Indonesian Journal of Agronomy

ISSN 2085-2916 | e-ISSN 2337-3652
Indonesian J. Agron., *51*(1), 109-120, April 2023
DOI: https://dx.doi.org/10.24831/ija.v51i1.45864

Research Article

Leaves production and its flavonoids content of moringa (*Moringa oleifera* Lam.) from fulvic acid treatment

Joan Joulanda Grace Kailola [1], Edi Santosa [2,*], Sandra Arifin Aziz [2], Diny Dinarti [2], and Winarso Drajad Widodo [2]

[1] Graduate School of Agronomy and Horticulture, Faculty of Agriculture, IPB University, Jl. Meranti, Kampus IPB Darmaga, Bogor 16680, INDONESIA
[2] Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University, Jl. Meranti, Kampus IPB Darmaga, Bogor 16680, INDONESIA
* Corresponding author (✉ edisang@gmail.com)

ABSTRACT

Moringa is drought tolerant and its leaf is traditionally used as a vegetable. Recently, the leaf is used commercially in traditional medicine and functional foods. Fulvic acid is a kind of plant growth regulator derived from humic acid and is considered a fertilizer in sustainable agriculture. The objective of the research was to determine the effect of fulvic acid concentration on the growth, biomass, phosphorus and flavonoids contents of moringa seedlings. The experiment was conducted from July 2020 to January 2021 at Leuwikopo Experimental Station, Bogor. The treatment was arranged in a randomized block design consisting of two factors, i.e., fulvic acid level (0, 1, 2, 3, and 4 mL L^{-1}) and moringa accessions (East Nusa Tenggara-ENT and Leuwikopo-LWK). Observation focused on growth and biomass, total flavonoids, and phosphorus contents of leaves. The results showed that fulvic acid enhanced leaf growth, and plant height. On the other hand, the fulvic acid application had no significant effect on biomass production, total flavonoids, and phosphorus contents of moringa leaves. Accessions expressed different responses to fulvic acid levels, i.e.e, the fulvic acid of 1 mL L^{-1} seemed favorable for ENT accession while 2 mL L^{-1} was favorable for LWK accession as indicated by the level of flavonoid content. Therefore, fulvic acid is beneficial in moringa cultivation, particularly for ENT accession. It is interesting to evaluate the fulvic acid application on moringa trees grown in ENT where soil moisture is considered low.

Keywords: accession; phosphorus; seedling; humic acid; East Nusa Tenggara; vegetable

Edited by:
Siti Marwiyah

***Received**:*
30 January 2023
***Accepted**:*
14 April 2023
***Published online**:*
27 April 2023

Citation:
Kailola, J. J. G., Santosa, E., Aziz, S. A., Dinarti, D., & Widodo, W. D. (2023). Leaves production and its flavonoids content of moringa (*Moringa oleifera* Lam.) from fulvic acid treatment. *Indonesian Journal of Agronomy*, *51*(1), 109-120

INTRODUCTION

Moringa (*Moringa oleifera* Lam.) named *kelor* by Indonesian is a hardy perennial tree that tolerant to saline and drought (Nouman et al., 2013; Azam et al., 2020). The tree is fast-growing, and deciduous, with small and sparse leaves (Orwa et al., 2009). In Indonesia, the moringa tree is mostly- abundant in drought-prone areas, especially in particular areas of Central and East Java, and West and East Nusa Tenggara. According to Orwa et al. (2009), moringa grows at altitudes 0 to 1,000 m above sea level, annual temperature of 12.6 to 40.0 °C, and annual precipitation of at least 500 mm. Therefore, the moringa tree is suitable as a fence, hedge, and afforestation (Dani et al., 2019).

All moringa parts, i.e., stem, leaves, pods, and seeds are used (Dani et al., 2019). Leaves and young pods are usually cooked as vegetables; and the leaves are utilized as tea and made into flour for broader food dishes (Dani et al., 2019). Moringa leaf is rich in flavonoids and recently has been used as herbal medicine and functional foods (Leone et al., 2015; Brodowska, 2017; Lin et al., 2018). According to Gonzalez-Romero et al. (2020)

in 100 g dry weight, moringa leaf contains total flavonoids 327.2±13.8 mg which is considered higher than those of existing commercial vegetables (3.8–191 mg). For that, WHO has declared moringa 'a miracle plant' because it has saved many lives, especially children in many poor countries (Kurniawan, 2019).

In Indonesia, increasing the importance of the moringa tree, resulted in many efforts have been done to introduce the tree to dry zone, such as the Nusa Tenggara Islands. Kefi et al. (2020) noted that Nusa Tenggara Islands, such as Timor Island have an annual precipitation of about 1,500 mm with many savannas. The introduction of moringa is aimed to increase affordable nutrition availability to poor farmers. Here, speeding up seedling tree establishment is important for such areas. Nevertheless, research on low-cost plant growth regulators is still limited in moringa.

Fulvic acid is a kind of plant growth regulator derived from humic acid and has been proven to increase production and quality in many crops (Canellas et al., 2015). Fulvic acid is extracted from humic substances; humic is an organic compound consisting of organic acids derived from the decomposition of plant and animal residues through microorganisms and geochemistries (Morales et al., 2014). Unlike humic acid which is already well-studied in tropical crops (Rohman et al., 2019), fulvic acid is still rare. Fulvic acid is believed to play as a fertilizer in sustainable agriculture promotion in developing countries because could be self-produced by farmers.

