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Effect of NPK and Actosol on the Growth, Essential Oil Production and Biochemical Composition of *Zanthoxylum piperitum* Plant.

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ABSTRACT

A pot experiment was carried out to study the effects of NPK and Actosol on plant growth , essential oil , chemical composition and antioxidant activity of *Zanthoxylum piperitum* plant, it was conducted under in saran greenhouse, at the Experimental Nursery of the Ornamental Horticulture Depart., Fac., of Agric. ,Cairo Univ., during 2018/2019-2019/2020.The plants were fertilized with NPK at 12g/plant (100% recommended dose), 9 and 6 g/ plant ,sprayed with Actosol at 0, 1.5 and 2.5 ml/L. Treating plants with 6 and 9 g/plant NPK + Actosol at 2.5 ml/L, gave tallest plants ,highest No. of branches and significantly increased the fresh and dry weights of plants, in both seasons. Reducing NPK from 12 to 9 or 6 g/plant increased significantly essential oil% ,and had a positive effect in both seasons. Actosol at 1.5 ml/L gave the highest oil % ,while at 2.5 ml/l gave the highest oil yield /plant. Twenty compounds were identified by GC-MS (β-phellandrene, cyclohexanol, germacrene-D , α-pinene, geranyl acetate and 1,8-cineole), were the dominant compounds, which increased with NPK (12 ,9 and 6 g/ plant) + Actosol 1.5 ml/L, in the first season, respectively. Decreasing NPK doses combined with high rate of Actosol increased the phenolic contents. The highest flavonoids content was recorded in plants treated with NPK 9g/plant + Actosol 1.5 ml/L(first season) and NPK 9 g/plant (second one). The antioxidant profile indicated that the crude extract in plants received 12g/plant NPK showed the highest IC50 in both seasons.

KEYWORDS: *Zanthoxylum piperitum* ,Korean pepper, NPK , Actosol , Essential oil and Antioxidant

1. INTRODUCTION

The genus of Zanthoxylum (family Rutaceae) comprises about 250 species, native to the temperate and subtropical regions of the world. It include an important group of plants which has much commercial and ethanobotanical importance, used as sources of pharmaceutical and cosmetics raw material. Okagu *et al.*, (2021) stated that many of the plant species are used as food ingredients. One of the most important species is *Z. piperitum* (Japanese pepper or Korean pepper), it is an aromatic spiny shrub (Ravindran 2017), native to Japanese, China and Korean . It has special aroma(all plant organs), is used as a spice, and employed in the traditional medicine , and as a vegetable (Imaizumi 1999 and Hashimoto *et al.,* 2001).

The essential oils of *Zanthoxylum piperitum* show a strong antimicrobial, insecticidal, antioxidant and anticancer activities (Kamsuk *et al.*, 2007and Lee and Lim 2008),as well as show a wide spectrum of biological activities (as anti-inflammatory, analgesic, antinociceptive, antibiotic, hepatoprotective, antiviral and antifungal due to the constituents, essential oil, alkaloids and cumarins (Kim *et al.,* 2003 and Negi *et al.,* 2011,the fruit extract has strong antioxidant (Yamazaki *et al.,* 2006).

Fertilization of NPK is one of the most important factors limiting the productivity of plants. It is the most striking practice in the plant nutrition (Marschner,2012). Nitrogen(N) is required in synthesis of proteins and enzymes, it is a component of amino acids, nucleic acids and chlorophyll . Phosphorus (P) is one of the most macro nutrients, it plays a vital role in growth and root development.(Jin *et al.*, 2005, Marschner,2012 and He *et al.*, 2019). Sharpley *et al.,* (1996) mentioned that P has the role of storage and transfer of energy through the plant (ADP& ATP) that control the processes of photosynthesis, respiration, protein and nucleic acid synthesis, and nutrient transport in plant cells. Potassium (K) is the most abundant cation in higher plants, it has pivotal role in plant physiology and enzymatic activity (Khalid and Shedeed 2015 and Hasan *et al.,* 2016).It is involved in plant cell membrane, regulation of stomata, maintenance of turgor, and enzyme activity (Wiedenhoeft, 2006).Hosseini (2012) reviewed the promoting roles of N,P and K on production of some important medicinal plants (basil, turmeric, black pepper, cardamom, fennel, fenugreek and aloe)..

Actosol is biostimulant, formulated with natural substances(20% humic and fulvic acids), it derived from leonardite (natural humus),it improves stress tolerance, water retention, enhances chelating of nutrients and root and plant growth. Several authors explained the positive effect of Actosol and other humic products, in improving the productivity of plants. In addition, Masciandaro *et al.,* (2002) confirmed its role in enhancing the uptake of nutrients, cell membrane permeability, oxygen uptake, respiration, photosynthesis and cell elongation. The major functional groups include carboxyl, phenolic hydroxyl, alcoholic hydroxyl and ketone that has a positive effect on plant growth and the nutrient uptake (Cacco and Agnolla,1984). Pouneva (2005) and Burkowska and Donderski (2007) reported that humic substances increased the growth rate by stimulating enzyme activities. EL-Gohary *et al.,* (2014) on Mentha piperita found that essential oil% and constituents increased with foliar nutrition of humic acid. Auimoviu *et al.,* (2015) found that bio-humus (Royal Ofert) significantly increased the oil% in anise. El-Sherif and Ismail (2017) stated that humic acid at 2g/l increased the growth , yield and curcumin%. On achillea Sani and Jodaeian (2017) and on Thymus kotschyanus ,Saber *et al.,* (2021) obtained similar results.

Many studies investigated the possibility of bio-fertilizers utilization instead of mineral fertilizers-NPK and their effects on growth ,oil yield and secondary metabolites, as previously reported by Mohamed and El-Ganaini (2003), Badran and Safwat (2004) on fennel plant and Swaefy *et al.,* 2007) on peppermint plant. Also, Abdelaziz *et al.,* (2007) indicated that biofertilizers could replace the conventional NPK fertilizers of rosemary ,they obtained a significant increase in growth, carbohydrate content and essential oil production. On fennel, Azzaz *et al.,* (2009) and Valiki and Ghanbari (2015) emphasized the possibility and enhancing effect of organic and bio-fertilizers utilization instead of NPK on vegetative growth and oils yield, giving significant increases growth and oil yield. Hassan *et al.,* (2015) on basil stated that NPK at 100% of recommended and bio fertilizer at 6 g l-1,ADY, increased vegetative growth ,total chlorophyll , oil% and linalool. Ahmed (2017) on celery and dill plants, revealed bio fertilizer + 50% NPK significantly increased vegetative growth,chlorophylls and essential oils% as well the main components(ß. pinene, limonene and ß-phellandrene%). Shehata (2019) on parsley recommended 200 mg/l humic acid + 50 % of recommended NPK for growth and chlorophyll content. Ghatas (2020) indicated that different treatments of NPK and bio-fertilization significantly increased chemical constituents and oil productivity.

Ahmed *et al.,* (2019) on chamomile plant stated that NPK alone increased vegetative growth and NBK+ bio-fertilizer significantly increased volatile oil%. El-Sayed *et al.,* (2021) on Camelina sativa found that 100% NPK and 75%NPK + Bio produced the highest values of vegetative growth ,chemical composition and oil productivity.

Therefore, the aim of present study was to investigate the effect different doses of NPK and Actosol as foliar application at different rates on the growth, essential oil production and biochemical composition of Japanese pepper *Zanthoxylum piperitum.* plant.

