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Environmental Effect of seed age, planting medium and humic addition on mango seeds germination and subsequent growth of seedlings

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Abstract: This experiment was carried out in shade of Department of Biology – Al-Rasheed University College for seasons 2019 and 2020 on mango seeds of Bull's Heart cultivar Three factors are used in this experiment, which are as follows: First: seed age factor, which includes one day after harvest (D_1), two days after harvest (D_2), and three days after harvest (D_3). Second: growing medium factor (M), which includes loamy soil: peat moss (3:1) M_1, loamy soil: peat moss (1:1) M_2, and loamy soil: peat moss (1:3) M_3. Third: humic acid addition factor, which includes adding 5 $g.L^{-1}$ (H_5) and adding 10 $g.L^{-1}$ (H_{10}). A factorial experiment was conducted within (RCBD) with five replicates for germination percentage (two seeds per experimental unit) and 3 replicates for vegetative traits (two seedlings per experimental unit). Thus, number of seeds planted in pots included in experiment was 180 seeds, and number of seedlings on which vegetative traits were studied was 108. Results showed that ($D_1M_2H_{10}$) treatment gave the highest percentage of germination of 85.00 and 95.00 %, 123.15 and 158.64 cm as plant height ,13.04 and 13.88 mm as stem diameter, 71.00 and 83.33 $leaf.seedling^{-1}$ as leaf number , 92.76 and 98.75 cm^2 as leaf area, 30.18 and 32.96 $mg.g^{-1}$ as leaves chlorophyll content , 0.425 and 0.449 % as leaves phosphor content and 1.928 and 2.108 % as leaves potassium content for two seasons, respectively.

Key words: *Mangifera indica* L , growth, peat moss, germination percentage.

INTRODUCTION

One of evergreen fruit trees, the mango tree (*Mangifera indica* L.), is native to South Asia, Indian Himalayas, and Burma. Its cultivation progressively expanded to nearly every hot region of world, as well as some tropical areas with warm winters and some Mediterranean regions (Paull and Duarte, 2011). Mango trees were first planted in Iraq, more precisely in Basra, prior to fourteenth century AD. They were primarily grown in palm groves that were dispersed along Shatt al-Arab banks in the neighborhoods of Abu al-Khaseeb and al-Salihiya. It is also known that there are mango trees in Basra home gardens (Hassan et al., 1991). Global production of mangoes is estimated at about 61,107,091 tons, with a cultivated area of 6,159,876 hectares. India is the first country in world in terms of mango production, followed by Indonesia, China, Mexico, and then China in fifth place. Egypt is the first Arab country in terms of mango production and seventh in world, with a production of 1,715,365 tons (FAO, 2024). Mango fruit is low in calories but full of nutrients as it provides the body with proteins, carbohydrates, fats, fiber, vitamin C, copper, folic acid, vitamin B-6, vitamin B-5, vitamin A, vitamin E, vitamin K, potassium, magnesium, manganese and others. It provides the body with essential antioxidants and works to enhance the immune system, support heart and circulatory health, reduce cholesterol and fat levels, and provide the body with vitamin A, lutein and other elements that support eye health and reduce the risk of cancer, especially colon, lung, prostate, breast and bone cancer (Maldonado-Celis et al., 2019 , Al-Naimi,2010). Techniques that produce more plants in order to maintain species or increase its cultivation are known as propagation. One method of plant propagation through seeds carrying embryo produced by pollination and fertilization is sexual reproduction. One of most popular and least expensive ways to create fruit rootstocks for budding or grafting onto commercial types is by seed propagation (Pinto et al., 2018). Mango seeds may be monoembryonic or polyembryonic, and

seeds are characterized by their rapid loss of vitality. Therefore, their seeds are used to obtain new cultivars of monoembryonic mango seeds, and are used to obtain seedlings (rootstocks) for grafting or budding onto commercial cultivars with desired characteristics. In addition, they are used to produce plants similar to the mother plant in their genetic characteristics from polyembryonic mango seeds. There are cultivars whose seeds contain multiple embryos, and when they germinate, several seedlings (1-11) are formed from them, and they are usually all vegetative embryos and thus similar to the mother plant in their genetic characteristics. This case occurs, for example, in Bull's Heart cultivar, which is successful in Iraq (Pinto et al., 2018, Naik and Kumar, 2020). Seed age after harvesting the fruits affects its germination rate and germination speed. Studies have been conducted to determine effect of seed age on germination rate and subsequent growth of seedlings. Supriya et al. (2015) found that germination rate decreases after a week of extracting mango seeds from fruits and continues to decrease the longer the seed storage period increases until it reaches its lowest level at age of 5 five weeks. In another experiment conducted by Ghani et al. (2018) to determine effect of mango seed size on its germination rate and subsequent seedling growth, this was: small (33.48 cm^3), Medium (36.00 cm^3) and Large (39.20 cm^3) result showed that Maximum germination, shoot length , stem diameter, number of leaves plant-1, were recorded for larger size seeds, as compared with small size.