Application of fulvic acid benefits plants by increasing drought resistance, increasing nutrient absorption, stabilizing soil pH, and reducing fertilizer leaching (Suh et al., 2014). The application also increases the availability of phosphorus in the soil (Yang et al., 2013; Abdelhamid et al., 2016). According to Abdelhamid et al. (2016) phosphorus uptake by plants increases after application of fulvic acid to the soil. Therefore, phosphorus status in the plant is an important indicator of the effectivity of fulvic acid application.

According to Sun (2020), fulvic acid increases drought tolerance in *Camelia sinensis* through the regulation of ascorbate metabolism and biosynthesis of total flavonoids. As mentioned above, total flavonoids is an important component of moringa leaves (Leone et al., 2015; Brodowska, 2017; Lin et al., 2018; Gonzalez-Romero et al., 2020). Thus, the evaluation of flavonoids level in the present experiment is important. The study aimed to determine the effect of fulvic acid concentration on the growth, biomass, phosphorus and flavonoids content of moringa seedlings.

MATERIALS AND METHODS

Experimental site

The research was conducted from July 2020 to January 2021 at the Leuwikopo Experimental Station, Department of Agronomy and Horticulture, IPB University, Bogor (-6.56416854,106.72477705; 186 m asl). Plants were maintained under a shading net with about 20% shading level. Observation of flavonoid content was carried out at the Post-Harvest Laboratory, and plant analysis was carried out at the Testing Laboratory of the Department of Agronomy and Horticulture, IPB University.

Moringa seeds were obtained from farmer fields in Timur Tengah Utara District of East Nusa Tenggara East (ENT) Province and Leuwikopo, Bogor West Java Province, named as ENT and LWK accessions, respectively. The seeds were collected from mature pods and directly shipped to IPB University.

Treatment method

A two-factor experiment was arranged in a randomized block design and replicated 3 times. Number of sample plants in one replication was 10. The first factor was accessions (ENT and LWK), while the second factor was fulvic acid concentration. The fulvic acid solution originated from commercial liquid fertilizer (Terra Novelgro®). Based on analysis, the fertilizer solution contained C 12.9%, N 0.51%, P 0.04%, K 109.00 ppm, Ca 8.23 ppm, Mg 4.08 ppm, Fe 44.85 ppm, Zn 4.05 ppm, and humic acid 0.55% and fulvic acid

74.26% with pH 9.4. The fulvic acid treatment consisted of five levels (0, 1, 2, 3, and 4 mL L^{-1}). The level of fulvic acid was based on total solution of Terra Novelgro®.

Moringa seeds were soaked, in water for 24 hours until swollen, then two seeds were planted in a polybag measuring 30 cm x 30 cm containing a mixture of soil:sand:compost (2:1:1; v/v). The seeds germinated within 5 – 7 days after planting. A single vigorous plant was maintained by removing an unvigorous one. The fulvic acid solution was applied through the soil to seedlings, starting 1 week after planting (WAP). About 100 mL solution was applied in a polybag using the designed treatment. The application was repeated every 2 weeks, and the final application was 7 WAP.

Plant maintenance included daily watering, weeding, and pest and disease control. Harvesting leaves was done at 8 WAP by picking all fresh leaves.

Growth analysis

Plant growth was monitored from plant height and the number of leaves. Plant height was measured from the soil surface to the tip of plant growth. The number of leaves was counted considering tripinnate leaves that had fully opened. Plant height and leaf number counting were carried out at 1-8 WAP.

Fresh and dry weight analysis

At harvest, the fresh weight of shoot, and roots were measured. The dry weight of the shoot and roots was measured after oven-dried at 105 ^{o}C for 24 hours. Shoot to root ratio was calculated based on the dry weight.

Phosphorus and total flavonoids analysis

Phosphorus status in plants was measured by ash-method wetted with a mixture of HNO_3 and $HClO_4$ following Eviati and Sulaeman (2009). Total flavonoids content was analyzed following Vongsak et al. (2013), and data were presented in % dry weight (DW). Phosphorus dan total flavonoids were evaluated from leaves. Before analysis, all leaves were harvested. Petioles were excluded from analysis.

A brief method for flavonoids content was as follows: the sample was dried at 60 ^{o}C for 24 hours, powdered, and sieved with 20 mesh. About 3 g sample was stored in a closed container plus silica gel to maintain powder humidity. Moringa leaf powder of about 2 g was oven-dried at 105 ^{o}C for 3 hours until constant weight. For extraction analysis, 0.01 g was dissolved in 4 mL ethanol 70%, incubated, and shaken for 72 hours in a dark room. After incubation, ethanol 70% was added to reach a final volume of 10 mL, mixed, and centrifuged at 5000 RPM for 7 minutes. A supernatant of 2 mL was transferred to a new tube and added 2 mL of aluminum chloride solution (2%). The solution then was homogenized and incubated for 10 minutes at dark and room temperature. Finally, the solution was read at a wavelength of 415 nm using a spectrophotometer, and data was presented in mg/100 g DW. Quercetin of 0.1 to 1.0 mg mL^{-1} was used as a standard of flavonoid measurement.

Statistical analysis

Analysis of variance (ANOVA) was performed with SAS 9.12 portable followed by Duncan's Multiple Range Test (DMRT) at α=0.05 or a level of confidence of 95%.

RESULTS AND DISCUSSION

Analysis of variance

ANOVA revealed interaction effect was found no significant between genotypes and fulvic acid treatments on observed variables, except for plant height 6 to 7 WAP and number of leaves at 2, 3 and 5 to 8 WAP (Table 1). In this study, fulvic acid was applied through the soil, so that the effect of its application had a greater role on the nutrients in the soil (El-Din et al., 2021). The finding indicates that the root system is likely to have similarities between moringa genotypes so that they do not respond differently to the different concentrations of fulvic acid applications.

