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Effects of Plant Spacing and Its Association with Humic Acid Application on the Growth, Yield and Active Composition in *Curcuma longa*

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Abstract: The effects of two plant spacing (20×20 cm) and (40×40 cm), Humic acid concentration (0, 1000 and 2000 ppm) and their interaction on growth, yield and principal components of ethanol extract of *Curcuma longa* under sand soil conditions were studied. The results showed that plan spacing (20×20cm) increased the growth and yield of *Curcuma longa*, while Humic acid had no effect on yield of *Curcuma longa* with the plant spacing (20×20 cm). With increased Humic acid concentration up to 2000 ppm in the case of plant spacing (40×40cm), the growth, yield and curcumin percentage were increased.

Keywords: Humic Acid, Turmeric, Plant Density, Vegetative Growth, Rhizome Yield, Active Compound

1. Introduction

The widespread usage of mineral fertilizers may leads to many negative consequences on the environment. Excessive chemical residues accumulated on plant tissue may cause potentially harmful health effects on human and animal consumers. Comparatively, organic fertilizers offer safer nutritional option because it is environmental friendly, and can releases their nutrients to the field crop in a slow and steady manner [1]. Humic acid (HA) is a natural product can improve the physical, chemical and biological properties of soil [2, 3]. Studies in potato [4], pepper and peas [5], wheat [6] have shown that application of HA increased the yield of these crops. Turmeric (*Curcuma longa*), a member of the Zingiberaceae family is an herbaceous perennial plants, which is commonly found in the tropical and subtropical regions of the world and have been widely grown in South East Asian countries. Turmeric grows well in humid and clay soils and its tuberous rhizomes have been used as spices and herbal medicine for the treatment of prostrate, breast, skin and colon cancer [7, 8], as well as anti-inflammatory and anti-bacteria agents. The active compounds found in the rhizomes refers as Curcuminoids, includes the Curcumin, Demethoxycurcumin, and Bisdemethoxycurcumin [9]. Curcumin is the major bio-active constituent [10]. Use of inappropriate plant spacing affects plant population and final yield [11]. Plant spacing is one factor that determines the efficiency of the use of land, light, water and nutrients and equally distant plants compete minimally for nutrients, light and other factors [12, 13]. [14] Showed the effects of plant spacing on turmeric growth and rhizome yield.

The aim of this work is to investigate the effect of different plant spacing, biofertilization (HA) concentration and their interaction on vegetative growth, chemical contents, rhizome yield and composition of active compounds of *curcuma longa* under sand soil conditions.

2. Matherials and Methods

2.1. Plant Material and Culture Conditions

Rhizome of *Curcuma longa* was obtained from Sekem Company, Cairo, Egypt. Field experiments were conducted during the two successive seasons of 2015 and 2016 at the Agricultural-Veterinarian Training and Research Station, King Faisal University, Al-Ahsaa, the Kingdom of Saudi Arabia, under greenhouse conditions. During the experiment, temperature between 32-36°C, relative humidity of 47-56%, and an average of 14 h photoperiod were recorded. Seedlings of turmeric (5 cm in length and carrying three pairs of leaves) were sown on Jun 1st in both 2015 and 2016, to study the response of *Curcuma longa* plants to the application of Humic acid (HA) (American Egyptian United Company, Cairo) and the planting spacing. The treatments contains three HA fertilizer concentration (0, 1000 and 2000 ppm) and two different plant spacing (20×20 and 40×40 cm). Compost ($10m^3$/ fed) was added and well-mixed with the soil during the preparation process, three weeks before planting. The NPK (as recommended by the Ministry of Agriculture) was used as the chemical fertilizer control to compare the effectiveness of the bio-fertilizer treatments. The first addition of HA was done one month after cultivation while the other additions were applied at two months intervals after the first application respectively. The experiment followed a split plot design with 12 replicates. The biofertilization (HA) at (0, 1000 and 2000 ppm) treatments were considered as main-plots, while the plant spacing at (20×20 and 40×40cm) represented the sub plots. The chemical contents of the irrigation water are tabulated in Table (1). After 24 weeks from sowing, the plant height (cm), number of leaves and roots/ plant, main root length/ plant and fresh and dry weight of leaves and roots/ plant (g) were recorded using six random plants from each treatment. In both seasons at harvest (30 weeks from sowing), number of rhizome/ plant, rhizome diameter (mm) and dry weight of rhizome / plant (g) were recorded.