growing medium is one of important factors for seed germination, so basic steps are to choose best suitable conditions that affect plant growth, including soil properties such as moisture retention efficiency, cation exchange efficiency, and soil fertility. When these properties are poor in some types of soil, they inevitably affect seed germination and plant growth, and they can be improved by adding some materials that improve their properties such as peat moss or compost, etc. (Pinto et al., 2018, Al-Hadethi et al., 2020). A number of studies were conducted to determine effect of growing medium on germination of fruit seeds in general and mango in particular. Kaur (2017) found in an experiment that included effect of growing media on germination and growth of mango seedlings sown in August in polythene bags containing different soil potting mixture treatments comprising of Soil + Sand + Farmyard manure (FYM) (1:1:1), Soil + Sand + Vermicompost (1:1:1), Soil +Sand + FYM (2:1:1), Soil +Sand + Vermicompost (2:1:1), Soil + Sand + FYM (1:2:1), Soil + Sand + Vermicompost (1:2:1), Soil + Sand + FYM (1:1:2), Soil + Sand + Vermicompost (1:1:2) and Soil as control treatment, found that growing media, Soil + Sand + Vermicompost (1 : 1 : 2) was most effective for better germination of mango seeds as well as growth of mango seedlings in terms of minimum number of days taken to seed germination with highest germination, survival, seedling height, leaves number, fresh and dry weight of seedling as compared with soil only growing media this is in his study of three mango cultivars Dusehri, Amrapali and Alphonso . In another study conducted in Ethiopia to evaluate germination and seedling growth response of mango cultivars to different combinations of nursery potting media on three mango cultivars Local, Dodo and Keitt were sown in full top soil; top soil: sawdust: sand (3:2:1); top soil: FYM (Farmyard manure): sand (3:2:1) and top soil: FYM: sawdust: (3:2:1) Gebregiorgs et al. (2021) found that potting media affected most of parameters except days to germination, germination percentage, root number, and shoot number especially in top soil :FYM: sand in ratio of 3:2:1.

A humic compound known as humic acids is drawn from soil by alkaline solutions or other nutrients. Dark brown solutions are their form, and their structural composition is not set in stone. They are a class of high molecular weight compounds having comparable compositions and characteristics. The variety of particle sizes and absence of uniformity in specifics of their plant composition are two of humic acids' inherent properties. These traits and attributes have made it extremely difficult to determine molecular weight of humic acids. From an agricultural standpoint, humic acids are crucial for soil development because they act as a storehouse of plant nutrients, particularly nitrogen. Humic acid's high acidity improves efficiency of

ammonium metabolism, decreases nitrogen volatilization in form of ammonia gas, and boosts soil with positive cations. Because humic and fulvic acids increase soil's ammonium content and decrease nitrogen loss in form of ammonia, researcher discovered an inverse relationship between nitrogen volatilization in form of ammonia gas and addition of humic acid to soil (Sharma and Chetani, 2017; Al-Mawsili et al, 2019; Mohammed et al., 2024) . Given the role of humic acid in soil properties, which is positively reflected in growth properties, many experiments were conducted on mango seedlings. (Al-Marsoumi and Al-Hadethi, 2020) In order to determine effects of humic acid and seaweed extract on growth, vegetative characteristics, and leaf nutritional content, researchers sprayed three different levels of humic acid (Disper Humic 85%) on one-year-old mango seedlings: zero, 1, and 2 $g.L^{-1}$. They found that humic acid significantly increased the amount of minerals in leaves, especially at concentrations of 2 $g.L^{-1}$.Ghani et al. (2018) found that adding humic acid at different concentrations led to a significant increase in germination rate and seedling growth on mango plants. Due to lack of studies on mango trees propagation by seeds in Iraqi conditions, as well as to avoid chemical fertilizers and growth regulators and their role in germination and growth, the study aimed to know effect of seed age, medium and adding humic acid on germination and growth of mango seedlings.

MATERIALS AND METHODS

This experiment was carried out in shade of Department of Biology – Al-Rasheed University College for seasons 2019 and 2020 on mango seeds of Bull's Heart cultivar taken from 33-year-old trees planted in gardens in Al-Saidiya city in Baghdad for seeds planted in 1986 and brought from Basra Governorate. Three factors are used in this experiment, which are as follows: First: seed age factor, which includes one day after harvest (D_1), two days after harvest (D_2), and three days after harvest (D_3). Second: growing medium factor (M), which includes loamy soil: peat moss (3:1) M_1, loamy soil: peat moss (1:1) M_2, and loamy soil: peat moss (1:3) M_3. Third: humic acid addition factor, which includes adding 5 $g.L^{-1}$ (H_5) and adding 10 $g.L^{-1}$ (H_{10}). A factorial experiment was conducted within a randomized complete block design (RCBD) with five replicates for germination percentage (two seeds per experimental unit) and 3 replicates for vegetative traits (two seedlings per experimental unit). Thus, number of seeds planted in pots included in experiment was 180 seeds, and number of seedlings on which vegetative traits were studied was 108. The date for planting seeds for first season began on 16/8/2019 and planting began in second season on 20/7/2020. Humic was added before planting and was added once a month throughout experiment. The results were analyzed according to L.S.D. test at a probability level of 0.05 (Elsahookie and Wuhaib, 1990). The following characteristics were studied: germination percentage by dividing number of germinated seeds by total number of seeds × 100. Some vegetative characteristics were measured, including plant height (cm), which was measured with a measuring tape, stem diameter (mm) by vernier, leaf area (cm^2) by digimizer and leaves number ($leaf.transplant^{-1}$). In addition to some chemical properties of the leaves, it was chlorophyll content of leaves (mg.g-1 fresh weight) in first week of June for both seasons total chlorophyll was determined in leaves of mango seedlings, according to method of Mackinney (1941) modified by Arnon (1949). At same time, measurements were made leaves nitrogen (%) content according Chapman and Pratt (1978), phosphor and potassium (%) according Estefan et al, (2013).

