGPR may work best on specific plant species or certain plant parameters. Besides, different crop species may respond differently toward selected PGPR. The UPMB10 may promote the crop yield on certain crop species, but not the others. It was reported that UPMB10 significantly increased the grain yield of rice through its ability of fixing atmospheric nitrogen and producing IAA (Razak et al., 2019).

3.6 Dry weight of pod

For the main effect of PA (Table 4), there was no significant influence of different PA dilution on the dry weight per pod of DLB. The highest pod dry weight was recorded with P400, and it has a mean value of 1.26 g, which was 16.6% higher than the control. Meanwhile, P200 had the lowest pod dry weight with a mean value of 1.06 g, and it was 4.2% lower than the control. For P600, it has a mean value of 1.20 g, which was 8.2% higher than control. A study on tomato also showed slight increases in fresh weight of fruit, but the total soluble solutes of fruit were significantly enhanced with PA application (Mungkunkamchao et al., 2013). A study also had found that PA had no significant effect on weight per 100 seeds of the soybean. But they showed that PA had better suppression of pest infestations (Pangnakorn et al., 2009). Also, the yield and yield components of peanut were not significantly increased with PA application (Jothityangkoon et al., 2008). However, they had found that the seed yield and shelling percentage were slightly increased. The insignificant difference between treatments in terms of dry weight per pod also indicates that each pod's weight did not influence the promotion effect of total yield (dry weight) of DLB. The result showed that the increments of whole pods dry weight per plant resulted from improvement toward the pod's number produced per plant. The environmental factors, application methods, and different test crop species were the possible reasons that cause an insignificant difference of PA toward dry weight per pod of DLB.

For the main effect of UPMB10 (Table 4), plants inoculated with UPMB10 had slightly higher dry weight per pod than uninoculated control plants, and the difference was not significant. With a mean value of 1.18 g, UPMB10 was only 2.6% higher over uninoculated control, which had a mean value of 1.15 g. The slight improvement of UPMB10 on pod dry weight might be due to the promoting effect by UPMB10, such as IAA production and nitrogen fixation abilities. A previous study done on common bean had found that a bio fertilizer composted with *Bacillus subtilis*, *Trichoderma* sp., and *Rhizobium* spp. increased pods weight (Pohan et al., 2019). Another study on groundnut showed significant improvement in pod weight when inoculated with *Bacillus licheniformis*, which has efficient spermosphere colonisation and IAA production traits (Prashanth and Mathivanan, 2010). Moreover, inoculation of *Bacillus subtilis* on peanut also promoted the dry weight of pods under greenhouse and open field condition (Ahmad et al., 2019). The insignificant effect of UPMB10 toward pod dry weight might be due to either environmental factor, application method, or fertilizer rate. For the environmental aspect, the possible threats that contribute to causing crop injuries on DLB were the herbicide drift, either physical or vapour drift (Schwartz-Lazaro et al., 2017), and the crop pest, either insect pests or pathogens (Hill, 2008). The infection of crop pest probably occurred after the damage by herbicide drift happened where the plants had low resistance during that state (Duke et al., 2007).

In terms of the application method of UPMB10, a higher volume of given inoculant might help promote the beneficial effect toward pods dry weight of DLB. A study on common bean had stated that plant treated with 1.00 and 1.25 g of the bio fertilizer experienced the highest mean value of pods weight (Pohan et al., 2019). Both bio fertilizer dosages were significantly higher than 0, 0.25, 0.50 and 0.75 g treatments, which indicated that a higher volume of inoculant given onto the test crop might promote yield's weight over lower inoculant volume. The 100% of the recommended rate of irrigated fertilizer given on plants might fade the beneficial effect of UPMB10 on pod weight of DLB. The amount of nutrients produced by PGPR is less than inorganic fertilizer. A low fertilizer rate might help to increase the significant difference between inoculated and uninoculated by UPMB10 on pod weight of DLB. A study on banana found no significant difference between inoculated and uninoculated by UPMB10 in terms of finger weight when applied with 100% N-fertilization, but not at 33% N-fertilization (Mia et al., 2005).