Table 1. *Chemical content of the irrigation water.*

Salinity Level ($mS\ cm^{-1}$)	Cations ($meq\ L^{-1}$)				Anions ($meq\ L^{-1}$)				SAR
	Ca^{+2}	Mg^{+2}	Na^{+}	K^{+}	CO_3^{-2}	HCO_3^{-}	SO_4^{-2}	Cl^{-}	
(1.35)	5.72	2.02	7.27	0.38	0.28	2.68	4.03	8.4	3.43

2.2. Chemical Analysis in Leaves

2.2.1. Photosynthetic Pigments

The fourth leaf from bottom was collected after 24 weeks from sowing and chlorophylls a and b and carotenoid contents were determined calorimetrically according to [15].

2.2.2. Determination of hhe Percentage of Nitrogen, Phosphorus and Potassium

Plant leaf samples at 24 weeks after sowing were collected and dried at 70 ^{0}C for 24 h. The obtained dry matter was ground and digested according to [16] to determine the nitrogen, phosphorus and potassium contents. The nitrogen (N) content was determined using the modified micro-Kjedahl method, as described by [17]. The percentage of phosphorus (P) was estimated calorimetrically as according to the method of [18]. The potassium (K) percentage was determined using atomic absorption flame photometry (3300) as according to [19].

2.3. GC/MS Analysis

The GC/MS analysis was performed at the Mass spectrometry Experimental Nuclear Physics Dept, NRC, Cairo Egypt.

2.3.1. Sample Preparation

One gram of the homogenized air-dried rhizome (from three plants in the second season) was added to a 28-ml stoppered culture tube and defatted by 30-ml of ethanol for one day with shaking at 100 rpm on a rotary shaker. The extracts were filtered through a 0.2 mm syringe filter and 2μL was injected into the GC/MS system.

2.3.2. Instrumentation and Separation Conditions

GC 1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–35MS (30 m x 0.25 mm x 0.25 μm film thickness) was used. The separation of active compounds was performed according to [20].

2.3.3. Identification of Components

Active compounds were identified using the database of National Institute Standard and Technology (NIST). The components were identified by comparison of their retention times and mass spectra with NIST 11 mass spectral database [21].

2.4. Statistical Analysis

Data were statistically analyzed using ANOVA/MANOVA of Statistica 6 software [22]. The significance of differences among means was detected using the Least Significant Test (L. S. D) at p =0.05.

3. Results and Discussion

3.1. Vegetative Growth

The effects of plant spacing, HA concentration and their interactions on plant height, leaves and roots number, longest root length and fresh and dry weight of leaves and root of turmeric in 2015 and 2016 seasons are shown in table (2 and 3). Data cited in Tables (2 and 3) reveal significant increase on growth of turmeric in terms of plant height, number of leaves, number of roots and fresh and dry weight of leaves due to the plant spacing treatments (20×20 cm). The longest root (27.0 and 21.5 cm) were recorded at the plant spacing (40×40 cm), the fresh weight (56.7 and 45.28 g) and dry weight (8.8 and 6.7 g) of roots were recorded at the plant spacing (40×40 cm) + 2000 ppm HA treatment in the first and second season respectively. The lowest plant height, number of leaves, number of roots and fresh and dry weight of leaves and root were obtained with plant spacing (40×40 cm) + HA at 1000 ppm treatment in both seasons and the lowest root length was produced in the plant spacing (40×40 cm) treatment in both seasons. The data showed that the plant height, number of leaves, number of roots, fresh and dry weight of leaves and root significantly decreased and the root length significantly increased with increase HA concentration, when the plant spacing was (20× 20 cm). In contrast, with increased of HA concentrations in the case of plant spacing (40×40 cm) treatments, all the above parameters except root length significantly decreased and then significantly increased with the 2000 ppm HA concentration as compared to the control treatments (plant spacing (40×40 cm) without HA (Table 2 and 3). The plant spacing (20× 20 cm) gave the best growth of turmeric plant compered to plant spacing (40×40 cm). This results is agreements with the finding of [14, 23, 24, 25, 26]. Data in Table (2 and 3) showed that the control treatments (NPK fertilization) significantly increased all vegetative growth of turmeric plant except the root length compared to HA treatments when the plant spacing was (20× 20 cm) but the HA concentration improve the plant growth when interacted with plant spacing (40× 40 cm). Many studies conducted with different plants indicated that plant growth increased with HA applications [27, 28, 29]. [30, 31] stated that the plant growth of tomato seedlings increased with the application of HA but decreased when the concentrations of HA exceeded.