Table 1. ANOVA summary for interaction between genotype and fulvic acid treatments on particular variables.

WAP	Plant height	Number of leaves	Root weight		Shoot weight		Ratio shoot/root[z]	Total flavonoids	Phosphorus
			Fresh	Dry	Fresh	Dry			
1	ns	ns	-[y]	-	-	-	-	-	-
2	ns	*	-	-	-	-	-	-	-
3	ns	*	-	-	-	-	-	-	-
4	ns	ns	-	-	-	-	-	-	-
5	ns	*	-	-	-	-	-	-	-
6	*	*	-	-	-	-	-	-	-
7	*	*	-	-	-	-	-	-	-
8	*	*	ns	ns	ns	ns	ns	-	ns

Note: ns-non significant, * significant at α = 5%; [z] Based on dry weight, [y] No data was taken/not suitable for statistical analysis.

Growth analysis

The level of fulvic acid significantly affected plant height at 3 to 8 WAP (Table 2). Application of 2 mL L^{-1} stimulated the highest height at 3, 4, 5, and 6 WAP, i.e., 19 cm, 25.33 cm, 31.58 cm, and 41.83 cm, respectively. At 7 and 8 WAP, the highest plant occurred from fulvic acid 1 mL L^{-1}, i.e., 50.00 cm and 55.50 cm.

The accessions had different heights at 1 and 2 WAP, while no difference was observed thereafter (Table 2). Leuwikopo (LWK) accession showed a higher shoot than the ENT accession in both weeks. Different plant heights at the initial growth stage are probably due to differences in the nutrients of seeds. In *Phaseolus vulgaris*, Singh et al. (2017) revealed that differences in early seedling growth are due to differences in primary nutrients reserved in cotyledons and seed size. The effect of propagule origin, size, and genetic factors are also known to affect early plant establishment in various crops (Kołodziejek, 2017; Singh et al., 2017; Hidayatullah et al., 2020; Maretta et al., 2020; Maghfirah et al., 2022). Kolodziejek (2017) noted that seed coming from the forest has higher viability than those from the roadside. However, in the present experiment, the seed size, nutrient content, genetic and ecological site of mother plants were not evaluated.

Furthermore, there was an interaction between fulvic acid levels and accessions on plant height at 5 to 8 WAP (Table 2). LWK accession produced taller seedlings when supplemented with 2 mL L^{-1} fulvic acid, while ENT accessions produced superior growth with 1 mL L^{-1}.

Fulvic acid of 1 to 2 mL L^{-1} seems the best in plant height of both LWK and ENT accessions. Abdel-Baky et al. (2019) studied *Vicia faba* L. and revealed that application of fulvic acid at a rate of 9 g L^{-1} results in the highest plant as compared to control without fulvic acid application; and the response is dependent on variety. Many authors describe the mechanism of fulvic acid to increase tree growth is through stimulating cell metabolism and nutrient absorption (Nardi et al., 2002; Yazdani et al., 2014, Akladious and Mohamed, 2018).

Number of leaves

The fulvic acid application significantly affected the number of leaves (Table 3). A fulvic acid level of 1 mL L^{-1} stimulated the plant to produce more leaves than at other levels. The highest number of leaves was 8 leaves at 3 WAP and 10 leaves at 5 WAP of the fulvic acid level of 1 mL L^{-1}. Table 3 shows that the accessions showed similar ability to produce leaves at 1 to 8 WAP. In general, 1-3 leaves were developed in a week, irrespective of accessions.

Table 2. Plant height of moringa from different fulvic acid treatments at 1-8 WAP.

Plant age	Fulvic acid level (mL L^{-1})	Plant height (cm)		
		LWK	ENT	Avg
1 WAP	0	6.96a	6.33a	6.65a
	1	10.66a	7.00a	8.83a
	2	10.96a	7.83a	9.40a
	3	8.16a	6.03a	7.10a
	4	9.33a	5.10a	7.21a
	Avg	9.22a	6.46b	
2 WAP	0	10.83a	9.83a	10.33a
	1	17.00a	12.00a	14.50a
	2	16.00a	12.83a	14.41a
	3	12.66a	11.33a	12.00a
	4	15.16a	8.16a	11.66a
	Avg	14.33a	10.83b	
3 WAP	0	12.16a	13.33a	12.75a
	1	21.16a	16.50a	18.83a
	2	21.50a	16.50a	19.00a
	3	16.00a	16.33a	16.17a
	4	18.83a	10.50a	14.67a
	Avg	17.93a	14.63a	
4 WAP	0	14.00a	16.00a	15.00b
	1	25.66a	21.16a	23.41a
	2	28.66a	22.00a	25.33a
	3	20.50a	21.16a	20.83ab
	4	22.33a	13.66a	18.00ab
	Avg	22.23a	18.80a	
5 WAP	0	17.16b	20.33a	18.75b
	1	29.33ab	28.00a	28.66a
	2	37.16a	26.00a	31.58a
	3	24.50ab	26.83a	25.66ab
	4	28.50ab	16.83a	22.66ab
	Avg	27.33a	23.60a	
6 WAP	0	22.00b	24.33c	23.17c
	1	39.33ab	38.33a	38.83ab
	2	46.66a	37.00ab	41.83a
	3	29.00ab	35.33abc	32.16abc
	4	35.66ab	22.00c	28.83bc
	Avg	34.53a	31.40a	
7 WAP	0	23.67b	29.16b	26.41b
	1	49.67ab	50.33a	50.00a
	2	53.17a	44.50ab	48.83a
	3	32.00ab	45.33ab	38.66ab
	4	41.83ab	28.33b	35.08ab
	Avg	40.06a	39.53a	
8 WAP	0	24.67b	31.66b	28.17b
	1	53.67ab	57.33a	55.50a
	2	57.67a	46.66ab	52.17ab
	3	35.33b	52.00a	43.67b
	4	43.00ab	32.33b	37.67b
	Avg	44.00a	42.86a	

Note: Values in a column of particular WAP followed by different alphabets are significantly different after DMRT test α = 5%; Avg-average; LWK-Leuwikopo, ENT-East Nusa Tenggara; WAP-week after planting.