RESULTS AND DISCUSSION

Seeds germination percentage: Table (1) and Figure 1 indicates that seed age had a moral effect on germination percentage, It was highest percentage of germination in seeds planted one day after their harvesting, which amounted to 77.50 and 86.67% compared to those planted three days after their harvesting of 66.67and 71.67% for two growth season, successive. Results of Table (1) and Figure (2) also indicated effect of growing medium in percentage of

germination of mango seeds, when seeds are grown in loamy soil: peat moss (1:1) (M_2), the highest percentage was that it reached a 75.00 and 85.00 % moral difference from other two mediums, as it was the lowest in loamy soil: peat moss (3:1) (M_1) of 70.83 and 76.67 % for two experiment season, respectively. As for addition of humic acid, it was found from results of table (1) and Figure (3) to superiority of addition at 10 $g.L^{-1}$ (H_{10}) by giving it highest germination percentage of 76.67 and 84.44 % compared to addition 5 $g.L^{-1}$ (H_5), which gave lowest germination percentage of 67.78 and 75.58 % for both seasons, respectively. The results shown in Table (1) also indicated that twice interaction between experience treatments had a significant impact on increasing percentage of germination, especially when interfering between seed age and growing medium, excellence was clear when (D_1M_2) treatment and gave highest percentage of 80.00 and 90.00 % for two seasons, respectively.

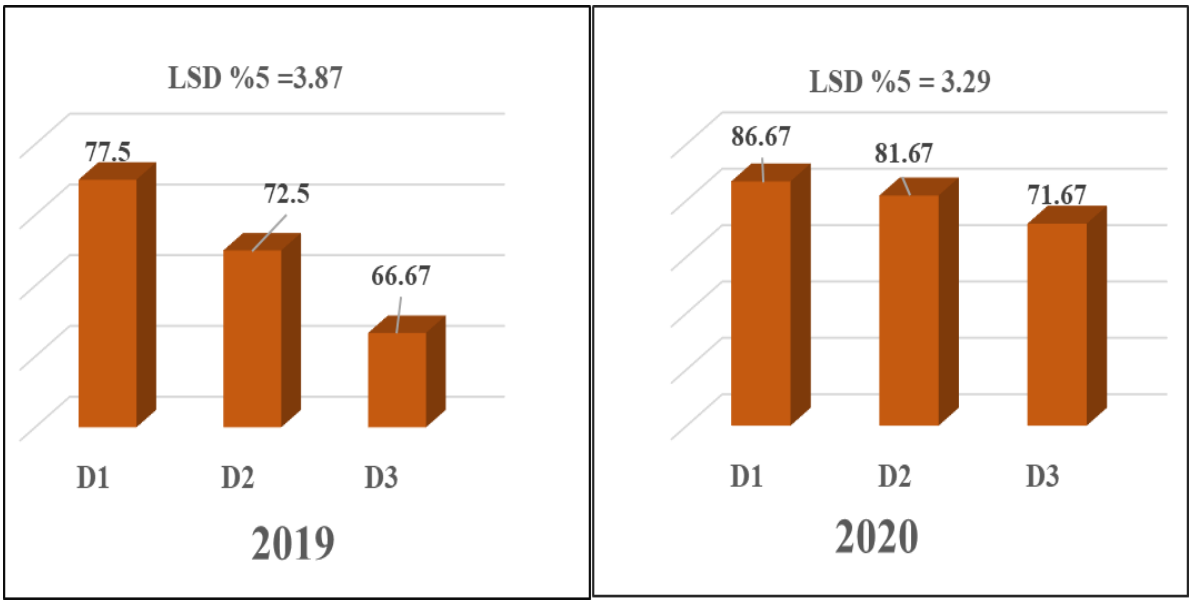

Fig (1) Effect of seed age on mango seeds germination percentage (%).

Also when interfering between seed age and humic acid, excellence was clear when (D_1H_{10}) treatment and gave highest percentage of 81.67 and 90.00 % for two seasons, respectively. As well as, interaction between growing medium and humic acid, excellence was clear when (M_2H_{10}) treatment and gave highest percentage of 80.00 and 90.00 % for two seasons, respectively. Likewise, when tripartite interaction between treatments, results of Table (1) show that percentage of germination has been significantly affected by experiment treatments and was highest germination in ($D_1M_2H_{10}$) of 85.00 and 95.00 % as compared with ($D_3M_1H_5$) treatment, which gave lowest seed germination percentage of 60.00, and 55.00 % for two seasons, respectively.