2. MATERIALS AND METHODS

2.1. Procedures and design

A pot experiment was conducted at the Experimental Nursery of Ornamental Horticulture Depart.,Fac. of Agric. ,Cairo Univ.,Giza (30E01' 39.36" N latitude and31E12' 36.50"E Longitude) during the seasons of 2018/19 and 2019/20 to study the effect of NPK fertilization and Actosol on growth, essential oil (EO) content and (EO)composition, as well as and chemical composition of *Zanthoxylum piperitum.* plant. On May 27th, 2018 and May 30th, 2019 during the first and second seasons, respectively, the age of 6 months uniform seedlings (20-25cm height-from private nursery) were individually transplanted into 40cm plastic pots filled with loamy clay soil Table (1). The experiment was conducted under partial shade conditions in saran house (50 % shade). The experimental design was randomized complete block design in split plot form with three replicates. The three levels of NPK (12 g/plant as 100 %addition, 9 g/plant as 75% and 6 g/plant as 50%) were allocated to the main plots, while the three concentrations of Actosol (0, 1.5 and 2.5 ml/L) were assigned as sub plots. Each level of sub plot was represented by five pots for each replicate. Each level of NPK was applied as a soil drenching at three equal splits; one, three and five months after transplanting, respectively, whereas, Actosol was bimonthly applied as foliar application four times; starting after a week from transplanting and spraying plants with Actosol was carried out up to run off point. Actosol was obtained from Egy-American Co.For Investment &

Table 1. The physical and chemical analysis of the experimental soil during the two seasons.

	pH	EC dS/m	Organic Matter %	N	P ppm	K
First season	7.60	1.42	1.35	19.42	11.25	122
Second season	7.32	1.58	1.42	21.75	13.15	131

pH: Soil acidity; EC: Electrical conductivity; N: Nitrogen; P: phosphorous; K: potassium.

Agriculture Devel. Heliopolis, Cairo. Spraying with tap water represented the control treatment. The plants were irrigated regularly.

2.2 Recorded Data:

In this study, we focused on the chemical and physiological responses of *Z. piperitum* to NPK and Actosol supplements. After 6 months of treatments, the following morphological measurements were determined: The data on the growth parameters were recorded on mid-May in 2019 and 2020 , in terms of plant height, stem diameter ,No. of branches/ plant, root length and roots No/plant , leaves, stem, and roots fresh weight/plant were determined at harvest of each season. The fresh leaves, stems and roots were dried at 70 C° for 48 hr. and the dry weights were recorded.

2.3.Essential oil extraction and GC-MS analysis:

The essential oil (EO)in the air dried leaves was obtained by hydro-distillation using the original Clevenger-type apparatus for EO, lighter than water (according to British Pharmacopoeia,(1963).Samples of leaves (100 g) were used to determine the EO % (v /w). The obtained oil was separated by extraction with Et_2O (Merck, Germany), maintained dry by subjecting samples to anhydrous sodium sulfate (Na_2SO_4, Aldrich, USA), and immediately analyzed. To determine the main constituents of the obtained EO, GC/MS analysis was performed according to Adams (1995) at the Central Laboratory of Fac.of Agric., Cairo Univ. The retention time (RT) of the components was used to identify the constituents of the EO and the report of GC/MS analysis, helped in calculation the of main components percentage of essential oil.

2.4.Total chlorophylls content:

It was determined using a digital chlorophyll meter (SPAD), model SPAD-502, which SPAD unit = 10mg/100g fresh weight of leaves (Netto *et al.*, 2005).

2.5. Total hydrolysable carbohydrate:

Samples of 0.5 g from dried ground leaves were placed in 20 ml test tubes, followed by addition of 10ml 1N sulfuric acid. The tube was sealed, and placed overnight in an oven at 100º C, then it was completed 100 ml with distilled water and filtered. The total hydrolysable carbohydrates were determined using the phenol- sulfuric acid method according to Dubois *et al.* (1956).

2.6. Determination of total phenol and flavonoids contents:

Polyphenolics contents were determined by the Folin–Ciocalteu method (Meda *et al.*, 2005). Flavonoids content was determined in each extract according to the aluminum chloride colorimetric method described by Chang *et al.*, (2002).

2.7. Antioxidant activity:

Total free radical scavenging capacity of the extract was evaluated by two different metheds using the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical as reported by Kedare and Singh, (2011).

2.8. Determination of N,P and K contents:

The digested solution was chemically analyzed to determine the concentration of N, P and K in the dry leaves according to the method recommended by Cottenie et al (1982).N-content was determined by the modified micro-Kjeldahl method as described by A. O. A. C (1990), P-content according to Jackson (1973) and K- content by using atomic absorption spectrophotometer Model SP 1900 with a boiling air-acetylene burner and recorded read out Issac and Kerber (1971).

2.9. Statistical analysis:

Data recorded on the growth parameters were subjected to analysis of variance based on split-plot arrangement in a randomized complete block design according to procedures outlined by Gomez and Gomez (1984) using MSTAT-C computer package Freed *et al.*, (1989). Treatment mean comparisons were performed using least significant difference (LSD) at 5% level of probability.

3. RESULTS AND DISCUSSION

3.1.Morphological Responses :

Data obtained on the morphological responses of *Zanthoxylum piperitum* plants to the application of NPK and Actosol, Table(2) revealed that treating plants with low and medium levels of NPK (6 and 9 g/plant) significantly affected plant height, comparing to the low one. The dose of 6g/plant gave the tallest plants (43.50 and 45.42cm) , in the first and second seasons, respectively. Under the treatment of Actosol, the high level (2.5 ml/L) gave the tallest plants (45.92-47.58cm) in both seasons, respectively. The combined treatment of NPK at 6g/plant with Actosol at 2.5ml/l, gave the tallest plants, giving 50.25and 51.0cm,in the first and second seasons respectively. The results proved that the lowest level of NPK (6g/plant) tended to promote the height growth in the presence of Actosol.

The response of stem diameter to NPK and Actosol (Table 2) shows that stem thickness of plants treated with NPK 6 g/plant, regardless the effect of Actosol, had the highest values (0.98 and 0.83 cm), in the first and second seasons, respectively. Spraying plants with Actosol at high level (2.5 ml/l) was the most effective in increasing it in both seasons.

Treating the plants with NPK at 6g/ plant gave the greatest No. of branches /plant (12.75 and 13.83) in both seasons, as compared to the other NPK doses(Table 2). Regardless ,the effect of NPK, spraying *Z. piperitum* plants with Actosol at 2.5 ml/l produced the highest No. of braches/plant(13.83and14.83 , in both seasons, respectively). The combination between NPK 6g /plant + Actosol 2.5 ml/L recorded the highest N0.of branches /plant (15.50 and16.75) in the two seasons , respectively, followed by the treatments of NPK 6g/plant + Actosol 1.5 and NPK 9 g/plant + Actosol 2.5 ml/l. Whereas, the treatment of NPK (6g/plant) without Actosol sprayings, resulted in the lowest No. of branches/plant(8.75 and 9.50) in both seasons. This means that the foliar spraying of Actosol enhancing the formation of branches. Regarding the responses of root length and roots No/ plant to the treatments of NPK and Actosol, as shown in Table(2) the data revealed that NPK at 9g/plant ,regardless Actosol treatments, insignificantly increase the root length (46.25and 48.08cm),over the other two doses ,in both seasons. But, Actosol 2.5 ml/l significantly increased it (50.83and 52.50cm, respectively) as compared with the control.

The interaction between NPK and Actosol had a significant effect in the 2nd season, and plants treated with 6g/plant NPK +2.5 ml/L Actosol gave the tallest roots (53.75and 55.00 cm) in the two seasons, respectively. The same Table.(2) shows that the No.of roots in plants treated with NPK at 6 g/plant was significantly more than that of NPK at 9 and 12 g/plant, only in the 2nd season, giving the greatest number of main roots /plant (4.33 and 4.92). Actosol at 2.5ml/L, in both seasons, gave the greatest number of main roots /plant (5.75).

The stimulation effects of applying NPK on vegetative growth may be attributed to the well known functions of N,P and K, which they are the most abundantly acquired mineral elements by plants, they play vital roles in many aspects of plant metabolism and plant life. NPK-fertilization is one of the most important factors limiting plant productivity(Jin *et al.*, 2005, Marschner,2012 and He *et al.*, 2019), as mentioned in Introduction. The sufficient supply of these macro-elements is required for their proper functions on activation of photosynthesis and metabolic processes of organic compounds in plants , consequently, the vegetative growth parameters increased . These findings are in agreement with results recorded by Hosseini (2012) on some economic medicinal plants (basil, turmeric, black pepper, cardamom, fennel, fenugreek and aloe), Khalid and Shedeed (2015) on *Nigella sativa* plant.