Table 3. Number of moringa leaves of different fulvic acid treatments at 1-8 WAP.

Plant age	Fulvic acid level ($mL L^{-1}$)	Number of leaves		
		LWK	ENT	Avg
1 WAP	0	2.33a	3.00a	2.66a
	1	3.33a	3.00a	3.16a
	2	3.00a	3.00a	3.00a
	3	2.66a	3.00a	2.83a
	4	2.33a	2.00a	2.16a
	Avg	2.73a	2.80a	
2 WAP	0	4.66a	6.33a	5.50a
	1	5.66a	5.66ab	5.66a
	2	6.00a	5.66ab	5.83a
	3	5.33a	6.00ab	5.66a
	4	5.33a	5.33b	5.33a
	Avg	5.40a	5.80a	
3 WAP	0	6.00a	7.66a	6.83b
	1	8.00a	8.00a	8.00a
	2	7.00a	7.66a	7.33ab
	3	6.66a	8.33a	7.50ab
	4	7.33a	6.33b	6.83b
	Avg	7.00a	7.60a	
4 WAP	0	7.66a	8.66a	8.16a
	1	9.66a	8.33a	9.00a
	2	8.33a	9.33a	8.83a
	3	8.33a	8.66a	8.50a
	4	9.00a	8.33a	8.66a
	Avg	8.60a	8.66a	
5 WAP	0	7.66b	8.66a	8.16b
	1	11.33a	8.66a	10.00a
	2	9.00ab	10.00a	9.50ab
	3	8.33b	9.00a	8.66ab
	4	10.33ab	8.33a	9.33ab
	Avg	9.33a	8.93a	
6 WAP	0	8.00b	8.66a	8.33a
	1	11.33a	8.66a	10.00a
	2	9.00ab	10.00a	9.50a
	3	8.33ab	9.00a	8.66a
	4	10.33ab	8.33a	9.33a
	Avg	9.40a	8.93a	
7 WAP	0	8.00b	8.66a	8.33a
	1	11.33a	8.66a	10.00a
	2	9.00ab	10.00a	9.50a
	3	8.33ab	9.00a	8.66a
	4	10.33ab	8.33a	9.33a
	Avg	9.40a	8.93a	
8 WAP	0	8.00b	8.66a	8.33a
	1	11.33a	8.66a	10.00a
	2	9.00ab	10.00a	9.50a
	3	8.33ab	9.00a	8.66a
	4	10.33ab	8.33a	9.33a
	Avg	9.40a	8.93a	

Note: Values in a column of particular WAP followed by different alphabets are significantly different after DMRT test α = 5%; Avg-average; LWK-Leuwikopo, ENT-East Nusa Tenggara; WAP-week after planting.

ANOVA indicated that the interaction between fulvic acid level and accessions on the number of leaves was significant (Table 1), except at 1 and 4 WAP. Non-consistent interaction effect between fulvic acid applications and accessions in the early growth of moringa genotypes is still unknown.

Application of fulvic acid at a rate of 4 mL L^{-1} on ENT accession produced the lowest number of leaves as compared to other treatments at 3 WAP (Table 3). It seems that 4 mL L^{-1} of fulvic acid had a detrimental effect on ENT accession at 3 WAP. Table 2 shows that ENT accession treated with 4 mL L^{-1} fulvic acid showed a tendency to have a lower leaf number starting in 2 WAP. It is probable that the low leaf number at 3 WAP of the accession is due to carry over growth suppression from the previous week.

Here, LWK accession is likely more responsive to the fulvic acid application than ENT accession, especially starting at 5 WAP (Table 3). On the other side, ENT accession response to the different levels of fulvic acid for the number of leaves only at 2 WAP. In terms of leaf number, the application of fulvic acid is more prominent in LWK accession. It is still unknown, why the LWK accession is more responsive than the ENT one. Since the number of leaves is the most economic value in the moringa tree, it is interesting to evaluate LWK and ENT accessions from the genetic and ecological background.

The finding in the number of leaves is unexpected because fulvic acid treatments generally stimulated a higher number of leaves since fulvic acid stimulates hormone activity involving photosynthesis, respiration, and protein synthesis (Schiavon et al., 2010, Fan et al., 2014, Dawood et al., 2019; Bayat et al., 2021). According to Bayat et al. (2021), the application of fulvic acid stimulates *Achillea millefolium* L. to produce 2 leaves higher than the control. Nevertheless, further statistical analysis using the level of confidence of 90% indicated that fulvic acid level of 1 to 2 mL L^{-1} produced the highest number of moringa leaves in both Leuwikopo and NTT accessions (data not shown).