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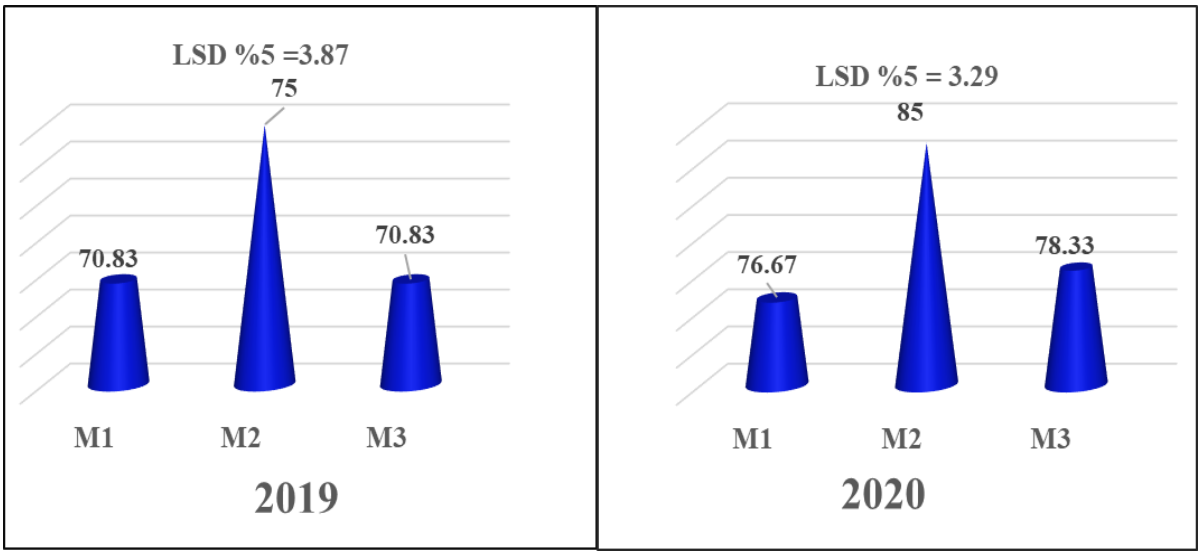

Fig (2) Effect of growing media on mango seeds germination percentage (%).

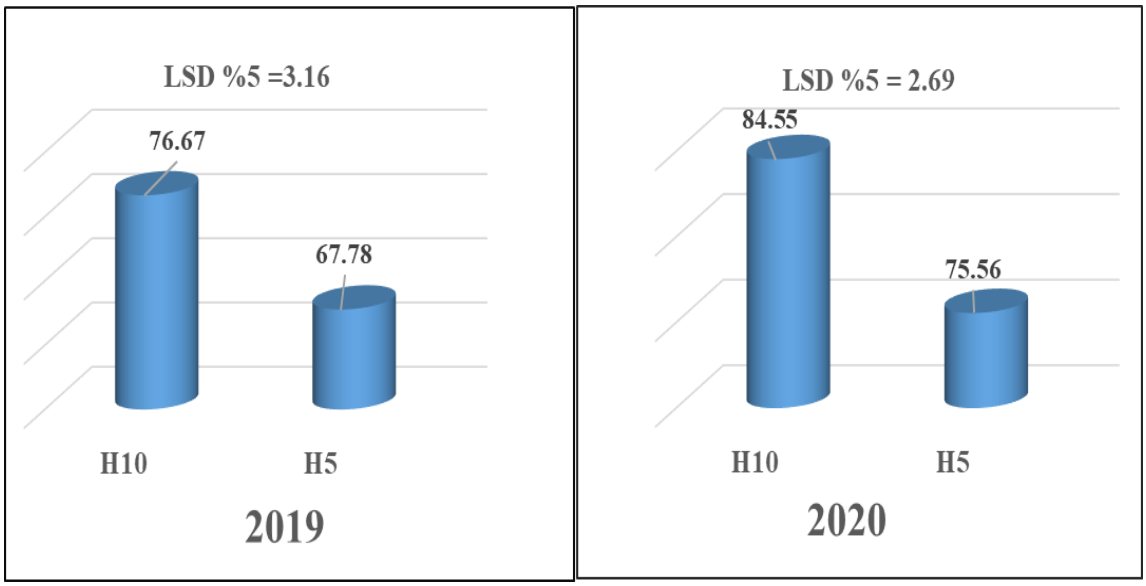

Fig (3) Effect of humic acid on mango seeds germination percentage (%).

Table 1. Effect of seed age, planting medium and humic acid addition on mango seeds germination percentage (%).

Humic acid (H)	Seed age (D)	2019				2020			
		Growing medium (M)			H × D	Growing medium (M)			H × D
		M_1	M_2	M_3		M_1	M_2	M_3	
H_5	D_1	70.00	75.00	75.00	73.33	80.00	85.00	85.00	83.33
	D_2	70.00	70.00	65.00	68.33	80.00	80.00	75.00	78.33
	D_3	60.00	65.00	60.00	61.67	55.00	75.00	65.00	65.00

H_{10}	D_1	80.00	85.00	80.00	81.67	90.00	95.00	85.00	90.00
	D_2	75.00	80.00	75.00	76.67	80.00	90.00	85.00	85.00
	D_3	70.00	75.00	70.00	71.67	75.00	85.00	75.00	78.33
LSD 5%		9.48			5.47	8.07			4.66
H × M					H	H × M			H
H_5		66.67	70.00	66.67	67.78	71.67	80.00	75.00	75.56
H_{10}		75.00	80.00	75.00	76.67	81.67	90.00	81.67	84.44
LSD 5%		5.47			3.16	4.66			2.69
D × M					D	D × M			D
D_1		75.00	80.00	77.50	77.50	85.00	90.00	85.00	86.67
D_2		72.500	75.00	70.00	72.50	80.00	85.00	80.00	81.67
D_3		65.00	70.00	65.00	66.67	65.00	80.00	70.00	71.67
LSD 5%		6.70			3.87	5.71			3.29
M		70.83	75.00	70.83		76.67	85.00	78.33	
LSD 5%		3.87				3.29			