3.7 Relative water content of pod

The dry weight of DLB pods was determined to access the relative water content (RWC) of pods from each treatment combinations. Relative water content is an indicator to measure the plant's water balance, which measures the plant water status in terms of physiological consequence of cellular water deficit (Barrs and Weatherley, 1962). The current experiment showed no significant difference among treatments for both of the main effect of PA and UPMB10 factors and no interaction between the factors (Table 4). For the main effect of PA (Table 4), the highest value of RWC was P400 with a mean value of 92.4% of RWC. The P400 was only 0.5% higher than control, with no significant difference. Meanwhile, the lowest RWC was P200 with a mean value of 91.5%, and it was slightly lower than the control at 0.4%. The P600, which had a mean value of 91.7%, was also somewhat lower than control at 0.2%. For the main effect of UPMB10 (Table 4), the RWC was similar on both inoculated and uninoculated treatments with a mean value of 91.9%. From the result, PA and UPMB10 were not adversely affecting the RWC of DLB pods. If the percentage of RWC was lower than usual, then the maturity of pods might interfere as well. Low RWC of pods will lead to faster pod maturity by increasing the levels of γ-TIP proteins (Egashira et al., 2016), although the pod size was still smaller than average. The RWC tended to decrease if stress happened on the plant, such as drought stress (Shirkhani and Nasrolahzadeh, 2016) and salinity stress (Shahverdi et al., 2020). The difference between the treated and control plants in terms of RWC of pods might be significant if the plant undergoes stresses, especially drought and salinity stress. Generally, plants treated with PA or inoculated with PGPR maintained higher RWC as compared to control. Rice seeds of two cultivars (KDML 105 and RD6) primed with PA had better maintain RWC under water shortage conditions (Simma et al., 2017). A study done on perennial ryegrass had found that PGPRs named Bacillus amyloliquefaciens strain GB03 and WRA had significantly improved RWC under severe drought stress (Su et al., 2017). According to a study, an increase in RWC could be the result of alterations in stomatal closure sensitivity (Dodd et al., 2010). Despite recent progress, the mechanisms underlying increased RWC with PGPR treatment have yet to be understood (Su et al., 2017).

4 Conclusion

Based on the result, the P400 was the most suitable PA dilution to promote the DLB yield. The application of P400 significantly improved the chlorophyll content during seed filling and the total yield dry weight during harvesting by increasing the number of pod production. Our study also found no significant difference between inoculated and uninoculated UPMB10 treatments on the DLB growth and yield, which probably due to the overlapping effect by the given fertilizer rate. The promotion effect of PA and UPMB10 on the DLB plant may be improved in a future study by applying both of them at different times, with an early and more frequent application using a larger volume of inoculant (by diluting), and also with reducing irrigated fertilizer rate.

Acknowledgments

We want to express the most profound appreciation to the Faculty of Agriculture, Universiti Putra Malaysia, Malaysia, for providing us with support and platform.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

Abad M, Noguera P, Puchades R, Maquieira A, Noguera V. 2002. Physico-chemical and chemical properties of some coconut coir dusts for use as a peat substitute for containerised ornamental plants. Bioresource Technology 82:241–245. doi: 10.1016/S0960-8524(01)00189-4.

Acosta JC, Petrache LM. 1960. The transfer of the bushy character from cowpea (Vigna sinensis [Linn.] Savi) to sitao (Vigna sesquipedalis Fruw.). Philippine Agriculturist 43:535–547.

Ahmad AGM, Attia AZG, Mohamed MS, Elsayed HE. 2019. Fermentation, formulation and evaluation of PGPR Bacillus subtilis isolate as a bioagent for reducing occurrence of peanut soil-borne diseases. Journal of Integrative Agriculture 18:2080–2092. doi: 10.1016/S2095-3119(19)62578-5.

Ali-Tan KZ, Radziah O, Halimi MS, Abdul Rahim KB, Abdullah M, Shamsuddin ZH. 2017. Growth and yield responses of rice cv. MR219 to rhizobial and plant growth-promoting rhizobacterial inoculations under different fertilizer-n rates. Bangladesh Journal of Botany 46:481–488.

Argenta G, Silva PRFd, Sangoi L. 2004. Leaf relative chlorophyll content as an indicator parameter to predict nitrogen fertilization in maize. Ciência Rural 34:1379–1387. doi: 10.1590/S0103-84782004000500009.