Plant dry weight

Application of different levels of fulvic acid did not show any significant effect on the fresh and dry weight of both root and shoot, irrespective of accessions (Table 4). Further analysis of ANOVA using a level of confidence of 90% showed that fresh and dry weight of root was significant at level fulvic acid 2 mL L^{-1} for LWK accession (data not shown). On the other hand, control treatment tended to produce the highest root biomass for ENT accession. Nevertheless, it is still unknown why the fulvic acid application tended to suppress dry weight accumulation in shoot of ENT accession. It is interesting in the future to study morpho-physiological effect of fulvic acid on ENT accession for a better understanding of the role of fulvic acid on dry weight accumulation.

Table 4. The fresh and dry weight of root and shoot of moringa of different fulvic acid treatments at 8 WAP.

Fulvic acid level (mL L^{-1})	Fresh weight (g)			Dry weight (g)		
	LWK	ENT	Avg	LWK	ENT	Avg
			Root			
0	49.45a	40.80a	45.13a	11.26a	8.21a	9.73a
1	71.99a	28.69a	50.34a	15.56a	5.39a	10.47a
2	97.95a	32.93a	65.44a	22.22a	6.46a	14.34a
3	62.48a	26.65a	44.56a	13.61a	5.16a	9.38a
4	78.62a	29.44a	54.03a	16.65a	5.85a	11.25a
Avg	72.10a	31.70b		15.86a	6.21b	
			Shoot			
0	27.57a	67.29a	47.43a	5.54a	13.40a	9.47a
1	48.87a	37.75a	43.31a	9.42a	7.47a	8.44a
2	46.49a	33.85a	40.17a	9.53a	6.91a	8.22a
3	32.13a	33.05a	32.59a	6.39a	6.20a	6.29a
4	33.76a	26.88a	30.32a	6.39a	5.35a	5.87a
Avg	37.76a	39.76a		7.45a	7.86a	

Note: Values in a column of particular WAP followed by different alphabets are significantly different after DMRT test α = 5%; Avg-average; LWK-Leuwikopo accession, ENT-East Nusa Tenggara accession; WAP-week after planting.

Irrespective of fulvic acid levels, LWK accession produced higher root fresh and dry weight than the ENT (Table 4). Interaction between fulvic acid levels and accessions was none for both weights of shoot and root biomass (Table 1). On average, the dry weight of LWK root was 15.86 g while ENT was 6.21 g. It is noticeable that the dry weight of LWK is nearly 2.6 times higher than that of ENT accession. However, shoot biomass was statistically similar among accessions. Interestingly, the control plant of ENT from fulvic acid treatment tended to have higher shoot dry mass than those of other treatments. As shown in Table 4, the dry weight of the control plants was 13.4 g almost 3 times higher than the treatment of 4 mL L^{-1} fulvic acid.

The finding of the present experiment is in contrary to Yasdani et al. (2014), where the application of fulvic acid stimulates root weight in *Gerbera jamesonii* L. Moreover, Yasdani et al. (2014) stated that plants treated with nutrient solution supplemented with 50 mg L^{-1} fulvic acid produce root dry weight 4.8 times heavier than the control. Different application method probably determines the effectivity as in the previous report. In the present experiment, the application of fulvic acid solution was through soil dressing.

Ratio shoot to root

Ratio shoot to root based on dry weight showed that the fulvic acid treatment did not have a significant effect, although 1 mL L^{-1} had a tendency to increase the average ratio shoot to root, irrespective of accessions (Table 5). Interestingly, on average ENT accession had a higher ratio shoot to the root (almost three times) than that of LWK accession, i.e., 1.23 and 0.49, respectively.

Table 5. Ration shoot to root, total flavonoids and phosphorus contents of moringa at 8 WAP.

Fulvic acid level (mL L^{-1})	Ratio shoot/root[z]			Total flavonoids (mg/100 g DW)[y]			Phosphorus (% DW)[y]		
	LWK	ENT	Avg	LWK	ENT	Avg	LWK	ENT	Avg
0	0.49a	1.33a	0.91a	754.36	1,361.11	1,057.74	0.35a	0.33a	0.34ab
1	0.61a	1.38a	1.00a	1,056.54	1,625.08	1,340.81	0.34a	0.50a	0.42a
2	0.44a	1.08a	0.76a	1,227.36	1,458.59	1,342.98	0.26a	0.23a	0.25b
3	0.44a	1.48a	0.96a	480.71	1,037.95	759.33	0.28a	0.28a	0.28ab
4	0.48b	0.89a	0.69a	1,066.12	843.33	954.73	0.22a	0.25a	0.23b
Avg	0.49b	1.23a		754.36	1,361.11	1,057.74	0.29a	0.32a	

Note: Values in a column followed by different alphabets are significantly different after DMRT test α = 5; Avg-average; LWK-Leuwikopo accessions, ENT-East Nusa Tenggara accessions; [z]Based on dry weight basis; [y]Based on leaves dry weight.

The higher ratio shoot to root is due to ENT accession having a smaller root dry weight as shown in Table 4. Ratio shoot to root is known as affected by genetics, agriculture practice, and environmental factors (Yulianti et al., 2018; Budiarto et al., 2019; Khasanova et al., 2019). Here, the different ratio shoot to root between LWK and ENT accessions is likely due to genetic factors. However, such speculation needs further genetic analysis.

Total flavonoids content

The total flavonoids content in leaves seemed to be affected by fulvic acid treatment and accession origin (Table 5). However, no statistical analysis was suitable for flavonoids content because the number of samples was not meet the number of replicates. Flavonoids content in LWK accession ranged from 480.71- 1,227.36 mg, while ENT accession had 843.33- 1,625.08 mg with an average 917.02 and 1,265.21 mg/100 g leaf dry weight, respectively.