Concerning the responses of fresh and dry weights of leaves and stems to the application of NPK and Actosol as shown in Table (3), it is obvious from the obtained data show that the application of 6g/plant NPK alone gave the least values for fresh and dry weights of leaves and stems. Raising the NPK rate from 6.0 to 9.0 and 12.0 g/plant gradually increased the fresh and dry weights of leaves and stems. Also, raising the Actosol rate from 1.5 to 2.5ml/l significantly increased the fresh and dry weights, recording the heaviest weights in both seasons. Regarding the combined treatments of NPK with Actosol, the data cleared that the application of 6 g/ plant of NPK + spraying plants with Actosol at 2.5 ml/l ,were the most effective in increasing the fresh and dry weights of both leaves and stems, in both seasons. Moreover, the treatments of Actosol at 2.5ml/l under NPK at 9 g/plant resulted in higher increases than those obtained under NPK at 6 g/plant, as compared with other treatments.

The fresh and dry weights of roots/plant, in response to NPK and Actosol treatments, had a similar trend to the responses of root length and number/plant, obtaining the heaviest weights with the treatment of 6g /plant NPK combined with 2.5 ml Actosol spraying, in both seasons. These results are in harmony with that obtained by Chen and Avaid (1990) they reported that

Table 2. Effect of NPK and Actosol and their interactions on plant height, stem diameter, branches No./plant ,root length and roots No/plant of *Zanthoxylum piperitum* plant during 2019and 2020seasons.

				Plant height (cm)					
		(2019)					**(2020)**		
Treatments	***NPK1**	**NPK2**	**NPK3**	**M(B)**		**NPK1**	**NPK2**	**NPK3**	**M(B)**
***Act 1**	39.50	38.00	35.25	**37.58**	**Act 1**	42.50	39.50	36.75	**39.58**
Act 2	40.75	42.00	45.00	**42.58**	**Act 2**	41.50	43.50	48.50	**44.50**
Act 3	42.50	45.00	50.25	**45.92**	**Act 3**	43.50	48.25	51.00	**47.58**
M(A)	**40.92**	**41.67**	**43.50**		**M(A)**	**42.50**	**43.75**	**45.42**	
L.S.D 0.05	A:1.46	B: 2.85	Ax B : 4.94			A: 2.34	B: 2.37	Ax B: 4.73	
				Stem diameter (cm)					
	0.73	0.70	0.65	**0.70**		0.70	0.65	0.63	**0.66**
	0.65	0.78	1.03	**0.82**		0.60	0.75	0.90	**0.75**
	0.70	0.85	1.25	**0.93**		0.68	0.83	0.98	**0.82**
	0.69	**0.78**	**0.98**			**0.65**	**0.74**	**0.83**	
L.S.D 0.05	A: 0.08	B: 0.07	A x B : 0.12			A: 0.11	B: 0.07	A x B: 0.12	
				Branches No/plant					
	10.50	9.75	8.75	**9.67**		11.00	10.25	9.50	**10.25**
	11.50	12.25	14.00	**12.58**		11.75	13.00	15.25	**13.33**
	12.00	14.00	15.50	**13.83**		12.50	15.25	16.75	**14.83**
	11.33	**12.00**	**12.75**			**11.75**	**12.83**	**13.83**	
L.S.D 0.05	A:0.90	B: 0.75	AxB: 1.29			A: 0.73	B: 0.69	A xB: 1.19	
				Root length(cm)					
	41.25	38.75	32.75	**37.58**		42.50	40.00	36.25	**39.58**
	43.75	48.75	51.25	**47.92**		46.25	50.50	52.50	**49.75**
	47.50	51.25	53.75	**50.83**		48.75	53.75	55.00	**52.50**
	44.17	**46.25**	**45.92**			**45.83**	**48.08**	**47.92**	
L.S.D 0.05	A: n.s	B: 4.96	AxB : n.s			A: n.s	B: 3.73	AxB: 6.47	
				Roots No/plant					
	3.75	3.50	3.25	**3.50**		4.00	3.75	3.50	**3.75**
	4.25	4.50	4.75	**4.50**		4.50	5.00	5.50	**5.00**
	4.50	5.00	5.00	**4.83**		4.75	5.50	5.75	**5.33**
	4.17	**4.33**	**4.33**			**4.42**	**4.75**	**4.92**	
L.S.D 0.05	A: n.s	B: 0.56	Ax B : n.s			A: 0.43	B: 0.44	A x B: 0.77	

humic substances had a very pronounced influence on the growth of roots and enhanced root initiation. Also, Hanafy et al., (2017) on Artemisia abrotanum who explained that Actosol affected on the cells elongation as will formation of new tissues more that the effected of NPK. Samavatipour et al., (2019) on Anethum graveolens stated that root fresh and dry weights of plants treated with fulvic acids was higher than the control, improving the root system, consequently allowed for nutrients and minerals to be absorbed readily. Nofal et al., (2020) Erantheumum pulchellum found that application of NPK and humic acid gave the best results than the control .

From the above mentioned results, treating plants with 6and 9 g/plant of NPK had significant effect on plant height ,branching ,stem thickness and weights of leaves and stems. ,On the other hand, spraying plants with the high level (2.5 ml/L) of Actosol gave the tallest plants ,the thickest stems, the highest values of No. of branches and weights. Moreover, the combined treatments of NPK at 6g /plant with Actosol at 2.5ml/l, gave the tallest plants, the highest No. of branches, the thickest stems. were the most effective on increasing the vegetative growth parameters , this means the possibility of bio-fertilizers Actosol utilization in reducing the amount of NPK doses required for better growth of Zanthoxylum plants.

Table 3. Effect of NPK and Actosol and their interactions on fresh and dry weight of leaves, stems and roots of *Zanthoxylum piperitum* plant during 2019and 2020seasons.

Leaves fresh weight (g/ plant)									
	2019					**2020**			
Treatments	***NPK1**	**NPK2**	**NPK3**	**M(B)**	**Treatments**	**NPK1**	**NPK2**	**NPK3**	**M(B)**
***Act 1**	25.62	22.98	19.85	**22.82**	**Act 1**	28.25	23.56	21.41	**24.41**
Act 2	28.75	35.99	39.52	**34.75**	**Act 2**	30.11	38.06	43.85	**37.34**
Act 3	40.09	48.17	48.59	**45.73**	**Act 3**	42.93	50.64	50.68	**48.08**
M(A)	**31.49**	**35.97**	**35.84**		**M(A)**	**33.76**	**37.42**	**38.65**	
L.S.D 0.05	A: 2.03	B:1.83	AxB :3.17			A:2.01	B :1 .56	AxB:2.07	
Leaves dry weight (g/ plant)									
	9.26	8.08	7.24	**8.19**		10.55	8.59	7.94	**9.03**
	9.82	12.31	13.80	**11.98**		10.76	13.11	15.87	**13.24**
	13.60	17.60	17.68	**15.99**		13.94	18.29	18.37	**16.87**
	10.89	**12.70**	**12.58**			**11.75**	**13.33**	**14.06**	
L.S.D 0.05	A: 1.0	B:0.61	AxB:1.06			A: 0.94	B: 0.87	AxB: 1.51	
Stems fresh weight (g /plant)									
	12.05	11.96	10.99	**11.66**		12.78	12.41	11.87	**12.35**
	12.65	17.34	20.78	**16.92**		13.27	19.69	23.83	**18.93**
	16.57	24.10	24.94	**21.53**		17.55	25.16	25.47	**22.72**
	13.76	**17.80**	**18.57**			**12.78**	**19.08**	**20.39**	
L.S.D 0.05	A: 1.34	B: 1.13	AxB: 1.96			A: 0.82	B: 0.78	AxB: 1.36	
Stems dry weight (g/ Plant)									
	5.36	5.20	4.35	**4.97**		5.77	5.43	4.97	**5.39**
	5.54	8.02	9.23	**7.59**		5.91	8.98	11.02	**8.64**
	7.52	11.72	11.80	**10.28**		8.50	12.33	12.68	**11.17**
	6.14	**8.31**	**8.39**			**6.73**	**8.92**	**9.56**	
L.S.D 0.05	A: 0.75	B:0.73	AxB: 1.27			A: 0.62	B: 0.56	AxB: 0.97	
Roots fresh weight (g/plant)									
	14.01	13.11	12.85	**13.32**		14.95	14.20	13.25	**14.13**
	15.10	18.35	19.78	**17.74**		15.53	19.04	20.62	**18.40**
	19.54	23.14	24.38	**22.35**		20.83	24.80	25.50	**23.71**
	16.22	**18.20**	**19.00**			**17.10**	**19.35**	**19.79**	
L.S.D 0.05	A: 1.49	B: 0.69	AxB: 1.19			A: 1.10	B: 0.57	AxB: 0.99	
Roots dry weight (g/plant)									
	9.54	8.94	8.77	**9.08**		10.16	9.68	9.05	**9.63**
	10.47	12.63	13.58	**12.22**		10.81	13.12	14.15	**12.69**
	13.83	16.23	17.11	**15.72**		14.72	17.42	17.98	**16.70**
	11.28	**12.60**	**13.15**			**11.89**	**13.41**	**13.73**	
L.S.D 0.05	A: 0.99	B: 0.46	AxB : 0.80			A: 0.73	B: 0.38	AxB: 0.66	