Table 2. *Effect of different plant spacing, Humic acid and their interaction on plant height, number of leaves, number of root and root length (cm) of turmeric plant.*

Treatments		Plant height (cm)		No. of leaves		No. of roots		Longest root length (cm)	
Spacing (cm)	Humic acid (ppm)	2015	2016	2015	2016	2015	2016	2015	2016
20×20		63.56 a*	57.13 a	7.08 a	7.25 a	19.83 a	17.25 a	17.75 a	15.69 a
40 ×40		62.17 a	56.88 a	6.33 a	6.19 a	16.22 b	15.63 a	18.56 a	16.88 a
	0	77.00 a	66.75 a	6.90 a	8.13 a	18.80 a	19.25 a	18.10 a	17.37 a
	1000	49.00 c	48.67 c	6.60 a	6.33 b	17.40 a	15.83 ab	19.40 a	15.83 a
	2000	63.00 b	58.83 b	6.40 a	6.17 b	16.80 a	15.17 b	17.20 a	16.00 a
20×20	0	80.50 a	77.50 a	9.25 a	7.75 ab	21.10 a	21.00 a	11.75 d	13.25 c
20×20	1000	58.00 b	47.00 c	7.00 b	6.00 cd	20.20 a	15.67 b	22.33a	16.00 bc
20×20	2000	48.00 bc	53.67 bc	5.00 c	5.33 d	17.50 ab	12.00 c	14.50 d	17.00 b
40 ×40	0	73.00a	56.00 bc	5.33 c	7.00 abc	16.33 b	17.50 b	27.00a	21.50 a
40 ×40	1000	43.00 c	50.33 c	6.33 bc	6.67 bcd	15.00 b	16.00 b	14.33 d	15.67 bc
40 ×40	2000	74.67 a	64.00 b	7.33 b	8.50 a	17.33 ab	18.33 ab	19.00 c	15.00 bc

*Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L. S. D. test

Table 3. *Effect of different plant spacing, Humic acid and their interaction on fresh and dry weight of leaves and root of turmeric plant.*

Treatments		Fresh weight of leaves (g/plant)		Fresh weight of roots (g/plant)		Dry weight of leaves (g/plant)		Dry weight of roots (g/plant)	
Spacing (cm)	Humic acid (ppm)	2015	2016	2015	2016	2015	2016	2015	2016
20×20		124.46 a*	133.66 a	32.36 a	40.13 a	11.08 a	12.98 a	4.25 b	5.25 a
40 ×40		98.30 a	121.61 a	37.24 a	27.90 b	10.18 a	11.51 a	6.91 a	4.57 a
	0	131.47 a	192.06 a	34.07 b	38.34 a	14.17 a	16.21 a	6.46 a	5.65 a
	1000	97.44 a	92.00 c	27.36 c	33.40 b	8.32 b	9.80 c	4.22 b	4.60 a
	2000	97.38 a	120.32 b	44.44 a	31.75 b	9.12 b	12.05 b	6.86 a	4.73 a
20×20	0	171.98 a	223.53 a	38.03 b	31.40 cd	15.18 a	18.33 a	4.70 b	4.90b
20×20	1000	122.25 b	104.80 c	33.00 bc	41.57 ab	10.85 abc	11.30 b	4.30 b	4.97 b
20×20	2000	79.15 c	102.60 c	26.05 cd	35.27 bc	7.20 c	11.10 bc	4.10b	4.57 b
40 ×40	0	104.47 bc	138.03 b	31.43 bcd	28.23 cd	10.40 bc	13.00 b	7.63 a	4.60 b
40 ×40	1000	80.90 c	79.20 c	23.60 d	25.23 d	6.63 c	8.30 c	3.03b	4.23 b
40 ×40	2000	109.53 bc	160.60 b	56.70 a	45.28 a	13.50 ab	14.10 b	8.80 a	6.70 a

*Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L. S. D. test

3.2. Rhizome Yield

Plant spacing of (20×20 cm) has recorded significantly highest number of rhizome per plant and dry weight of rhizome, followed by the plant spacing (40× 40 cm) + 2000 ppm HA treatment. On the other hand, the significant lowest rhizome number per plant (2.67 and 3.67) and dry weight of rhizome (8.65 and 6.7 g) were recorded at the plant spacing (40× 40 cm)+1000 ppm HA in both seasons (Table 5 and Figure 1). The rhizome diameter in both seasons significantly decreased with increase HA concentration as compared to control treatments when the plant spacing was (20x20cm). The highest rhizome diameter (18.8 and 17.7 mm) were obtained by plant spacing (40× 40 cm) + 2000 ppm HA treatments in both seasons (Table 5 and Figure 1). These results are in line with the earlier findings of [14, 25, 32] in turmeric, ginger and potato crop respectively. The results showed that HA application did not affect turmeric yield, yield decreased with increased concentration of HA compared to the control at plant spacing (20×20 cm) in both seasons (Table 4 and Figure1). Similar results were reported on grape [33], olive [34] and milk thistle [35].

Table 4. *Effects of plant spacing, Humic acid and their interaction on the growth and rhizome yield of turmeric plant.*

Treatments		**No. of rhizome**		**Rhizome diameter (mm)**		**Dry weight of rhizome (g/plant)**	
Spacing (cm)	**Humic acid (ppm)**	**2015**	**2016**	**2015**	**2016**	**2015**	**2016**
20×20		9.25 a*	9.69 a	13.97 a	15.10 a	32.45 a	31.43 a
40 ×40		7.33 a	8.50 a	14.82 a	15.46 a	22.9 b	25.52 b
	0	13.50 a	15.38 a	17.52 a	16.03 a	51.68 a	46.98 a
	1000	4.40 b	5.17 c	12.52 b	13.78 a	46.4 b	22.35 b
	2000	6.40 b	8.83 b	13.40 b	16.28 a	14.98 b	16.1 b
20×20	0	15.75 a	17.25 a	15.60 b	16.20 ab	54.5 a	50.1 a
20×20	1000	7.00 b	6.67 d	12.71 c	14.60 bc	24.15 c	25.5 b
20×20	2000	5.00 bc	7.67 cd	13.60 c	14.87 bc	18.7 d	18.7 b
40 ×40	0	7.33 b	10.00 c	13.27 c	15.85 ab	11.25 e	26.0 b
40 ×40	1000	2.67 c	3.67 e	12.40 c	12.97 c	8.65 e	6.7 c
40 ×40	2000	12.00 a	13.50 b	18.80 a	17.70 a	48.87 b	43.85 a

*Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L. S. D. test

Table 5. *Effects of plant spacing, Humic acid and their interaction on chlorophyll a (mg\100g F. W.), chlorophyll b (mg\100g F. W.) and carotenoids (mg\100g F. W.) of turmeric plant.*