This result may be due to availability of many important and specific environmental factors and conditions for successful germination process, including water, heat, air, light, and others, which were affected by experimental treatments and led to an increase in percentage of seed germination. Water is considered one of basic environmental factors necessary for germination. Stimulating enzymatic activity and processes of demolition and construction of various nutrients (proteins - carbohydrates - fats) requires an aqueous medium, growing medium used in experiment played a role in increasing water content, as ability of seeds to absorb water depends on several important factors, most important of which are degree of permeability of seed coats to water, water available in external medium surrounding seed, and finally temperature of medium or environment. We find that increasing external temperature to certain limits leads to an increase in rate of seed absorption of water. Which is positively reflected in increasing germination rate. The vitality of seed also affects increase in germination percentage. Therefore, we note that seed age after maturity had a significant effect on increasing germination percentage, in addition to role of humic acid in maintaining water content and reducing soil alkalinity, which led to an increase in germination percentage (Al-Azzawi and Ghassan, 2024, Al-Douri and Al-Douri, 2024).

Effect of seed age, planting medium and humic addition on vegetative characteristics:

Tables (2-4) indicates that seed age had a significant effect on plant height, stem diameter and leaves number. It was highest plant height, stem diameter and leaves number in seeds planted one day after their harvesting, which amounted to 100.47 and 120.87 cm, 11.98 and 12.43 mm and 61.00 and 67.83 leaf.seedling^{-1} for above-mentioned traits, respectively compared to those planted three days after their harvesting of 91.63 and 112.22 cm and 10.42 and 10.86 mm and 50.83 and 60.00 leaf.seedling^{-1} for two growth season, successive, while seed age did not affect leaf area for two experimental seasons. Results of Tables (2-5) also indicated effect of growing medium in plant height, stem diameter, leaves number and leaf area, when seeds are grown in loamy soil: peat moss (1:1) (M_2),highest plant height was that it reached a 101.77 and 134.23 cm, highest stem diameter of 11.93 and 12.44 mm, highest leaf number of 61.50 and 73.83 leaf.seedling^{-1} and highest leaf area of 85.69 and 93.85 cm^2 moral difference from other two mediums, for two experiment season, respectively.

Table 2. Effect of seed age, planting medium and humic acid addition on plant height (cm) of mango seedlings.

Humic acid (H)	Seed age (D)	2019				2020			
		Growing medium (M)			H × D	Growing medium (M)			H × D
		M_1	M_2	M_3		M_1	M_2	M_3	
H_5	D_1	83.14	87.69	91.60	87.48	103.62	125.48	106.88	111.99
	D_2	78.32	83.14	77.45	78.53	105.68	116.15	102.30	108.04
	D_3	75.70	80.80	74.18	76.89	96.96	116.77	98.07	103.93
H_{10}	D_1	106.46	123.15	110.78	113.46	114.58	158.64	116.00	129.74
	D_2	102.68	120.24	100.57	107.83	117.06	147.97	109.34	124,79
	D_3	100.18	115.62	103.30	106.37	110.24	140.39	110.87	120.50
LSD 5%		13.05			7.35	11.46			6.62
H × M					H	H × M			H
H_5		79.05	83.88	81.08	81.33	102.09	119.47	102.42	107.99
H_{10}		103.11	119.67	104.88	109.22	113.96	149.00	112.07	125.01
LSD 5%		7.53			4.35	6.62			3.82
D × M					D	D × M			D
D_1		94.80	105.42	101.19	100.47	109.10	142.06	111.44	120.87
D_2		90.50	101.69	89.01	93.73	111.37	132.06	105.82	116.42
D_3		87.94	98.21	88.74	91.63	103.60	128.58	104.47	112.22
LSD 5%		9.23			5.33	8.10			4.68
M		91.08	101.77	93.94		108.02	134.23	107.42	
LSD 5%		5.33				4.68			

As for addition of humic acid, it was found from results of tables (2-5) to superiority of addition at 10 $g.L^{-1}$ (H_{10}) by giving it highest plant height was that it reached a 109.22 and 125.01 cm, highest stem diameter of 11.84 and 12.20 mm, highest leaf number of 59.28 and 68.78 $leaf.seedling^{-1}$ and highest leaf area of 84.00 and 89.38 cm^2 compared to addition 5 $g.L^{-1}$ (H_5), which gave lowest plant height of 81.33 and 107.99 cm, stem diameter of 10.68 and 11.12 mm, leaf number of 52.44 and 59.22 $leaf.seedling^{-1}$ and lowest leaf area of 74.79 and 80.40 $cm^2$for both seasons, respectively. The results shown in Table (2-5) also indicated that twice interaction between experience treatments had a significant impact on increasing plant height, stem diameter, leaf number and leaf area, especially when interaction between seed age and growing medium, excellence was clear when (D_1M_2) treatment and when interaction between seed age and humic acid, excellence was clear when (D_1H_{10}) treatment and interaction between growing medium and humic acid, excellence was clear when (M_2H_{10}) treatment and gave highest values for above mentioned attributes for two seasons. Likewise, when tripartite interaction between treatments, results of Tables (2-5) show that plant height, stem diameter, leaf number and leaf area has been significantly affected by experiment treatments and was highest values in ($D_1M_2H_{10}$) of 123.15 and 158.64 cm as plant height ,13.04 and 13.88 mm as stem diameter, 71.00 and 83.33 $leaf.seedling^{-1}$ as leaf number and 92.76 and 98.75 cm^2 as leaf area for two seasons, respectively.

Table 3. Effect of seed age, planting medium and humic acid addition on stem diameter (mm) of mango seedlings.