***NPK1**: NPK 12g /plant , **NPK2**: NPK 9g /plant, **NPK3**: NPK 6g /plant , **Act1** : Actosol 0ml/L , **Act2** :Actosol 1.5 ml/L and **Act3** :Actosol 2.5 ml/L.

Many studies investigated the possibility of bio-fertilizers utilization instead of mineral fertilizers especially NPK, as previously reported by Ahmed (2017) on celery and dill, Shehata (2019) on parsley, Ahmed et al (2019) on chamomile Nofal et al., (2020)on Erantheumum pulchellum plant and El-Sayed et al., (2021) on Camelina sativa plant. The results clearly demonstrated the benefits of using solid HS as a management input to improve transplant quality in these crop species.

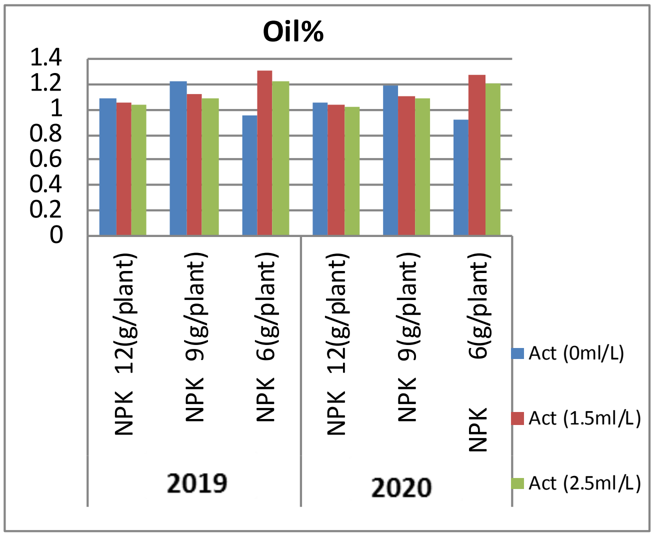
The pronounced effect of Actosol and other humic acid products on vegetative growth may be attributed to its effect on improving water retention, enhancing chelating of nutrients stimulating cells enlargement and elongation, leading to production of higher vegetative growth and greater proliferation of roots. The superiority of the plants that received Actosol at 2.5 ml/l , in producing the heaviest total plant fresh and dry weights may be attributed (according to Masciandaro et al., 2002) to its role in enhancing the uptake of nutrients and oxygen , respiration, photosynthesis and cell elongation, due to the major functional groups of humic acid that has a positive effect on plant growth and the nutrient uptake ,specially nitrate (Cacco and Agnolla, 1984) and by stimulating enzyme activities (Pouneva 2005) and Burkowska and Donderski 2007) .In this regard, El Sherif and Ismail (2017) on Curcuma longa, Sani and Jodaeian (2017) on Achillea millefolium and Saber et al., (2021) on Thymus kotschyanus, they found that humic acid significantly improved the growth development and yield and had a positive effect on root growth. Hanafy et al., (2017) on Artemisia abrotanum, found that application of Actosol at the rate of 1 and 2 ml/pot, significantly increased the plant growth. El-Khateeb et al., (2017) reported increases in the fresh and dry weights of marjoram as a result of humic acid treatments. Mahmoud (2019) on artemisia found that the highest values for plant height were obtained from plants fertilized with actosol in both seasons

3.2.Essential oil % and yield/plant:

The data in Fig.(1&2) revealed that the essential oil (EO) % and yield/plant were affected significantly with NPK in both seasons. Reducing NPK levels from 12 g/plant (100% ,recommended) to 9 g/plant (75%) and 6 g/plant (50%) increased significantly essential oil% and yield/plant and NPK at 12 g/plant gave the lowest oil% (1.06% and 1.03%) and oil yield/plant (0.115and 0.121 ml/plant) in the first and second seasons, respectively. Reducing NPK rates from 100% up to 50% increased significantly essential oil % by 9.43% and 9.71% and essential oil yield/plant by 34.78 and 36.36% in the first and the second seasons, respectively. The reduction in essential oil productivity due to the effect of high levels NPK might be attributed to the production of more vegetative growth rather than the production of secondary products, i.e. essential oil. This result agreed with those reported by Puttanna *et al.,* (2010) on *Rosmarinus officinalis*, Juárez *et al.,* (2011) on *Thymus vulgaris*, El-Sayed et al (2015) on Ocimum sp, Hendawy *et al.,* (2015) on *Mentha piperita*, Cáceres *et al.,* (2017), Heikal and Helmy (2018), Hanafy *et al.,* (2018) on *Majorana hortensis* and Hanafy *et al.,* (2019) on *Artemisia abrotanum*.

The foliar application of Actosol had a positive and significant effect on essential oil productivity of *Z.piperitum* plant in both seasons. The control treatments had the lowest EO%. The foliar application of Actosol at 1.5 ml/l. gave the highest oil % (1.16% and 1.13%), while Actosol at 2.5 ml/l. gave the highest oil yield/plant (0.18 and 0.19 ml/plant), in the first and second seasons, respectively. The high value of oil yield accompanying high concentration of actosol indicates its significant role in enhancing the uptake of nutrients and oxygen, respiration, photosynthesis (Cacco and Agnolla, 1984) and by stimulating enzyme activities (Pouneva 2005 and Burkowska and Donderski 2007),which in turn increased the oil yield. Similar results were reported by El-Sayed *et al.,* (2016), Hanafy *et al.,* (2018 and 2019).

The interaction between NPK and Actosol had a significant effect on essential oil% and oil yield/plant. The highest essential oil% (1.30% and 1.27%) were recorded in plants fertilized with 6 g NPK and sprayed with 1.5 ml/l, whereas oil yield/plant (0.216 and 0.220 ml)was the highest with 6 g/plant NPK +Actosol 2.5 ml/l. EL-Gohary *et al.,* (2014)on *Mentha piperita* L found that essential oil content (% or ml plant-1) and its major constituents increased with the foliar nutrition of humic acid. Also, Auimoviu *et al.,* (2015) mentioned that the application of bio-humus (Royal-Ofert) significantly increased it in anise plant. Barghamadi and Najafi (2015) on Ajowan (*Carum copticum*) reported that humic acid increased essential oil% and yield.



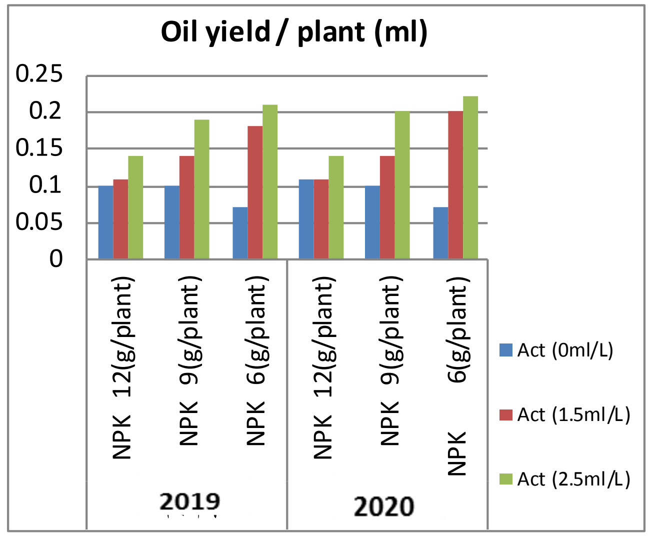


Fig.1 **Fig.2**

Fig.1&2. Effect of NPK and Actosol and their interactions on essential oil % and yield/plant of *Zanthoxylum piperitum* plant during 2019and 2020 seasons .