Treatments		**Chl a mg\100g F. W.**		**Chl b mg\100g F. W.**		**Carotenoids pigments mg\100g F. W.**	
Spacing (cm)	**Humic acid (ppm)**	**2015**	**2016**	**2015**	**2016**	**2015**	**2016**
20×20		37.59 a*	30.17 a	12.55 a	11.93 a	35.36 a	35.76 a
40 ×40		38.48 a	38.11 a	6.15 a	10.29 a	30.21a	30.41 a
	0	29.58 a	37.38 a	7.94 b	10.56 a	29.26 a	32.17a
	1000	47.13 a	47.45 a	15.49 a	12.71 a	35.60 a	34.83 a
	2000	37.40 a	17.58 a	4.63 c	10.06 a	33.49 a	32.25 a
20×20	0	28.08 a	29.03 ab	9.07 b	13.51 a	33.13 a	33.63 a
20×20	1000	52.32 a	48.91 a	21.12 a	13.93 a	33.86 a	40.34a
20×20	2000	29.38 a	12.56 b	7.48 b	8.34 a	31.39 a	33.31 a
40 ×40	0	31.08 a	45.74 a	8.41 b	7.20 a	25.39 a	29.31 a
40 ×40	1000	41.95 a	22.60 ab	9.19 b	11.78 a	33.13 a	30.72 a
40 ×40	2000	45.43 a	45.99 a	9.86 ab	11.99 a	39.81 a	31.19 a

*Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L. S. D. test

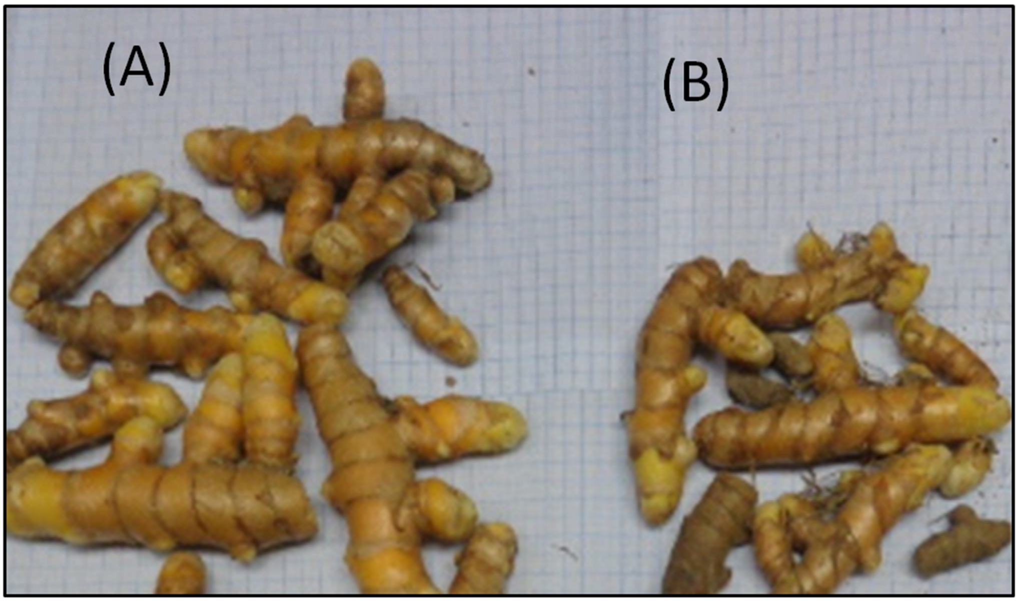


Figure 1. *Effect of plant spacing on the rhizome number of Curcuma longa plant (A) plant spacing 20x20cm and (b) plant spacing 40x40 cm.*