In absence of statistical analysis, Table 5 shows that the flavonoids value of plants treated with 2 mL L^{-1} fulvic acid was the highest in LWK accession and while the highest value in ENT accession was from 1 mL L^{-1} fulvic acid. It is immature to conclude the positive effect of fulvic acid on flavonoids of moringa. Nevertheless, from average data across accessions, 2 mL L^{-1} fulvic acid level was considered as the best level for both accessions with flavonoids content 1227.36 mg/100 g leaf dry weight.

A study by Santiago et al. (2008) shows the positive effect of the fulvic acid application on secondary metabolites of saffron (*Crocus sativus*) due to increasing nutrient uptake. Moreover, Schiavon et al. (2010) show fulvic acid stimulates phenylalanine and tyrosine ammonia-lyase enzymes, total phenolics, and flavonoids in maize leaves. The present experiment shows that fulvic acid application at the rate of 1 to 2 mL L^{-1} could be sufficient to obtain high flavonoids content of moringa. Nevertheless, it needs further clarification due to the insufficient statistical evidence in the present experiment.

Phosphorus content

No phosphorus variation was found among LWK and ENT accessions by different fulvic acid levels (Table 5). The interaction effect between fulvic acid levels and accessions on phosphorus content did not exist (Table 1). Table 5 shows that irrespective of fulvic acid level, phosphorus content in LWK accession was 0.22-0.35% (average 0.29%), and in ENT accession was 0.23-0.50% (average 0.32%). The finding is contrary to Yazdani et al. (2014) where the application of fulvic acid increases phosphorus levels in the leaves and stem of *Gerbera jamesonii* L.

Average phosphorus content across accessions showed that fulvic acid application had a significant effect (Table 5). Application of 1 mL L^{-1} fulvic acid stimulated moringa to accumulate 0.42% phosphorus; the highest among fulvic acid levels. Fulvic acid application larger than 1 mL L^{-1} tended to reduce the phosphorus content in moringa leaves. The finding in the present experiment is a little bit strange. It is widely studied that fulvic acid increases nutrient absorption through induce the activity plasma membrane (Nardi et al., 2002; Khaled and Fawy, 2011) and increases phosphorus availability in soil (Marshner, 1986; Stevenson, 1991). According to Yang et al. (2013) application of fulvic acid to soil stimulates the population of microbes, organic content, and capacity of cation exchange.

The non-significant effect of the fulvic acid application for both accessions (Table 5) could be due to the soil factor or incomplete data for statistical analysis. Du et al. (2013) and Yang et al. (2013) stated that the application of fulvic acid is effective in soil with a sufficient level of phosphorus status. Moreover, according to Ibrahim et al. (2022), the phosphorus uptake is optimum in soil pH 6.0–6.5. Unfortunately, soil analysis was not done in the present analysis. According to data published by Santosa et al. (2017), the soil of latosol Darmaga at Leuwikopo Experimental Station generally has low pH (H_2O) of 4.5 and total phosphorus (Bray I) of 5.6 ppm. It is possible that such a status of the phosphorus and pH level could determine the effectivity of the fulvic acid application in the present experiment.

CONCLUSIONS

The fulvic acid application significantly increased plant height and leaf number, but the application had no effect statistically on flavonoids, and phosphorus contents of moringa leaves. The dry weight of the root and shoot was not affected by fulvic acid treatments, irrespective of LWK and ENT accessions. Application of 1 to 2 mL L^{-1} fulvic acid solution seemed sufficient to support moringa growth at the seedling stage. In the future, it is interesting to study morpho-physiology and soil properties to increase the effectivity of fulvic acid application in a wider agroecological condition.

ACKNOWLEDGEMENTS

Thanks to the Education Fund Management Institute (LPDP), Ministry of Finance the Republic of Indonesia for research funding support.

REFERENCES

Abdel-Baky, Y.R., Abouziena, H.F., Amin, A.A., Rashad El-Sh, M., & Abd El-Sttar, A.M. (2019). Improve quality and productivity of some faba bean cultivars with foliar application of fulvic acid. *Bulletin of the National Research Centre*, *43*(2), 1-12. Https://doi.org/10.1186/s42269-018-0040-3

Abdelhamid, M.T., Elsayed, M.A., Abo-Elwafa, M.A., & El-Mogy, M.M. (2016). Effects of fulvic acid on soil properties and wheat yield in a sandy soil. *Journal of Soil Science and Plant Nutrition*, *16*(3), 731-741.

Akladious, S.A., & Mohamed, H.I. (2018). Ameliorative effect of calcium nitrate and humic acid on the growth, yield component and biochemical attribute of pepper (*Capsicum annuum*) plants grown under salt stress. *Scientia Horticulturae*, *236*, 244-250. Http://dx.doi.org/10.1016/j.scienta.2018.03.047

Azam, S., Nouman, W., Rehman, U-U., Ahmed, U., Gull, T., & Shaheen, M. (2020). Adaptability of *Moringa oleifera* Lam. under different water holding capacities. *South African Journal of Botany*, *129*, 299-303. Http://dx.doi.org/10.1016/j.sajb.2019.08.020

Bayat, H., Fatemeh, S., Mohamad, H.A., & Saeid, D. (2021). Comparative effect of humic and fulvic acids as biostimulants on growth, antioxidant activity and nutrient content of yarrow (*Achillea millefolium* L). *Scientia Horticulturae*, *279*, 1-9. Http://dx.doi.org/10.1016/j.scienta.2021.109912

Brodowska, K.M. (2017). Natural flavonoids: classification, potential role, and application of flavonoid analogues. *European Journal of Biological Research*, *7*(2), 108-123. Http://dx.doi.org/10.5281/zenodo.545778