Awang Y, Shaharom AS, Mohamad RB, Selamat A. 2009. Chemical and physical characteristics of cocopeat-based media mixtures and their effects on the growth and development of celosia cristata. American Journal of Agricultural and Biological Science 4:63–71. doi: 10.3844/AJAB.2009.63.71.

Barrs H, Weatherley P. 1962. A Re-Examination of the Relative Turgidity Technique for Estimating Water Deficits in Leaves. Australian Journal of Biological Sciences 15:413. doi: 10.1071/BI9620413.

Benzon HRL, Lee SC. 2016. Potential of Wood Vinegar in Enhancing Fruit Yield and Antioxidant Capacity in Tomato. Korean Journal of Plant Resources 29:704–711. doi: 10.7732/kjpr.2016.29.6.704.

Benzon HRL, Lee SC. 2017. Pyroligneous Acids Enhance Phytoremediation of Heavy Metal-Contaminated Soils Using Mustard. Communications in Soil Science and Plant Analysis 48:2061–2073. doi: 10.1080/00103624.2017.1406102.

Berahim Z, Panhwar QA, Ismail MR, Saud HM, Mondal MMA, Naher UA, Islam MR. 2014. Rice yield improvement by foliar application of phytohormone. Journal of Food, Agriculture and Environment 12:399–404.

Brathwaite RAI. 1978. Chemical Weed Control in Bodie Bean in Trinidad. Tropical Pest Management 24:177–180. doi: 10.1080/09670877809411608.

Cardelli R, Becagli M, Marchini F, Saviozzi A. 2020. Soil biochemical activities after the application of pyroligneous acid to soil. Soil Research doi: 10.1071/SR19373.

Deyn GD, Gattinger A, Lori M, Symnaczik S, Ma P. 2017. Organic farming enhances soil microbial abundance and activity — A meta-analysis and meta-regression. Plos One :1–25.

Dodd IC, Zinovkina NY, Safronova VI, Belimov AA. 2010. Rhizobacterial mediation of plant hormone status. Annals of Applied Biology 157:361–379. doi: 10.1111/j.1744-7348.2010.00439.x.

Duke SO, Wedge DE, Cerdeira AL, Matallo MB. 2007. Herbicide effects on plant disease. Outlooks on Pest Management 18:36–40. doi: 10.1564/18feb13.

Egashira C, Yamauchi T, Miyamoto Y, Yuasa T, Ishibashi Y, Iwaya-Inoue M. 2016. Physiological Responses of Cowpea (Vigna unguiculata (L.) Walp) to Drought Stress during the Pod-filling Stage. Cryobiology and Cryotechnology 62:71–77.

FAO. 1996. Malaysia : Country Report To the Fao International Technical Conference.

FAO. 2004. Fertilizer use by crop in Malaysia.

Goswami D, Thakker JN, Dhandhukia PC. 2016. Portraying mechanics of plant growth promoting rhizobacteria (PGPR): A review. Cogent Food & Agriculture 2. doi: 10.1080/23311932.2015.1127500.

Grewal A, Abbey L, Gunupuru LR. 2018. Production, prospects and potential application of pyroligneous acid in agriculture. Journal of Analytical and Applied Pyrolysis 135:152–159. doi: 10.1016/j.jaap.2018.09.008.

Hgaza VK, Diby LN, Aké S, Frossard E. 2009. Leaf growth and photosynthetic capacity as affected by leaf position , plant nutritional status and growth stage in Dioscorea alata L . Journal of Animal and Plant Sciences 5:483–493.

Hill DS. 2008. Pest damage to crop plants. In: Pests of Crops in Warmer Climates and Their Control. Dordrecht: Springer Netherlands. p. 59–80. doi: 10.1007/978-1-4020-6738-9_5.

Hingole SS, Pathak AP. 2016. Saline Soil Microbiome : A Rich Source of Halotolerant. Journal of Crop Science and Biotechnology 19:231–239. doi: https://doi.org/10.1007/s12892-016-0035-2.

Ibrahim IZB. 2008. Application Of Bacillus Sphaericus Upmb10 For Growth Enhancement Of Banana (Musa Spp. Var. Berangan) And Its Effect On Fusarium Wilt. [[Doctoral thesis]]: .