3.3. Essential oil composition :

It was performed by GC-MS analysis, the essential oil composition obtained from the leaves of *Z .piperitum* plants subjected to NPK+ Actosol treatments are shown in Table (4). About Twenty compounds were identified, representing : β-phellandrene, cyclohexanol ,germacrene-D , geranyl acetate , α-pinene and 1,8-cineole,were found to be the dominant compounds. These results were in agreement with those reported by Jiang and Kubota (2004) and Chang and Kim (2008). In our study plants treated with T2 (NPK 12g/plant+ Actosol 1.5 ml/L) , T5(NPK 9g/plant+ Actosol 1.5 ml/L and T8 (NPK 6g/plant+ Actosol 1.5 ml/L) in the first season were the most effective treatment in increasing the percentages of β-phellandrene, cyclohexanol and germacrene-D, the total amount reached 21. 6, 13. 7and 12. 6 %, with T2, 21.6, 13.8and 12.6 % with T5 and 21.3 , 13.4 and 12.3 % with T8. whereas in the second season the highest percentages these components were 23.1% with T8, 13.3% with T7and 11.8 % with T3,respectively. The percentage of geranyl acetate was the highest with T9 and T5 , the percentage of α-pinene reached the highest value with T9, whereas, the 1,8-cineole % was the highest with T9 and T3 , in the first and the second seasons, respectively. The nerolidol % reached to the maximum values in both seasons, with the treatment T9 . These results were in agreement with those reported by Jiang and Kubota (2004) and Chang and Kim (2008). In our observations, the percentages of some main components showed seasonal variation as affected with the same treatments. As reported by Jiang and Kubota(2004) the essential oils of fruits and dried pericarp of Japanese pepper contained citronellal, linalool, and methyl cinnamate and geraniol. large amount of d-limonene, beta-phellandrene, and myrcene. Yang (2008) revealed that the essential oil of many species of Zanthoxylum contained linalyl acetate, linalool ,limonene, sabinene , alpha-terpineol, myrcene, 1,8-cineole. Whereas , Chang and Kim (2008) on *Zanthoxylum pipperitum* revealed that EO % from Korean and Chinese plant were 2.0 and 1.2% (w/w) respectively, and β-phellandrene, sabinene, terpinen-4-ol and linalool were the most abundant compound in the Korean and limonene , geranyl acetate, citronellal, and phellandral (each >5%), in Chinese plant . Moreover, Bisht and Chanotiya (2011) stated that the leaf essential oils of *Z. armatum* (GC-MS) contained high proportion of non-terpenic acyclic ketones(2-undecanone and 2-tridecanone) and low of undec-10-en-1-al and p-phellandren-8-ol,and significant amounts of oxygenated monoterpenes(1,8-cineole, linalool, terpinen-4-ol, and alpha-terpineol) and sesquiterpene hydrocarbons (trans-caryophyllene, a-humulene and germacrene D). Kim (2012) analyzed essential oil in *Z.piperitum* by GC-MS in both leaves , he revealed that the amount of monoterpenes at different developmental stages were significant, the major compounds were β-phellandrene, citronella , β-myrcene, α-pinene , trans-caryophyllene, and fanesyl acetate. Fujita *et al.*, (2017) stated that volatile terpenes of Japanese pepper (*Z. piperitum*) accumulated inside the secretory cavities of leaves, the sesquiterpenes β-caryophyllene and germacrene- D and the monoterpene β-phellandrene were the major.

Table 4. Effect of NPK and Actosol and their interactions on the essential oil constituents of *Zanthoxylum piperitum* plant during 2019 and 2020 seasons.

Concentration %	Farnesyl acetate	α-pinene	Sabinene	1,8-cineole	Linalool	β-Citronellol	2-Undecanone	Citronella	α-Humulene	β-Phellandrene	β-Myrcene	α-Terpineol	Cyclohexanol	Nerolidol	Citronellyl acetate	Germacrene-D	Geranyl acetate	Trans-caryophyllene	2-Tridecane	2-Pentedecanone	Total identified
2019																					
T1*	1.1	5.5	1.7	2.9	1.4	1.5	1.3	1.5	0.8	19.2	1.8	1.9	11.6	2.3	1.4	9.6	2.1	1.4	3.0	2.1	74.1
T2	1.6	6.0	1.4	3.3	1.4	1.9	1.3	1.6	1.1	21.6	1.9	1.9	13.7	2.3	1.7	12.6	3.4	1.8	3.2	2.9	86.6
T3	0.7	5.6	0.6	3.7	0.5	1.0	0.5	0.7	0.2	20.7	1.1	1.0	12.8	1.4	0.8	11.7	2.3	0.9	2.7	2.0	70.9
T4	2.2	6.7	2.1	3.5	2.0	3.2	2.0	2.2	1.8	17.2	2.4	2.4	11.3	2.7	2.2	10.4	3.4	2.3	3.9	3.2	87.1
T5	1.5	6.1	1.4	3.6	1.3	2.0	1.4	1.3	1.2	21.6	2.1	1.8	13.8	2.1	1.8	12.6	3.7	1.8	3.3	3.02	87.4
T6	1.9	6.0	2.5	3.2	2.2	2.6	2.1	2.2	1.6	18.3	2.6	2.6	11.4	3.0	2.1	9.6	2.8	2.2	2.0	1.8	82.7
T7	0.9	5.7	0.8	3.0	0.8	1.3	0.7	0.9	0.4	20.9	1.3	1.3	13.0	1.7	1.0	11.9	2.6	1.2	2.9	2.2	74.5
T8	1.3	5.9	1.2	3.1	1.2	1.7	1.1	1.3	0.8	21.3	1.7	1.7	13.4	2.1	1.4	12.3	3.3	2.4	3.0	2.6	82.8
T9	2.6	7.6	2.6	4.0	2.5	5.0	2.5	2.6	2.4	13.8	2.8	2.8	9.4	3.1	2.7	8.8	3.5	2.8	6.8	2.3	90.6
2020																					
T1	1.5	4.6	2.0	2.3	2.9	1.9	1.8	1.2	1.2	15.1	2.0	2.4	12.6	2.1	1.8	10.0	2.1	1.7	3.2	2.5	74.9
T2	1.5	4.8	1.9	2.4	2.7	1.9	2.0	2.0	2.1	20.3	1.3	2.3	11.3	2.5	2.9	10.7	2.2	2.5	2.5	3.2	83.0
T3	3.1	5.3	1.7	2.7	1.7	1.5	2.0	1.6	1.8	20.1	1.2	2.0	12.8	2.0	2.0	11.8	1.8	3.2	2.1	3.7	84.1
T4	3.4	4.1	2.2	2.1	2.9	2.2	2.3	2.3	2.4	16.8	1.8	2.6	9.7	2.7	3.0	9.0	2.4	2.7	3.6	3.5	81.7
T5	1.1	4.1	2.9	2.1	2.9	2.8	3.1	2.8	2.9	14.2	2.6	3.1	9.7	3.1	3.1	9.1	2.9	3.8	5.3	2.0	83.6
T6	1.9	4.9	1.7	2.4	4.2	3.2	2.0	1.6	1.8	18.3	1.3	2.0	11.8	2.0	2.0	10.8	1.8	3.0	2.3	2.9	81.9
T7	1.0	4.7	2.0	2.3	4.5	3.5	2.0	1.7	2.0	14.8	1.3	2.3	13.3	2.3	2.7	10.6	2.3	2.4	2.9	3.1	81.7
T8	1.3	5.2	1.1	2.6	2.1	1.2	1.3	1.3	1.4	23.1	0.5	1.6	12.3	1.8	2.3	11.3	1.5	1.8	1.3	3.6	78.6
T9	2.0	5.4	2.2	2.6	2.0	2.2	2.2	1.7	1.4	21.8	2.5	2.6	12.1	3.8	1.7	10.0	1.6	2.0	6.7	3.4	89.9