Budiarto, R., Poerwanto, R., Santosa, E., & Efendi, D. (2019). A review of root pruning to regulate citrus growth. *Journal of Tropical Crop Science*, *6*(1), 1-7. Http://dx.doi.org/10.29244/jtcs.6.01.1-7

Canellas, L.P., Olivares, F.L., Aquiar, N.O., Jones, D.L., Nebbioso, A., Mazzei, P., & Piccolo, A. (2015). Humic and fulvic acids as biostimulants in horticulture. *Scientia Horticulturae*, *196*, 15-27. Http://dx.doi.org/10.1016/j.scienta.2015.09.013

Dani, B.Y.D., Wahidah, B.F., & Syaifudin, A. (2019). Ethnobotany of kelor (*Moringa oleifera* Lam.) in Kedungbulus village, Gembong Pati. *Al-Hayat: Journal of Biology and Applied Biology*, *2*(2), 44-52. Http://dx.doi.org/10.21580/ah.v2i2.4659

Dawood, M.G., Abdel-Baky, Y.R., El-Wadi, M.E.S., & Bakhoum, G.S. (2019). Enhancement quality and quantity of Faba bean plant grown under sandy soil conditions by nicotinamide and/or humic acid application. *Bulletin of the National Research Centre*, *43*(1), 1-8. Https://doi.org/10.1186/s42269-019-0067-0

Du, Z-Y., Wang, Q-H., Liu, F-C., Ma, H-L., Ma, B-Y., & Malhi, S.S. (2013). Movement of phosphorus in a calcareous soil as affected by humic acid. *Pedosphere*, *23*(2), 229-235. Https://doi.org/10.1016/S1002-0160(13)60011-9

El-Din, A.A.T., Hafez, H.M., & Elraheem, O.A.Y.A. (2021). Effect of foliar applied for humic and fulvic acids as a partial substitute for mineral nitrogen on some characteristics of two hybrids of grain sorghum. *Scientific Journal of Agricultural Sciences*, 3(2), 116-122. Https://doi.org/10.21608/SJAS.2021.91616.1145

Eviati, & Sulaeman. (2009). *Analysis of Soil Chemistry, Plants, Water and Fertilizers*. (In Indonesian.). Bogor : Balai Penelitian Tanah.

Fan, H.M., Wang, X.W., & Sun, X. (2014). Effect of humic acid derived from sediments on growth photosynthesis and chloroplast ultrastructure in *Chrysanthemum*. *Scientia Horticulturae*, *177*, 188-123. Https://doi.org/10.1016/j.scienta.2014.05.010

González-Romero, J., Arranz-Arranz, S., Verardo, V., García-Villanova, B., & Guerra- Hernández, E.J. (2020). Bioactive compounds and antioxidant capacity of *Moringa* leaves grown in Spain versus 28 leaves commonly consumed in pre-packaged salads. *Processes*, 8, 1297. Http://dx.doi.org/10.3390/pr8101297

Hidayatullah, C.S.R., Santosa, E., & Sopandie, D. (2020). Genotypic response of *Colocasia esculenta* var *esculenta* and var *antiquorum* on different watering intervals. (In Indonesian.). *Jurnal Agronomi Indonesia*, *48*(3), 249-257. Https://dx.doi.org/10.24831/jai.v48i3.33136

Ibrahim, M., Iqbal, M., Tang, Y-T., Khan, S., Guan, D-X., & Li, G. (2022). Phosphorus mobilization in plant–soil environments and inspired strategies for managing phosphorus: A review. *Agronomy*, *12*, 2539. Http://dx.doi.org/10.3390/agronomy12102539

Kefi, A., Guntoro, D., & Santosa, E. (2020). Weed abundance and seed bank on maize field from different history of cropping pattern in dry land. (In Indonesian.). *Jurnal Agronomi Indonesia*, *48*(1), 22-29. Http://dx.doi.org/10.24831/jai.v48i1.28383

Khaled, H., & Fawy, H.A. (2011). Effect of different levels of humic acids on the nutrient content, plant growth and soil properties under conditions of salinity. *Soil & Water Research*, *6*(1), 21-29. Http://dx.doi.org/10.17221/4/2010-SWR

Khasanova, A., Lovell, J.T., Bonnette, J., Weng, X., Jenkins, J., Yoshinaga, Y., Schmutz, J., & Juenger, T.E. (2019). The genetic architecture of shoot and root trait divergence between mesic and xeric ecotypes of a perennial grass. *Frontiers in Plant Science*, *10*, 366. Http://dx.doi.org/10.3389/fpls.2019.00366

Kołodziejek, J. (2017). Effect of seed position and soil nutrients on seed mass, germination and seedling growth in *Peucedanum oreoselinum* (Apiaceae). *Scientific Reports*, *7*, 1959. Http://dx.doi.org/10.1038/s41598-017-02035-1

Kurniawan, H. (2019). Growth of kelor (*Moringa oleifera*) seedling origin of East Nusa Tenggara with growing medium treatment. (In Indonesian). *Wahana Forestra: Jurnal Kehutanan*, *14*(1), 1-9.