Humic acid (H)	Seed age (D)	2019				2020			
		Growing medium (M)			H × D	Growing medium (M)			H × D
		M_1	M_2	M_3		M_1	M_2	M_3	
H_5	D_1	11.28	12.34	11.16	11.59	11.40	12.94	11.55	11.96
	D_2	10.25	11.68	10.42	10.78	10.69	12.00	10.71	11.13
	D_3	9.42	9.96	9.57	9.65	10.05	10.63	10.14	10.27
H_{10}	D_1	12.16	13.04	11.92	12.37	12.64	13.88	12.17	12.90
	D_2	11.47	12.82	11.56	11.95	11.75	13.12	11.85	12.24
	D_3	10.84	11.76	11.00	11.08	11.19	12.09	11.08	11.45
LSD 5%		1.38			0.80	1.23			0.71
H × M					H	H × M			H
H_5		10.32	11.33	10.38	10.68	10.71	11.86	10.80	11.12
H_{10}		11.49	12.54	11.49	11.84	11.86	13.03	11.70	12.20
LSD 5%		0.80			0.46	0.71			0.41
D × M					D	D × M			D
D_1		11.72	12.69	11.54	11.98	12.02	13.41	11.86	12.43
D_2		10.86	12.25	10.99	11.37	11.22	12.56	11.28	11.69
D_3		10.13	10.86	10.29	10.42	10.62	11.36	10.61	10.86
LSD 5%		0.98			0.56	0.87			0.50
M		10.90	11.93	10.94		11.29	12.44	11.25	
LSD 5%		0.56				0.50			

These results may be due to seed vitality and its high stored of nutrients, which led to greater growth of seedlings (Al-Azzawi and Ghassan, 2024, Abdullah and Khalaf, 2023).These results are due to role of medium and humic acid in physiological processes, and this role comes through encouraging work of enzymes and transferring the products of photosynthesis, in addition to its role in cell division and elongation, which leads to increased growth, including plant height, stem diameter, number and area of leaves. Or reason for increase in all of the studied vegetative traits may be due to the role of humic acid, which has a physiological effect on plant similar to auxin, which affects plant growth, as humic acid can play a role similar to cytokinin and gibberellin in terms of cell division and elongation, which is reflected in increased seedling vegetative growth. In addition to this acid and growing medium containing a number of nutrients that play an important role in growth, especially nitrogen. It may also be due to the content of this medium of major and minor nutrients and plant hormones that play an effective role in increasing growth and stimulating plant height stem diameter and leaf area (Al-Hadethi et al., 2020; Al-Douri and Al-Douri, 2024; Mohammed et al., 2024; Al-Marsoumi and Al-Hadethi, 2025).

Table 4. Effect of seed age, planting medium and humic acid addition on leaves number (leaf.seedling^{-1}) of mango seedlings.

Humic acid (H)	Seed age (D)	2019				2020			
		Growing medium (M)			H × D	Growing medium (M)			H × D
		M_1	M_2	M_3		M_1	M_2	M_3	
H_5	D_1	53.33	60.00	56.67	56.67	59.00	68.67	61.33	63.00
	D_2	51.00	57.33	50.33	52.89	55.67	68.00	54.67	59.45
	D_3	45.67	51.67	46.00	47.78	50.33	64.33	51.00	55.22

H_{10}	D_1	61.67	71.00	63.33	65.33	66.00	83.33	68.67	72.67
	D_2	54.50	66.67	54.67	58.61	64.33	81.00	61.33	68.89
	D_3	50.33	62.33	49.00	53.89	59.67	77.67	57.00	64.78
LSD 5%			3.72		2.15		3.27		1.89
H × M					H	H × M			H
H_5		50.00	56.33	51.00	52.44	55.00	67.00	55.67	59.22
H_{10}		55.50	66.67	55.67	59.28	63.33	80.67	62.33	68.78
LSD 5%			2.15		1.24		1.89		1.09
D × M					D	D × M			D
D_1		57.50	65.50	60.00	61.00	62.50	76.00	65.00	67.83
D_2		52.75	62.00	52.50	55.75	60.00	74.50	58.00	64.17
D_3		48.00	57.00	47.50	50.83	55.00	71.00	54.00	60.00
LSD 5%			2.63		1.52		2.31		1.33
M		52.75	61.50	53.33		59.17	73.83	59.00	
LSD 5%			1.52				1.33		

Table 5. Effect of seed age, planting medium and humic acid addition on leaf area (cm^2) of mango seedlings.

Humic acid (H)	Seed age (D)	2019				2020			
		Growing medium (M)			H × D	Growing medium (M)			H × D
		M_1	M_2	M_3		M_1	M_2	M_3	
H_5	D_1	72.14	82.90	73.15	76.06	75.77	88.939	74.68	79.85
	D_2	70.67	80.72	71.65	74.35	73.96	90.17	75.18	79.77
	D_3	71.36	78.60	71.90	73.95	77.80	91.60	75.55	81.65
H_{10}	D_1	79.82	92.76	80.31	84.30	84.65	98.75	85.06	89.49
	D_2	78.55	88.80	82.93	83.43	86.62	96.61	85.82	89.68
	D_3	81.04	90.34	81.46	84.28	83.72	97.02	86.13	88.96
LSD 5%			9.39		5.42		5.58		3.22
H × M					H	H × M			H
H_5		71.39	80.74	72.23	74.79	75.84	90.23	75.14	80.40
H_{10}		79.80	90.63	81.57	84.00	85.00	97.46	85.67	89.38
LSD 5%			5.42		3.13		3.22		1.86
D × M					D	D × M			D
D_1		75.98	87.83	76.73	80.18	80.21	93.84	79.87	84.64
D_2		74.61	84.76	77.29	78.89	80.29	93.39	80.50	84.73
D_3		76.20	84.47	76.68	79.12	80.76	94.31	80.84	85.30
LSD 5%			6.64		N.S		3.95		N.S
M		75.60	85.69	76.90		80.42	93.85	80.40	
LSD 5%			3.83				2.28		