* **T1**: NPK 12g /plant , **T2**: NPK 12g/plant+ Actosol 1.5 ml/L , **T3**: NPK 12g/plant+ Actosol 2.5 ml/L , **T4**: NPK 9g/plant, **T5**: NPK 9g/plant+ Actosol 1.5 ml/L , **T6**: NPK 9g/plant+ Actosol 2.5 ml/L , **T7**: NPK 6g/plant, **T8**: NPK 6g/plant+ Actosol 1.5 ml/L and **T9**: NPK 6g/plant+ Actosol 2.5 ml/L

3.4. Chemical constituents:

3.4.1.Total chlorophylls and total carbohydrates :

Data illustrated in Fig. (3) show that the application of NPK had a slight effect on the content of total chlorophylls of *Z. piperitum* , in both seasons. But,a positive effect had been realized as the combination between 6g/plant NPK and 2.5ml/L Actosol resulting in the highest value (57.6 and 58.9 spad unit) in the two seasons, respectively. There was a marked increase in chlorophyll content with the increase in Actosol level. This result agreed with Hassan *et al.,* (2015) on *Ocimum basilicum* L. who stated that biofertilizer at 4 and 6 g /l (ADY),with NPK greatly chlorophyll content. El-Khateeb *et al.,* (2017) on *Majorana hortensis* obtained the highest content with humic acid (2.5 ml /l). Ahmed (2017) on celery and dill plants, revealed that bio fertilizer + 50% NPK significantly increased total chlorophylls. Shehata (2019) on parsley recommended the treatment of 200 mg/l humic acid + 50 % of recommended NPK for growth and chlorophyll content.

The response of total carbohydrates content to the application of NPK and Actosol (Fig .4), revealed that, the carbohydrates content didn't differ markedly among the different NPK doses, but NPK 75% (9g/ plant) showed a significant increase in the second season. Moreover, the plants treated with NPK at 9g/ plant gave a slight increase in carbohydrates content. Masciandaro *et al.*, (2002) attributed this effect to its role in enhancing the uptake of nutrients, oxygen uptake, respiration and photosynthesis. Khalid and Shedeed (2015) reported that NPK at the high level had a positive total carbohydrate in *Nigella sativa* plant. Also, Abdelaziz *et al.*, (2007) indicated that bio-fertilizers could replace conventional NPK fertilizers in rosemary cultivation with a significant increase in carbohydrate content. Ghatas (2020) indicated that NPK and bio-fertilization significantly increased the chemical constituents. Abou- El Salehein etal (2021) found that application of NPK fertilization reflected a significant difference on chemical contents of herb plants total carbohydrates and pigments (chlorophyll a, b, a+ b, and carotenoids) of *Ocimum basilicum* L ,.El-Sayed *et al.,* (2021) on *Camelina sativa* reported that the contents of chlorophyll and total carbohydrates increased by the use of 75%NPK + Bio.fertilizer.

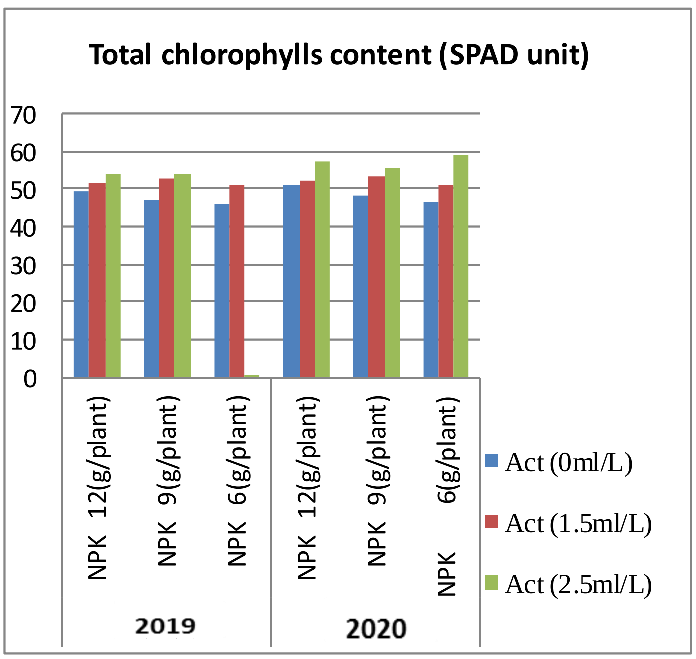


Fig.3

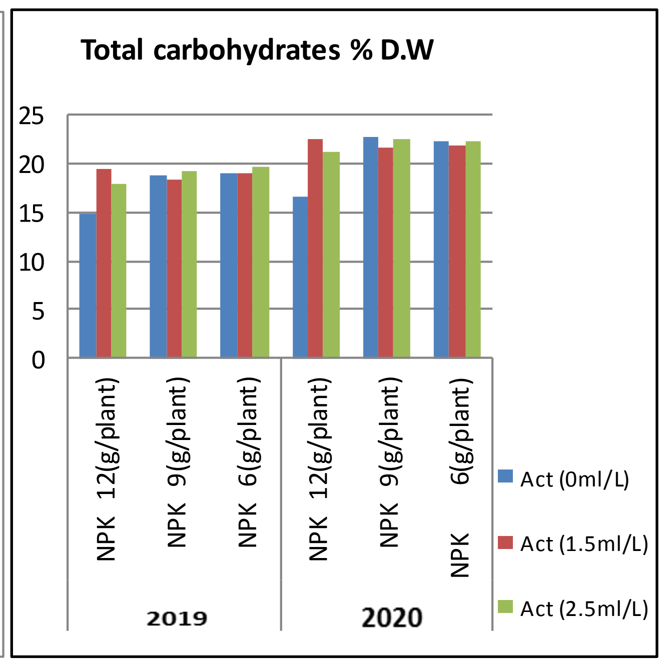


Fig.4

Fig3&4.The response of total chlorophylls (spad uint) and total carbohydrates(%DW) in leaves of *Z. piperitum* plant to NPK and Actosol treatments during 2019 and 2020 seasons.

3.4.2. Total phenolic and flavonoid contents:

The total phenolic contents in *Z. piperitum* leaves of the treated plants Fig. (5) were measured according to the Folin-Ciocalteu method. The determined values, in the first season, ranged from 4.45 mg GAEg-1 DW in plants treated with NPK12 g/plant +Actosol 1.5ml/l to 7.94 mg GAE g-1 DW with the treatment of NPK 9 g/plant +Actosol 1.5ml/l, whereas in the second season, it ranges between 4.70 and 8.45 for the plants treated with NPK12 g/plant +Actosol 1.5ml/l and NPK6 g/plant +Actosol 1.5ml/l, respectively. In both seasons, decreasing the dose of NPK from 12g/plant to 9 or 6 g /plant markedly increased the total phenolic contents in leaves .The same trend was also recorded with the increase in Actosol concentration. This indicating that plants treated with NPK 9g/plant + Actosol at 1.5 or 0 ml/L or NPK at 6 g/plant+1.5 ml/L Actosol was highly recommended for increasing the contents of phenolic compounds .