Leone, A., Spada, A., Battezzati, A., Schiraldi, A., Aristil, J., & Bertoli, S. (2015). Cultivation, genetic, ethnopharmacology, phytochemistry and pharmacology of *Moringa oleifera* leaves: An overview. *International Journal of Molecular Science*, *16*(1), 12791-12835. Https://doi.org/10.3390/ijms160612791

Lin, M., Zhang, J., & Chen, X. (2018). Bioactive flavonoids in *Moringa oleifera* and their health-promoting properties. *Journal of Functional Foods*, *47*, 469-479. Https://doi.org/10.1016/j.jff.2018.06.011

Maghfirah, Santosa, E., & Suwarto. (2022). Morpho-physiological characterization and genetic diversity of cocoyam accessions (*Xanthosoma sagittifolium* (L.) Schott). (in Indonesian.). *Jurnal Agronomi Indonesia*, *50*(2), 155-163. Http://dx.doi.org/10.24831/jai.v50i2.41872

Maretta, D., Sobir, Helianti, I., Purwono, & Santosa, E. (2020). Genetic diversity in eddoe taro (*Colocasia esculenta* var *antiquorum*) from Indonesia based on morphological and nutritional characteristics. *Biodiversitas*, *21*(8), 3525-3533. Http://dx.doi.org/10.13057/biodiv/d210814

Marshner, H. (1986). *Mineral nutrition of higher plants.* Academic Press, New York.

Morales, J., Manso, J.A., Cid, A., & Mejuto, J.C. (2012). Degradation of carbofuran and carbofuran-derivatives in presence of humic substances under basic conditions. *Chemosphere*, *89*(11), 1267-1271. Https://doi.org/10.1016/j.chemosphere.2012.05.018

Nardi, S., Pizzeghello, D., Muscolo, A., & Vianello, A. (2002). Physiological effect of humic substances on higher plants. *Soil Biology and Biochemistry*, *34*, 1527-1536. Https://doi.org/10.1016/S0038- 0717(02)00174-8

Nouman, W., Siddiqui, M.T., Basra, S.M.A., Farooq, H., Zubair, M., & Gull, T. (2013). Biomass production and nutritional quality of *Moringa oleifera* as a field crop. *Turkish Journal of Agriculture and Forestry*, *37*, 410-419. Http://dx.doi.org/10.3906/tar-1206-29

Rohman, F., Wachjar, A., Santosa, E., & Abdoellah, S. (2019). Humic acid and biofertilizer applications enhanced pod and cocoa bean production during the dry season at Kaliwining Plantation, Jember, East Java, Indonesia. *Journal of Tropical Crop Science*, *6*(3), 153-163.

Santiago, A., Quintero, J.M., Carmona, E., & Delgado, A. (2008). Humic substances increase the effectiveness of iron sulfate and vivianite preventing iron chlorosis in white lupin. *Biology and Fertility of Soils*, *44*, 875-883. Http://dx.doi.org/10.1007/s00374-008-0272-8

Santosa, E., Kurniawati, A., Sari, M., & Lontoh, A.P. (2017). Agronomic manipulation on flowering of Iles-iles (*Amorphophallus muelleri* Blume) to enhance seed production. (In Indonesian.). *Jurnal Ilmu Pertanian Indonesia*, *21*(2), 133-139. Http://dx.doi.org/10.18343/jipi.21.2.133

Schiavon, M., Pizzeghello, D., Muscolo, A., Vaccaro, S., Francioso, O., & Nardi, S. (2010). High molecular size humic substances enhance phenylpropanoid metabolism in maize (*Zea mays* L.). *Journal of Chemical Ecology*, *36*, 662-669. Https://doi.org/10.1007/s10886-010-9790-6

Stevenson, F.J. (1991). Organic matter-micronutrients reaction in soil. In J.J. Mortvedt et al. (Eds). *Micronutrients in Agriculture 2nd*. Soil Science Society of America, Madison, Wisconsin.

Suh, H.Y., Yoo, K.S., & Sang, G.S. (2014). Effect of foliar application of fulvic acid on plant growth and fruit quality of tomato (*Lycopersicon esculentum* L.). *Horticulture, Environment, and Biotechnology*, *55*(6), 455-461. Https://doi.org/10.1007/s13580-014-0004-y

Sun, J., Qiu, C., Ding, Y., Wang, Y., Sun, L., Fan, K., Gai, Z., Dong, G., Wang, J., Li, X., Song, L., & Ding, Z. (2020). Fulvic acid ameliorates drought stress-induced damage in tea plants by regulating the ascorbate metabolism and flavonoids biosynthesis. *BMC Genomics*, *21*, 411-423. Https://doi.org/10.1186/s12864-020-06815-4

Vongsak, B., Sithisarn, P., Mangmool, S., Thongpraditchote, S., Wongkrajang, Y., & Gritsanapan, W. (2013). Maximizing total phenolics, total flavonoids contents and antioxidant activity of *Moringa oleifera* leaf extract by the appropriate extraction method. *Industrial Crops and Products*, *44*, 566-671. Https://doi.org/10.1016/j.indcrop.2012.09.021

Yang, S., Zhang, Z., Cong, L., Wang, X., & Shi, S. (2013). Effect of fulvic acid on the phosphorus availability in acid soil. *Journal of Soil Science and Plant Nutrition*, *13*(3), 526-533. Http://dx.doi.org/10.4067/S0718-95162013005000041

Yazdani, B., Nikbakht, A., & Etemadi, N. (2014). Physiological effects of different combinations of humic and fulvic acid on *Gerbera*. *Communications in Soil Science and Analy*sis, *45*(10), 1357-1368. Http://dx.doi.org/10.1080/00103624.2013.875200

Yulianti, N., Santosa, E., & Susila, A.D. (2018). Production of fruits and leafy vegetables *Solanum nigrum* Linn under different shade levels. *Journal of Tropical Crop Science*, *5*(2), 64-72. Http://dx.doi.org/10.29244/jtcs.5.2.64-72