Effect of seed age, planting medium and humic addition on leaves chemical characteristics:

The results of Tables (6-9) indicate that seed age had no significant effect on chemical characteristics of leaves. Results of Tables (6-9) indicated effect of growing medium in leaves chlorophyll, N, P and K content when seeds are grown in loamy soil: peat moss (1:1) (M_2), highest leaves chlorophyll content was that it reached a 27.77 and 30.07 $mg.g^{-1}$, highest leaves nitrogen content of 1.732 and 1.801 % , highest leaves phosphor content of 0.401 and 0.421

% and highest leaves potassium content of 1.849 and 1.970 % moral difference from other two mediums, for two experiment season, respectively. As for addition of humic acid, it was found from results of tables (6-9) to superiority of addition at 10 $g.L^{-1}$ (H_{10}) by giving it highest leaves chlorophyll content was that it reached a 25.95 and 27.62 $mg.g^{-1}$, highest leaves nitrogen content of 1.739 and 1.766 % , highest leaves phosphor content of 0.385 and 0.401 % and highest leaves potassium content of 1.810 and 1.885 % compared to addition 5 $g.L^{-1}$ (H_5), which gave lowest leaves chlorophyll content of 23.07 and 24.84 $mg.g^{-1}$, leaves nitrogen content of 1.513 and 1.575 % , leaves phosphor content of 0.361 and 0.375 % and leaves potassium content of 1.685 and 1.726 % for both seasons, respectively.

Table 6. Effect of seed age, planting medium and humic acid addition on leaf Chlorophyll content ($mg.g^{-1}$) of mango seedlings.

Humic acid (H)	Seed age (D)	2019				2020			
		Growing medium (M)			H × D	Growing medium (M)			H × D
		M_1	M_2	M_3		M_1	M_2	M_3	
H_5	D_1	21.67	25.46	22.26	23.13	22.86	27.80	23.55	24.74
	D_2	21.16	25.94	22.61	23.24	22.54	28.35	23.85	24.91
	D_3	20.33	26.82	21.36	22.84	22.15	28.76	23.70	24.87
H_{10}	D_1	23.79	30.18	24.40	26.12	25.00	32.96	25.81	27.92
	D_2	24.00	28.96	24.55	25.84	24.58	31.87	25.73	27.39
	D_3	24.67	29.28	23.74	25.90	25.53	30.68	26.44	27.55
LSD 5%		1.59			0.92	1.35			0.78
H × M					H	H × M			H
H_5		21.05	26.07	22.08	23.07	22.52	28.30	23.70	24.84
H_{10}		24.15	29.47	24.23	25.95	25.04	31.84	25.99	27.62
LSD 5%		0.92			0.53	0.78			0.45
D × M					D	D × M			D
D_1		22.73	27.82	23.33	24.63	23.93	30.38	24.68	26.33
D_2		22.58	27.45	23.58	24.63	23.56	30.11	24.79	26.15
D_3		22.50	28.05	22.55	24.37	23.84	29.72	25.07	26.21
LSD 5%		1.12			N.S	0.95			N.S
M		22.60	27.77	23.15		23.78	30.07	24.85	
LSD 5%		0.65				0.55			

The results shown in Table (6-9) also indicated that twice interaction between experience treatments had a significant impact on leaves chlorophyll, N, P and K content, especially when interaction between seed age and growing medium, excellence was clear when (D_1M_2) treatment in leaves phosphor and potassium, while there was no significant effect on nitrogen content of leaves and when interaction between seed age and humic acid, excellence was clear when (D_1H_{10}) and (D_2H_{10}) treatment and interaction between growing medium and humic acid, excellence was clear when (M_2H_{10}) treatment and gave highest values for above mentioned attributes for two seasons. Likewise, when triple interaction between treatments, results of Tables (6-9) show that leaves chlorophyll, N, P and K content has been significantly affected by experiment treatments and was highest values in ($D_1M_2H_{10}$) of 30.18 and 32.96 $mg.g^{-1}$ as leaves chlorophyll content ,1.818 and 1.870 % as leaves nitrogen content, 0.425 and 0.449 % as leaves phosphor content and 1.928 and 2.108 % as leaves potassium content for two seasons, respectively.

Table 7. Effect of seed age, planting medium and humic acid addition on leaf nitrogen content (%) of mango seedlings.