The obtained data Fig. (6) regarding the response of total flavonoid content to the application of NPK and Actosol, showed that the values ranged from 1.75 to 4.74 mg/g dry weight (Quercetin equivalents),in the first season and from 1.91 to 4.41 mg/g dry weight (Quercetin equivalents),in the second season. The highest value in the first season (4.74mg) was determined in the plants treated with NPK 9gm/plant + Actosol 1.5 ml/L, followed by the treatment of NPK 6g/plant + Actosol 1.5 ml/L. The highest value in the second one (4.41) was determined in the plants treated with NPK 9g/plant ,followed by the treatment of NPK6 g/plant + Actosol 1.5 ml/L. It is worthy to mention that the treating plants with NPK(6g/plant) + Actosol (1.5 ml/L) seemed to increase the medicinal activity and the contents of phenolic and flavonoid contents in this plant. In addition, represent a rich source of naturally occurring flavonoids

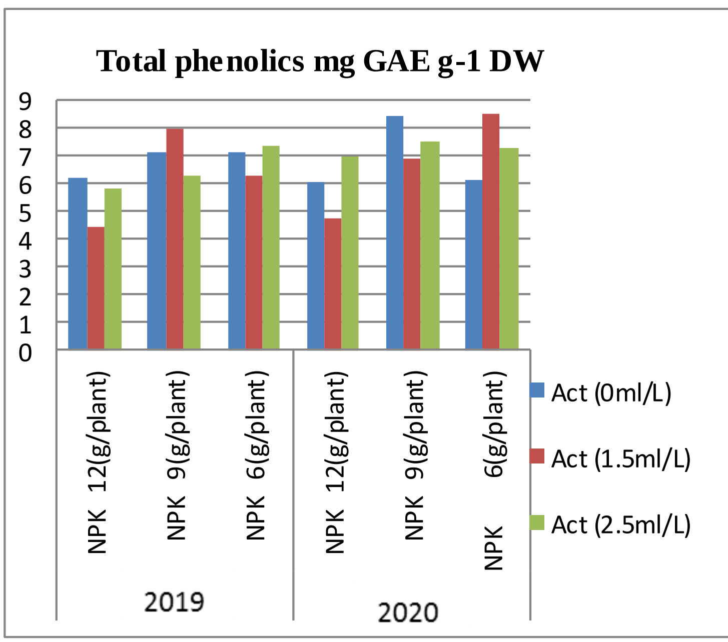


Fig.5

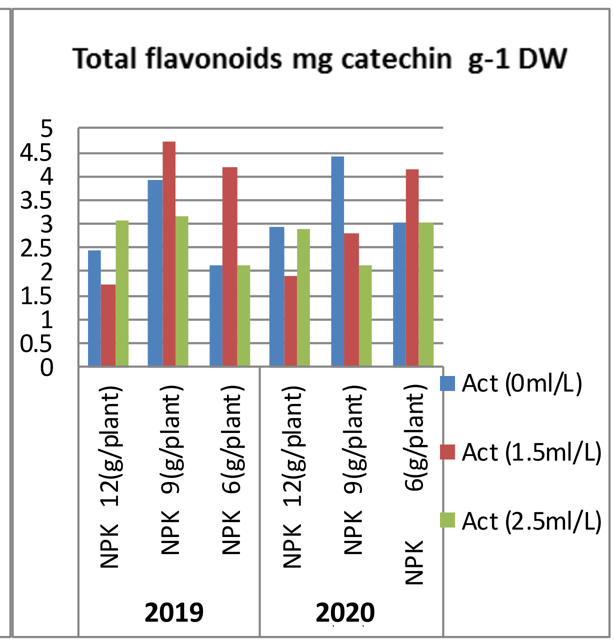


Fig.6

Fig.5&6 .Effect of NPK and Actosol and their interactions on contents of total phenolic and total flavonoid in leaves of *Z. piperitum* plant during two seasons 2019and 2020.

3.4.3. Antioxidant activity:

In both seasons, the methanolic extracts of *Z. piperitum* leaves from the different treatments were prepared to evaluate their antioxidant activity by DPPH method. The IC50 values (a midpoint of 50% between zero and full inhibition of diazo dye formation) of superoxide (O2–)-scavenging activity. The determined values , in the first season , ranged from 3.04 to 13.40, and from 3.85 to 13.45(µg/ml methanol Extract) in the second one. Data in Fig. (7) revealed a great variation in antioxidants scavenging activities against DPPH assay. The crude extract was able to scavenge 50% of both DPPH radicals ranging from 3.04 to 13.40 in the first season, as the plants received 12g/plant N PK showed the highest IC50, while that treated with 6 g/plant NPK + Actosol 1.5 ml/L exerted the lowest IC50values (3.04) ,this was the case in the first season. The crude extract in the second season, was able to scavenge 50% of both DPPH radicals ranging from 3.85 to 13.45 (µg/ml methanol Extract) , as the plants received 12gm/plant NPK showed the highest IC50, while that treated with 6 gm/plant NPK + Actosol 1.5 ml/L exerted the lowest IC50value (3.85).

In this regared, Pandey *et al.*, (2015) found that organic amendments in combination with chemical fertilizer significantly improved the antioxidant capacity of the marigold leaf extracts(DPPH activity) than the control . Also, Sheikh and Ishak (2016) showing that nitrogen application improves antioxidant activity. Bistgani, *et al.*, (2018) reported that the application of combined fertilizers (NPK +CM+VC) significantly increased the biomass and improved the antioxidant properties.

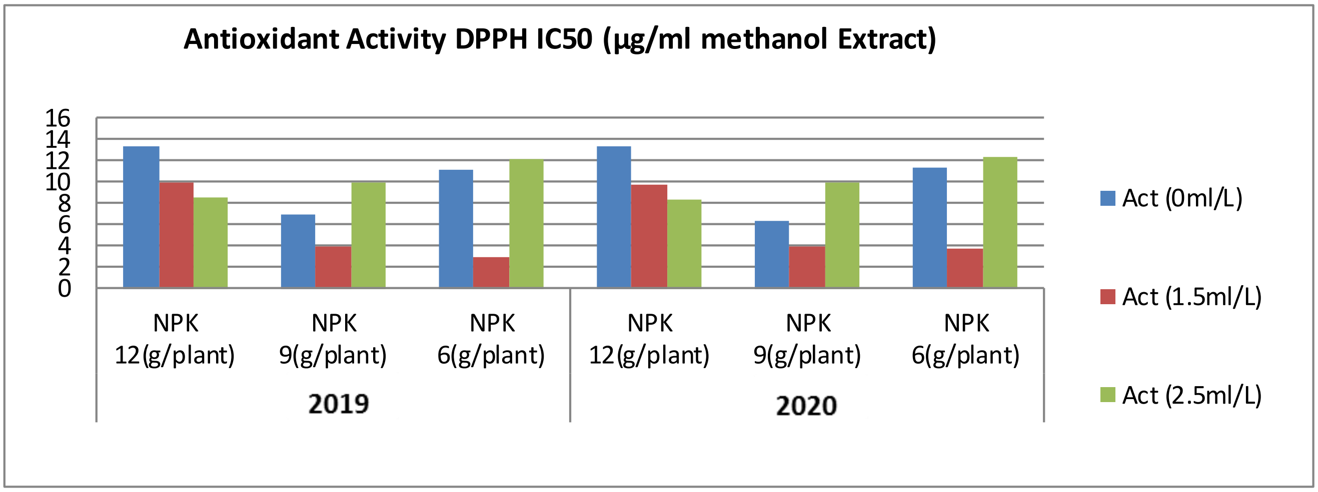


Fig.7
GAE DPPH IC_{50}

Fig.7. Effect of NPK and Actosol and their interactions on the antioxidant activity in the methanolic extracts of *Z. piperitum* leaves during two seasons 2019and 2020.

3.4.4. N, P and K contents:

The contents of N,P and K in leaves slightly differed between the different NPK dose in two growth seasons. Fig (8). The N-content (%) in the leaves of plants treated with the low dose of NPK and sprayed with Actosol at 1.5ml/l. was the highest, in both seasons.(3.05 and 3.07 % D.W),which followed by the treatment of NPK at 9g/plant without Actosol ,in the first season , and the treatment of NPK at 12g/plant + Actosol at 2.5ml/l, in the second one. Also, the plants treated with 6g/plant NPK +2.5ml/L Actosol contained higher P % in the first season as compared with the second one, giving 0.14 and 0.3 % D.W. However, there was a pronounced reduction in potassium content in plants treated with 9g/plant NPK +1.5ml/l Actosol. . Enhancement in the antioxidant activity may be due to an overall promoting effect of organic and NPK regimes on plant metabolic activities, as humic substances improves water retention, enhances chelating of plant nutrients and stimulates root mass and plant growth (Masciandaro *et al.*, 2002). In addition, Pouneva (2005) and Burkowska and Donderski (2007) confirmed the role of humic substances in enhancing the uptake of nutrients and photosynthesis that has a positive effect on plant growth. Abdelaziz *et al.*, (2007) indicated that biofertilizers could replace conventional NPK fertilizers in rosemary cultivation with a significant increase in growth, total N, P ,carbohydrate content. Samy, *et al.*, (2015) stated that the foliar application of 0.5 and 1 g/l humic acid significantly affected the chemical composition (N, P, K, Total carbohydrates and inulin contents) of Jerusalem artichoke . than control treatment. On *Thymus kotschyanus* ,Saber *et al.*, (2021) stated that humic acid had a positive effect increasing nutrient uptake due to its effect on root growth.