Ismail K, Husin MA, Zawawi NZ. 2020. Growing media effects on the performance of coconut seedlings (Cocos nucifera L.) at nursery stage. International Journal of Agriculture, Forestry and Plantation 10:1–8.

Jeong KW, Kim BS, Ultra Jr VU, Lee CL. 2015. Effects of rhizosphere microorganisms and wood vinegar mixtures on rice growth and soil properties.pdf. Korean Journal of Crop Science 60:355–365. doi: http://dx.doi.org/10.7740/kjcs.2015.60.3.355.

Jothityangkoon D, Koolachart R, Wanapat S, Wongkaew S, Jogloy S. 2008. Using wood vinegar in enhancing peanut yield and in controlling the contamination of aflatoxin producing fungus. International Crop Science 4:253–253.

Jun M, Zhi-ming Y, Wen-qiang W, Qing-li W. 2006. Preliminary study of application effect of bamboo vinegar on vegetable growth. Forestry Studies in China 8:43–47. doi: 10.1007/s11632-006-0023-6.

Kadota M, Niimi Y. 2004. Effects of charcoal with pyroligneous acid and barnyard manure on bedding plants. Scientia Horticulturae 101:327–332. doi: 10.1016/j.scienta.2004.01.002.

Kamaruddin MH. 2016. Respone of different sweet corn varieties to application of chemical fertilizer, poultry manure and Basillus sphaericus (UPMB10). [[Doctoral thesis]]: .

Kang MY, Heo KH, Kim JH, Cho SS, Seo PD, Rico CM, Lee SC. 2012. Effects of carbonized rice hull and wood charcoal mixed with pyroligneous acid on the yield, and antioxidant and nutritional quality of rice. Turkish Journal of Agriculture and Forestry 36:45–53. doi: 10.3906/tar-1001-640.

Khan N, Bano AMD, Babar A. 2020. Impacts of plant growth promoters and plant growth regulators on rainfed agriculture. PLoS ONE 15:1–32. doi: 10.1371/journal.pone.0231426.

Kuan KB, Othman R, Abdul Rahim K, Shamsuddin ZH. 2016. Plant growth-promoting rhizobacteria inoculation to enhance vegetative growth, nitrogen fixation and nitrogen remobilisation of maize under greenhouse conditions. PloS one 11:e0152478. doi: 10.1371/journal.pone.0152478.

Kurokura T, Hiraide S, Shimamura Y, Yamane K. 2017. PGPR improves yield of strawberry species under less-fertilized conditions. Environmental Control in Biology 55:121–128. doi: 10.2525/ecb.55.121.

Lashari MS, Ye Y, Ji H, Li L, Kibue GW, Lu H, Zheng J, Pan G. 2015. Biochar-manure compost in conjunction with pyroligneous solution alleviated salt stress and improved leaf bioactivity of maize in a saline soil from central China: A 2-year field experiment. Journal of the Science of Food and Agriculture 95:1321–1327. doi: 10.1002/jsfa.6825.

Ling Q, Huang W, Jarvis P. 2011. Use of a SPAD-502 meter to measure leaf chlorophyll concentration in Arabidopsis thaliana. Photosynthesis Research 107:209–214. doi: 10.1007/s11120-010-9606-0.

Lu X, Jiang J, He J, Sun K, Sun Y. 2019. Effect of Pyrolysis Temperature on the Characteristics of Wood Vinegar Derived from Chinese Fir Waste: A Comprehensive Study on Its Growth Regulation Performance and Mechanism. ACS Omega 4:19054–19062. doi: 10.1021/acsomega.9b02240.

Luo X, Wang Z, Meki K, Wang X, Liu B, Zheng H, You X, Li F. 2019. Effect of co-application of wood vinegar and biochar on seed germination and seedling growth. Journal of Soils and Sediments 19:3934–3944. doi: 10.1007/s11368-019-02365-9.

Mahmud KN, Yahayu M, Sarip SHM, Rizan NH, Min CB, Mustafa NF, Ngadiran S, Ujang S, Zakaria ZA. 2016. Evaluation on efficiency of pyroligneous acid from palm kernel shell as antifungal and solid pineapple biomass as antibacterial and plant growth promoter. Sains Malaysiana 45:1423–1434.