Humic acid (H)	Seed age (D)	2019				2020			
		Growing medium (M)			H × D	Growing medium (M)			H × D
		M_1	M_2	M_3		M_1	M_2	M_3	
H_5	D_1	1.448	1.636	1.455	1.513	1.496	1.752	1.487	1.578
	D_2	1.453	1.642	1.442	1.512	1.484	1.767	1.492	1.581
	D_3	1.459	1.631	1.450	1.513	1.503	1.700	1.497	1.567
H_{10}	D_1	1.696	1.818	1.711	1.742	1.714	1.870	1.725	1.770
	D_2	1.677	1.824	1.690	1.730	1.722	1.863	1.710	1.765
	D_3	1.701	1.839	1.694	1.745	1.737	1.854	1.705	1.765
LSD 5%		0.345			0.199	0.261			0.151
H × M					H	H × M			H
H_5		1.453	1.636	1.449	1.513	1.494	1.740	1.492	1.575
H_{10}		1.691	1.827	1.698	1.739	1.724	1.862	1.713	1.766
LSD 5%		0.199			0.115	0.151			0.087
D × M					D	D × M			D
D_1		1.572	1.727	1.583	1.627	1.605	1.811	1.606	1.674
D_2		1.565	1.733	1.566	1.621	1.608	1.815	1.601	1.675
D_3		1.580	1.735	1.572	1.629	1.620	1.777	1.601	1.666
LSD 5%		N.S			N.S	N.S			N.S
M		1.572	1.732	1.574		1.611	1.801	1.603	
LSD 5%		0.141				0.107			

The increase in content of chlorophyll and elements in mango seedlings leaves may be due to role of the growing medium and addition of humic acid in physiological processes, as these treatments encouraged the work of enzymes and transfer of the products of photosynthesis, in addition to role of these treatments in cell division and elongation, which leads to increased growth, including plant height, stem diameter, number of leaves, and leaf area, as in Tables (2-5), which leads to the absorption of these elements to meet the needs of the leaf system of seedlings, or reason for the increase in content of chlorophyll and elements in the leaves may be due to the fact that this medium and this acid contain a number of nutritional elements, and thus the concentration of these elements in the leaves increased (Al-Silmawy and Abdul-Ratha , 2023; Khalil, 2023; Abdulrhman and Al-Atrushy, 2024; Mohammed et al., 2024).

Table 8. Effect of seed age, planting medium and humic acid addition on leaf P content (%) of mango seedlings.

Humic acid (H)	Seed age (D)	2019				2020			
		Growing medium (M)			H × D	Growing medium (M)			H × D
		M_1	M_2	M_3		M_1	M_2	M_3	
H_5	D_1	0.348	0.387	0.350	0.362	0.355	0.413	0.361	0.376
	D_2	0.342	0.379	0.361	0.361	0.349	0.392	0.382	0.374
	D_3	0.345	0.384	0.357	0.362	0.355	0.400	0.368	0.374
H_{10}	D_1	0.362	0.425	0.368	0.385	0.371	0.449	0.375	0.398
	D_2	0.366	0.419	0.377	0.387	0.383	0.438	0.390	0.404
	D_3	0.371	0.410	0.371	0.384	0.393	0.432	0.382	0.402
LSD 5%		0.033			0.019	0.027			0.016

H × M				H	H × M			H
H_5	0.345	0.383	0.356	0.361	0.353	0.402	0.370	0.375
H_{10}	0.366	0.418	0.372	0.385	0.382	0.440	0.382	0.401
LSD 5%	0.019			0.011	0.016			0.009
D × M				D	D × M			D
D_1	0.355	0.406	0.359	0.373	0.363	0.431	0.368	0.387
D_2	0.354	0.399	0.369	0.374	0.366	0.415	0.386	0.389
D_3	0.358	0.397	0.364	0.373	0.374	0.416	0.375	0.388
LSD 5%	0.023			N.S	0.019			N.S
M	0.356	0.401	0.364		0.368	0.421	0.376	
LSD 5%	0.013				0.011			

Table 9. Effect of seed age, planting medium and humic acid addition on leaf K content (%) of mango seedlings.

Humic acid (H)	Seed age (D)	2019				2020			
		Growing medium (M)			H × D	Growing medium (M)			H × D
		M_1	M_2	M_3		M_1	M_2	M_3	
H_5	D_1	1.615	1.790	1.655	1.687	1.634	1.844	1.693	1.724
	D_2	1.610	1.769	1.674	1.684	1.621	1.857	1.705	1.728
	D_3	1.588	1.781	1.683	1.684	1.613	1.870	1.699	1.727
H_{10}	D_1	1.733	1.928	1.751	1.804	1.756	2.108	1.815	1.893
	D_2	1.746	1.919	1.780	1.815	1.779	2.077	1.801	1.886
	D_3	1.768	1.907	1.761	1.812	1.745	2.062	1.823	1.877
LSD 5%		0.123			0.071	0.150			0.087
H × M					H	H × M			H
H_5		1.604	1.780	1.671	1.685	1.623	1.857	1.699	1.726
H_{10}		1.749	1.918	1.764	1.810	1.760	2.082	1.813	1.885
LSD 5%		0.071			0.041	0.087			0.050
D × M					D	D × M			D
D_1		1.674	1.859	1.703	1.745	1.695	1.976	1.754	1.808
D_2		1.678	1.844	1.727	1.750	1.700	1.967	1.753	1.807
D_3		1.678	1.844	1.722	1.748	1.679	1.966	1.761	1.802
LSD 5%		0.087			N.S	0.106			N.S
M		1.677	1.849	1.717		1.691	1.970	1.756	
LSD 5%		0.050				0.061			

CONCLUSIONS

It was concluded from this experiment that seed vitality is very important in increasing germination percentage of mango seeds, in addition to role of the balanced medium between peat moss and loamy soil and the addition of humic acid had excellent results in growth characteristics and chemical leaf characteristics.

CONFLICT OF INTEREST The authors declare no conflicts of interest associated with this manuscript.

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