3.3. Chemical Analysis

3.3.1. Chlorophyll Pigments Determination

Data concerning the effect of plant spacing in the chlorophyll a, chlorophyll b and carotenoids pigments contents of turmeric were presented in Table (5). The chl a increased with increased plant spacing in both seasons. In contrast, the ch b and carotenoids increased with decreased plant spacing. The plant spacing (20×20 cm) + HA (1000 ppm) and plant spacing (40×40 cm) + HA (2000 ppm) treatments increased the chlorophyll a, b and carotenoids pigments content in the leaves of turmeric as compared to control treatments in both seasons. HA increased the photosynthetic area of the plant by increase in number of leaves per plant on the plant spacing (40×40 cm) + HA (2000 ppm). This could be possible due to the physiological activation of sink by the major nutrients [36]. The data indicate that HA concentrations caused enhances in chlorophyll a, b and carotenoids as compared with control treatment in both seasons. These results were in agreement with those obtained by [37, 38, 39, 40]on different plants. This positive effect of HA on photosynthetic pigments could be attributed to an increased in CO_2 assimilation and photosynthetic rate which increased mineral uptake by the plant [40].

3.3.2. Mineral Element Contents

The data in Table (6) showed that the plant spacing (20×20 cm) increased the N, P and K percentage in the leaves of turmeric compared to plant spacing (40×40 cm) in both seasons. The N, P and K content statistically significant decreased with the application of HA when interact with plant spacing (20×20 cm) in both seasons. Significantly higher N, P and K content were observed in the plant under plant spacing (40×40cm) + HA (2000 ppm) treatments as compared to control (plant spacing 40×40 cm) in both seasons. The stimulatory effect of HA due to increase permeability of plant membranes and enhance uptake of nutrients by building complex forms or chelating agents of HA matter with metallic cations, thereby increasing their availability to plants [41, 42]. Promoted growth and nutrient uptake of plants due to the addition of humic substances is reported by [27, 28, 43, 44]

Table 6. *Effects of plant spacing, Humic acid and their interaction on nitrogen (%), potassium (%) and phosphorus (%) in the leaves of turmeric plant.*

Treatments		**N%**		**K%**		**P%**	
Spacing (cm)	**Humic acid (ppm)**	**2015**	**2016**	**2015**	**2016**	**2015**	**2016**
20×20		1.51 a*	1.41 a	2.25 a	2.24 a	0.22 a	0.23 a
40 ×40		1.33 a	1.31 a	2.19 a	2.18 a	0.19 a	0.21 a
	0	1.62 a	1.51 a	2.24 a	2.11 a	0.19 b	0.23 a
	1000	1.39 b	1.34 a	2.03 a	2.07 a	0.20 ab	0.21 a
	2000	1.25 c	1.23 a	2.40 a	2.46 a	0.22 a	0.22 a
20×20	0	1.68 a	1.64 a	2.43 ab	2.37 ab	0.23 a	0.25 a
20×20	1000	1.65 a	1.47 ab	2.10 bc	2.05 ab	0.20 a	0.21 a
20×20	2000	1.19 b	1.12 cd	2.23 bc	2.30 ab	0.22 a	0.022 a
40 ×40	0	1.32 ab	1.33 bc	2.05 c	1.92 b	0.17 a	0.21 a
40 ×40	1000	1.13 b	1.05 d	1.95 c	2.08 ab	0.20 a	0.20 a
40 ×40	2000	1.56 ab	1.55 ab	2.58 a	2.54 a	0.21 a	0.23 a

*Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L. S. D. test

3.3.3. GC/MS Analysis of Essential Oils

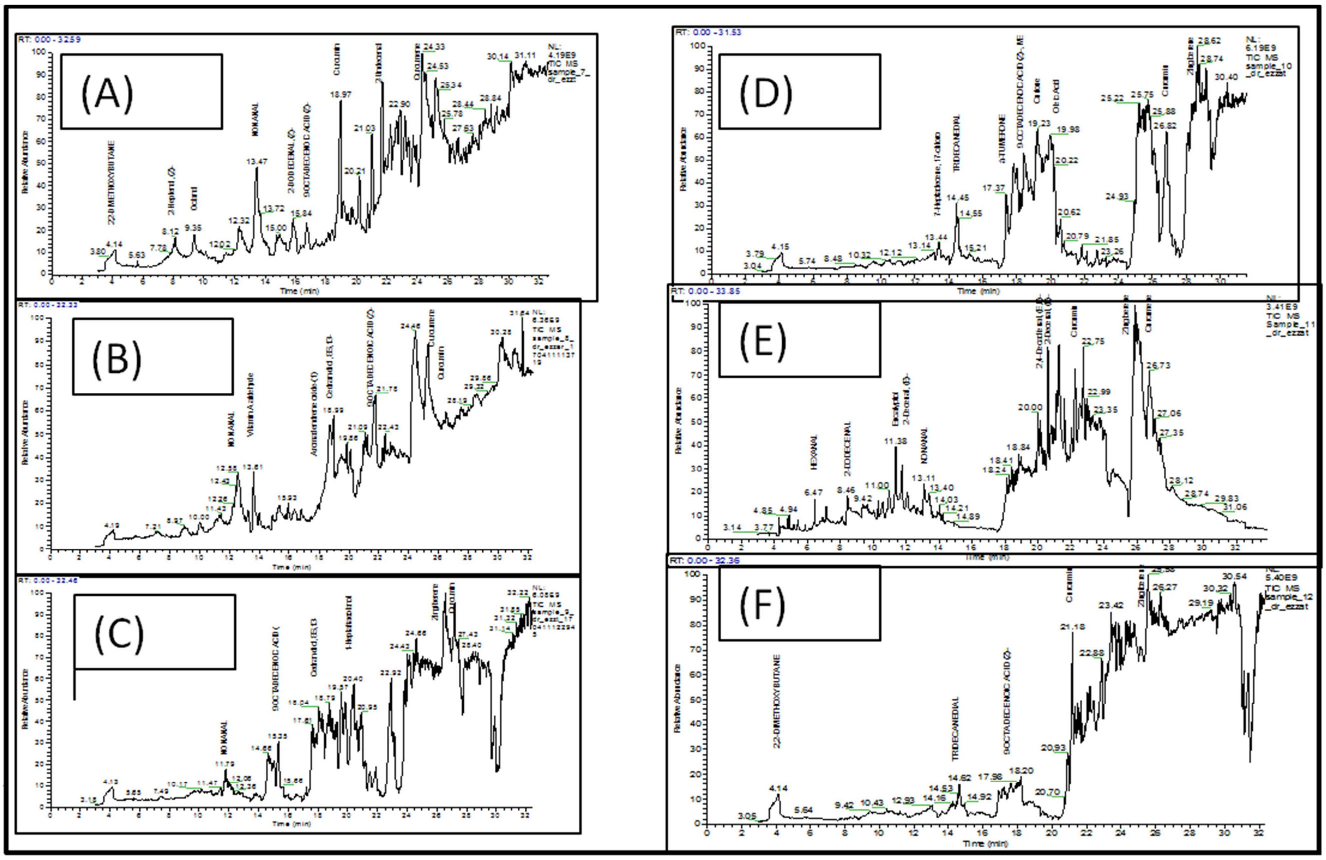
Results of GC-MS analysis of ethanol extract of turmeric rhizome is shown in (Table 7 Figures 2). The main chemical compounds were curcumin, curcumene and Zingiberene in ethanol extract of turmeric rhizome (Table 7 and Figures 2). The sample analysis showed the curcumin peaks in all treatments at different retention time, the curcumene and zingiberene peaks were presented only on some treatments at different retention time (Table 7and Figures 2). The plant spacing (20×20cm) treatment had curcumin 9.46%, curcumin percentage was reduced in the plant spacing (20×20 cm) treatments exposed to HA (Table 7 and Figs 2). However plant spacing (40×40 cm) treatments had curcumin 6.24% with increased the HA concentration to 2000 ppm + plant spacing (40×40 cm), the curcumin percentage increased to 11.73%, indicating an enhancement effect of high HA on the target compound. HA concentration decreased curcumin content at plant spacing (20×20cm). Similar results were reported by [33, 35]. In contrast, the HA increased the curcumin content at the plant spacing (40×40 cm). These results support previous observations of [45, 46, 47, 48].