MARDI. 2008. Kacang panjang renek.

Mia MA, Shamsuddin ZH, Wahab Z, Marziah M. 2010. Effect of plant growth promoting rhizobacterial (PGPR) inoculation on growth and nitrogen incorporation of tissue-cultured Musa plantlets under nitrogen-free hydroponics condition. Australian Journal of Crop Science 4:85–90.

Mia MAB, Shamsuddin ZH, Wahab Z, Marziah M. 2005. High-yielding and quality banana production through plant growth-promoting rhizobacterial (PGPR) inoculation. Fruits 60:179–185. doi: 10.1051/fruits:2005024.

Mia MB, Shamsuddin Z, Mahmood M. 2012. Effects of rhizobia and plant growth promoting bacteria inoculation on germination and seedling vigor of lowland rice. AFRICAN JOURNAL OF BIOTECHNOLOGY 11:3758–3765. doi: 10.5897/AJB09.1337.

Mmojieje J. 2016. Pyroligneous Acid: A Farmer's Flexible Friend? Journal of Crop Improvement 30:341–351. doi: 10.1080/15427528.2016.1160462.

Mmojieje J, Hornung A. 2015. The Potential Application of Pyroligneous Acid in the UK Agricultural Industry. Journal of Crop Improvement 29:228–246. doi: 10.1080/15427528.2014.995328.

Mungkunkamchao T, Kesmala T, Pimratch S, Toomsan B, Jothityangkoon D. 2013. Wood vinegar and fermented bioextracts: Natural products to enhance growth and yield of tomato (Solanum lycopersicum L.). Scientia Horticulturae 154:66–72. doi: 10.1016/j.scienta.2013.02.020.

Naihati YF, Taolin RICO, Rusae A. 2018. Pengaruh Takaran dan Frekuensi Aplikasi PGPR terhadap Pertumbuhan dan Hasil Tanaman Selada (Lactuca sativa L.). Savana Cendana 3:1–3. doi: 10.32938/sc.v3i01.215.

Ofori K, Klogo P. 2005. Optimum Time for Harvesting Yardlong Bean (Vigna sesquipedalis) for High Yield and Quality of Pods and Seeds. Journal of Agrciukture and Socail Sciences 1:86–88.

Olawuni I, Ojukwu M, Iwouno J, Amandikwa C, Ibeabuchi C, Kpaduwa S. 2013. Effect of pH and temperature on functional physicochemical properties of asparagus bean (Vigna sesquipedalis) flours. International Journal of Basic and Applied Sciences 2:1–16.

Pampana S, Masoni A, Arduini I. 2016. Grain legumes differ in nitrogen accumulation and remobilisation during seed filling. Acta Agriculturae Scandinavica Section B: Soil and Plant Science 66:127–132. doi: 10.1080/09064710.2015.1080854.

Pangnakorn U, Watanasorn S, Kuntha C, Chuenchooklin S. 2009. Application of wood vinegar to fermented liquid bio-fertilizer for organic agriculture on soybean. Asian Journal Of Food & Agro-industry 2:189–196.

Petter FA, Silva LB, Souza IJ, Magionni K, Pacheco LP, Almeida FA, Pavan BE. 2013. Adaptation of the Use of Pyroligneous Acid in Control of Caterpillars and Agronomic Performance of the Soybean Crop. Journal of Agricultural Sciences 5:27–36. doi: 10.5539/jas.v5n8p27.

Pohan SD, Silaban LM, Amrizal, Pusptasari WD, Masni E. 2019. The Induction of Root Nodule and Increasing of Nitrogen Content of Common The Induction of Root Nodule and Increasing of Nitrogen Content of Common Bean (Phaseolus vulgaris L .) by Using Rhizobium Plus Fertilizer. In: The 3rd SATREPS Conference. February. p. 129–136.

Polthanee A, Kumla N, Simma B. 2015. Effect of Pistia stratiotes, cattle manure and wood vinegar (pyroligneous acid) application on growth and yield of organic rainfed rice. Paddy and Water Environment 13:337–342. doi: 10.1007/s10333-014-0453-z.

Prashanth S, Mathivanan N. 2010. Growth promotion of groundnut by IAA producing rhizobacteria Bacillus licheniformis MML2501. Archives of Phytopathology and Plant Protection 43:191–208. doi: 10.1080/03235400802404734.

Razak MH, Brahim Z, Saud HM. 2019. Effects of inoculation of plant growth promoting rhizobacteria to minimize panicle grain shattering habit for increased yield of rice (Oryza sativa L.). African Journal of Microbiology Research 13:256–263. doi: 10.5897/ajmr2017.8734.

Razali M, Aminuddin H, Azizah AN, Habsah M. 2006. Effect of packaging on the quality of minimally processed long bean (Vigna sesquipedalis L.). Journal of Tropical Agriculture and Food Science 34:309–319.

Robledo-Buriticá J, Aristizábal-Loaiza JC, Ceballos-Aguirre N, Cabra-Cendales T. 2017. Influence of plant growth-promoting rhizobacteria (PGPR) on blackberry (Rubus glaucus Benth. cv. thornless) growth under semi-cover and field conditions. Acta Agronomica 67:258–263. doi: 10.15446/acag.v67n2.62572.

Rocha I, Souza-Alonso P, Pereira G, Ma Y, Vosátka M, Freitas H, Oliveira RS. 2020. Using microbial seed coating for improving cowpea productivity under a low-input agricultural system. Journal of the Science of Food and Agriculture 100:1092–1098. doi: 10.1002/jsfa.10117.

Rodriguez IR, Miller GL. 2000. Using a chlorophyll meter to determine the chlorophyll concentration,nitrogen concentration, and visual quality of St. Augustinegrass. HortScience 35:751–754.

Rui Z, Wei D, Zhibin Y, Chao Z, Xiaojuan A. 2014. Effects of wood vinegar on the soil microbial characteristics. Journal of Chemical and Pharmaceutical Research 6:1254–1260.

Schwartz-Lazaro LM, Miller MR, Norsworthy JK, Scott RC. 2017. Comparison of Simulated Drift Rates of Common ALS-Inhibiting Rice Herbicides to Florpyrauxifen-Benzyl on Soybean. International Journal of Agronomy 2017:1–5. doi: 10.1155/2017/9583678.

Seo PD, Ultra Jr VU, Rubenecia MRU, Lee SC. 2015. Influence of Herbicides-pyroligneous Acids Mixtures on Some Soil Properties, Growth and Grain Quality of Paddy Rice. International Journal of Agriculture and Biology 17:499–506. doi: 10.17957/IJAB/17.3.14.349.

Seufert V, Ramankutty N, Mayerhofer T. 2017. What is this thing called organic? – How organic farming is codified in regulations. Food Policy 68:10–20. doi: 10.1016/j.foodpol.2016.12.009.

Shahverdi MA, Omidi H, Damalas CA. 2020. Foliar fertilization with micronutrients improves Stevia rebaudiana tolerance to salinity stress by improving root characteristics. Revista Brasileira de Botanica 43:55–65. doi: 10.1007/s40415-020-00588-6.

Shirkhani A, Nasrolahzadeh S. 2016. Vermicompost and Azotobacter as an ecological pathway to decrease chemical fertilizers in the maize, Zea mays. Bioscience Biotechnology Research Communications 9:382–390. doi: 10.21786/bbrc/9.3/7.

Shuang SUN, Zi-Ting G, Zhan-Chao L, Yue L, Jun-Li G, Yuan-jun C, Hao L, Xing-Yu L, Zi-Ming W. 2020. Effect of Wood Vinegar on Adsorption and Desorption of Four Kinds of Heavy (loid) Metals Adsorbents. Chinese Journal of Analytical Chemistry 48:e20013–e20020. doi: 10.1016/S1872-2040(19)61217-X.

Silva CJD, Karsburg IV, Dias PC, Arruda TPMD. 2017. Pyroligneous liquor effect on in and Ex Vitro prodution of Oeceoclades maculata (Lindl). Lindl. Revista Caatinga 30:947–954. doi: 10.1590/1983-21252017v30n415rc.

Simma B, Siri B, Promkhambut A, Polthanee A. 2017. Influence of seed priming and soil water content on growth and yield of two rice cultivars grown under greenhouse conditions. Maejo International Journal of Science and Technology 11:175–188.