Table 7. *Effects of plant spacing, Humic acid and their interaction on curcumin, curcumene and zingiberene content in the rhizome of turmeric plant.*

Treatments		**Effect**	**peak**	**Rt***	**Name**	**Peak**	**Molecular**	**Molecular**
Spacing (cm)	**Humic acid (ppm)**	**compound**				**area %**	**Weight**	**formula**
20×20	0	Curcumin	9	18.97	1, 6-Heptadiene-3, 5 dione, 1, 7-bis(4-hydroxy-3 methoxyphenyl)	9.46	368	C21H20O6
		Curcumene	17	24.33	Benzene, 1-(1, 5-dimethyl-4-hexenyl)-4-methyl	4.68	202	C15H22
20×20	1000	Curcumin	19	27.17	1, 6-Heptadiene-3, 5-dione, 1, 7-bis(4-hydroxy-3-methoxyphenyl)	9.32	368	C21H20O6
		Curcumene	18	26.48	Benzene, 1-(1, 5-dimethyl-4-hexenyl)-4-methyl	5.68	202	C15H22
20×20	2000	Curcumin	15	27.17	1, 6-Heptadiene-3, 5-dione, 1, 7-bis(4-hydroxy-3-methoxyphenyl)	7.32	368	C21H20O6
		Zingiberene)	14	26.48	1, 3-Cyclohexadiene, 5-(1, 5-dimethyl-4-hexenyl)-2-methyl	6.46	204	C15H24

Treatments		Effect	peak	Rt*	Name	Peak	Molecular	Molecular
Spacing (cm)	Humic acid (ppm)	compound				area %	Weight	formula
40×40	0	Curcumin	14	26.82	1, 6-Heptadiene-3, 5-dione, 1, 7-bis(4-hydroxy-3-methoxyphenyl)	6.24	368	$C_{21}H_{20}O_{6}$
		Zingiberene	18	28.62	1, 3-Cyclohexadiene, 5-(1, 5-dimethyl-4-hexenyl)-2-methyl-,	9.12	204	$C_{15}H_{24}$
40×40	1000	Curcumin	13	22.24	1, 6-Heptadiene-3, 5-dione, 1, 7-bis(4-hydroxy-3-methoxyphenyl	5.41	368	$C_{21}H_{20}O_{6}$
		Zingiberene	18	25.78	1, 3-Cyclohexadiene, 5-(1, 5-dimethyl-4-hexenyl)-2-methyl	4.32	204	$C_{15}H_{24}$
		Curcumene	19	25.85	Benzene, 1-(1, 5-dimethyl-4-hexenyl)-4-methyl	6.43	202	$C_{15}H_{22}$
40×40	2000	Curcumin	4	21.18	1, 6-Heptadiene-3, 5-dione, 1, 7-bis(4-hydroxy-3-methoxyphenyl)	11.73	368	$C_{21}H_{20}O_{6}$
		Zingiberene	17	25.57	1, 3-Cyclohexadiene, 5-(1, 5-dimethyl-4-hexenyl)-2-methyl-,	6.73	204	$C_{15}H_{24}$

*R. t, retention time (min).



(A) Plant spacing 20×20 cm treatment, (B) Plant spacing 20×20 cm +1000 ppm HA (C) Plant spacing 20×20 cm +2000 ppm HA, (D) Plant spacing 40×40 cm, (E) Plant spacing 40×40 cm +1000 ppm HA, (F) Plant spacing 40×40 cm +2000 ppm HA

Figure 2. *The chemical compositions of ethanol extract from Curcuma longa rhizome analyzed by gas chromatography–mass spectrometry.*

4. Conclusion

A field experiment was conducted to evaluate the effect of two plant spacing (20×20 and 40×40 cm) and its association with HA application on the growth, yield and active composition in Curcuma longa. Plant spacing at (20×20cm) increased the growth and yield of Curcuma longa, while HA had no effect on yield of Curcuma longa at the plant spacing of (20×20 cm). However, increasing HA concentration up to 2000 ppm in the case of plant spacing (40×40cm), increased the growth, yield and curcumin percentage.

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