Siriwardena BP, Subasinghe S, Vidanapathirana NP, Dhanushka TGB. 2019. Study the Pest Repellant Action of Abelmoschus esculentus (Okra) and *Solanum melongena* (Egg- Plant) as Affected by Application of Different Concentration of Wood Vinegar / Pyroligneous Acid Produced by Using Different Wood Species. International Journal of Research & Review 6:137–140.

Stagnari F, Maggio A, Galieni A, Pisante M. 2017. Multiple benefits of legumes for agriculture sustainability: an overview. Chemical and Biological Technologies in Agriculture 4:1–13. doi: 10.1186/s40538-016-0085-1.

Steiner C, Das KC, Garcia M, Förster B, Zech W. 2008. Charcoal and smoke extract stimulate the soil microbial community in a highly weathered xanthic Ferralsol. Pedobiologia 51:359–366. doi: 10.1016/j.pedobi.2007.08.002.

Su AY, Niu SQ, Liu YZ, He AL, Zhao Q, Paré PW, Li MF, Han QQ, Khan SA, Zhang JL. 2017. Synergistic effects of bacillus amyloliquefaciens (GB03) and water retaining agent on drought tolerance of perennial ryegrass. International Journal of Molecular Sciences 18:1–13. doi: 10.3390/ijms18122651.

Sumithra K, Jutur PP, Carmel BD, Reddy AR. 2006. Salinity-induced changes in two cultivars of Vigna radiata: Responses of antioxidative and proline metabolism. Plant Growth Regulation 50:11–22. doi: 10.1007/s10725-006-9121-7.

Sun H, Feng Y, Xue L, Mandal S, Wang H, Shi W, Yang L. 2020. Responses of ammonia volatilization from rice paddy soil to application of wood vinegar alone or combined with biochar. Chemosphere 242:125247. doi: 10.1016/j.chemosphere.2019.125247.

Tan Kz, Radziah O, Halimi MS, Khairuddin AR, Habib SH, Shamsuddin ZH. 2014. Isolation and Characterization of Rhizobia and Plant Growth-Promoting Rhizobacteria. American Journal of Agricultural and Biological Sciences 9:342–360. doi: 10.3844/ajabssp.2014.342.360.

Tan Kz, Radziah O, Halimi MS, Khairuddin AR, Shamsuddin ZH. 2015. Assessment of plant growth-promoting rhizobacteria (PGPR) and rhizobia as multi-strain biofertilizer on growth and N 2 fixation of rice plant. Australian Journal of Crop Science 9:1257–1264.

Tantasawat P, Trongchuen J, Prajongjai T, Seehalak W, Jittayasothorn Y. 2010. Variety identification and comparative analysis of genetic diversity in yardlong bean (Vigna unguiculata spp. sesquipedalis) using morphological characters, SSR and ISSR analysis. Scientia Horticulturae 124:204–216. doi: 10.1016/j.scienta.2009.12.033.

Tindall HD. 1983. Vegetables in the tropics. doi: 10.1016/0304-4238(87)90006-9.

Travero JT, Mihara M. 2016. Effects of Pyroligneous Acid to Growth and Yield of Soybeans (Glycine max). IJERD-International Journal of Environmental and Rural Development :7–8.

Ujang Z. 2011. Potensi kacang panjang renek.

Wang Y, Qiu L, Song Q, Wang S, Wang Y, Ge Y. 2019. Root proteomics reveals the effects of wood vinegar on wheat growth and subsequent tolerance to drought stress. International Journal of Molecular Sciences 20:1–23. doi: 10.3390/ijms20040943.

Yasmin F, Othman R, Sijam K, Saad MS. 2007. Effect of PGPR Inoculation on Growth and Yield of Sweetpotato. Journal of Biological Sciences 7:421–424. doi: 10.3923/jbs.2007.421.424.

Zulkarami B, Ashrafuzzaman M, Husni M, Ismail MR. 2011. Effect of pyroligneous acid on growth, yield and quality improvement of rockmelon in soilless culture. Australian Journal of Crop Science 5:1508–1514.

The Official Journal of the
Farm to Fork Foundation
ISSN: 2518–2021 (print)
ISSN: 2415–4474 (electronic)
http://www.f2ffoundation.org/faa