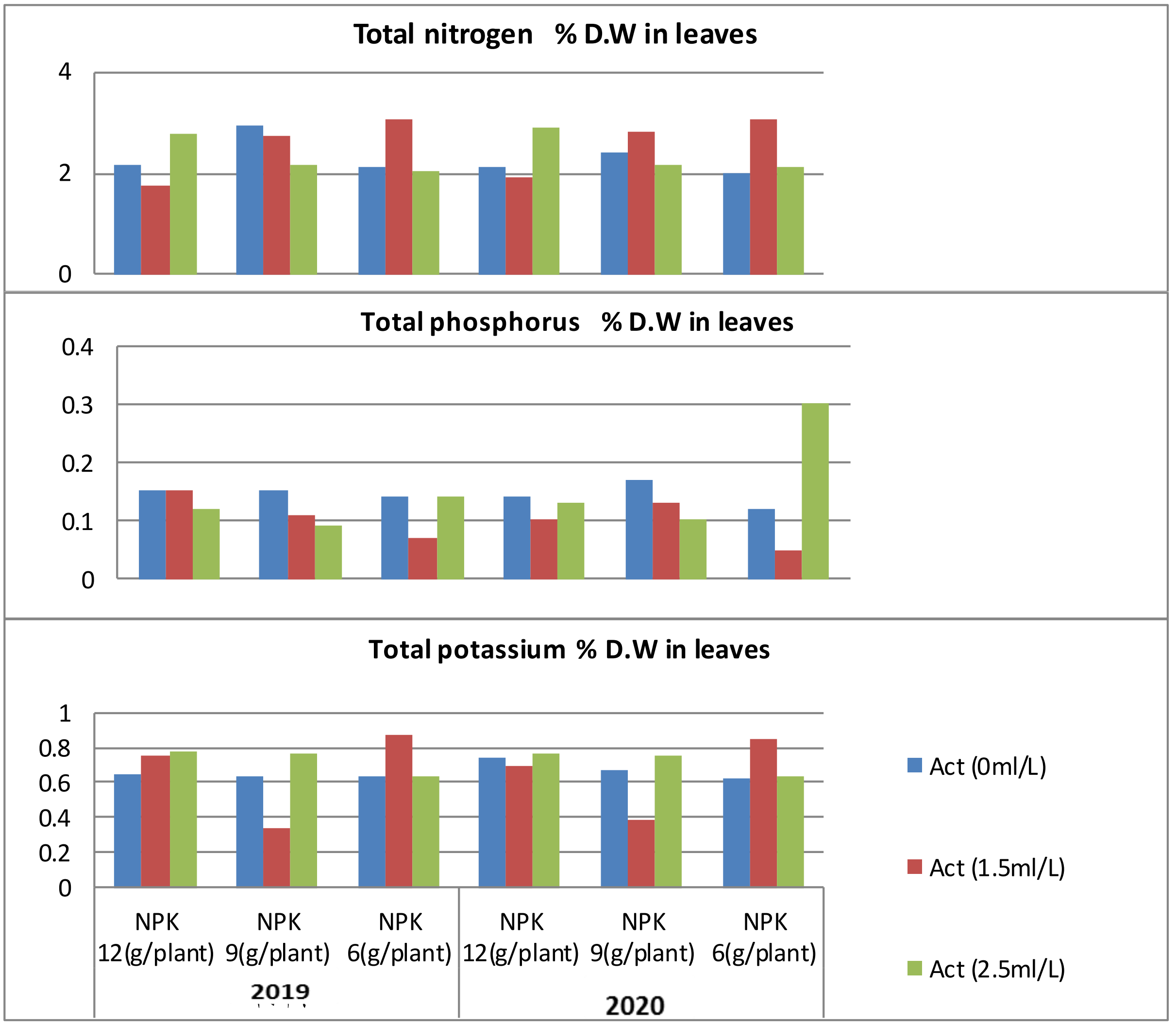


Fig.8. Effect of NPK and Actosol and their interactions on contents of N,P and K (%DW) in leaves of *Z. piperitum* plant during two seasons 2019and 2020.

4 .CONCLUSION

We could conclude that, *Zanthoxylum piperitum* treated with NPK at 6 and 9 g/plant + Actosol 2.5 ml/L in both seasons affected significantly on improving the plant growth, contents of phytochemicals and essential oil production. Also we cannot exclude that other treatments were found to improve plant behavior, but it was only in one direction. Actosol at 1.5 ml/l gave the highest oil %,while 2.5 ml/l gave the highest oil yield/plant. Howevre, *Zanthoxylum piperitum* plants shows great ability to grow under the Egyptian conditions which will open series of under studies on their bioactive derived compounds in the pharmaceutical industries.

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الملخص العربى

تاثير NPK , Actosol على النمو وانتاج الزيت العطري و التركيب الكيميائي لنبات الزانثوكسيلم

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اجريت تجربة اصص علي نبات *Zanthoxylum piperitum* بهدف دراسة تاثيرالتسميد ب NPK والاكتوسول علي النمو الخضري ،و الزيت الطيار ،والمحتوي الكيماوي ومضادات الاكسدة داخل الصوبة الساران بالمشتل التجريبى – قسم بساتين الزينة – كلية الزراعة –جامعة القاهرة– خلال عامي ٢٠١٨ / ٢٠١٩– ٢٠١٩ /٢٠٢٠. وقد سمدت النباتات ب NPK بمعدل ١٢ جم / نبات(١٠٠% جرعة موصي بها)، ٩و٦ جم /نبات اضافة للتربة وتم رش النباتات بالمنشط الحيوي اكتوسول بتركيزات صفر و ٥و١ و ٥و٢ مل/ لتر واظهرت النتائج ان التسميد بـ NPK ٦ او ٩ جم /نبات مع الرش بالاكتوسول ٥و٢ مل /لتر اعطي اطول النباتات مع اكبر عدد من الافرع علي النبات كما ادي الي زيادة الاوزان الطازجة والجافة معنويا في كلا الموسمين واوضحت النتائج ان خفض معدل التسميد من ١٢ الي ٩ او٦ جم /نبات ادي الي زيادة معنوي في النسبة المئوية للزيت الطيار خلال موسمي الدراسة ومع رش نباتات الزانثوكسيلم بالاكتوسول ٥و١ مل /لتر ادي زيادة النسبة المئوية للزيت– بينما الرش بالاكتوسول ٥و٢ مل /لتر ادي الي زيادة انتاج الزيت الطيار للنبات وقد تم التعرف علي حوالي ٢٠ مركب من مكونات الزيت بـ GC–MS واهم هذة المركبات (بيتا–فيلاندرين ، سيكلوهكسانول ، جرماكرين– د ، الفا– بينين ،أسيتات جيرانيل ثم ١,٨ – سينول) عند التسميد ب NPK ١٢، ٩، ٦ جم /نبات مع رش الاكتوسول بتركيز ٥و١ مل/لتر في الموسم الاول .كما وجد ان المعاملة بالمعدل المنخفض من ال NPK مع المعدل المرتفع من الاكتوسول ادي الي زيادة النسبة المئوية للمركبات الفينولية بينما محتوي المواد الفلافونيدية حقق اعلي نسبة مئوية مع المعاملة NPK ٩ جم/نبات + ٥و١ مل /لتر اكتوسول في الموسم الاول ومع المعاملةNPK بـ ٩ جم /نبات في الموسم الثاني. واوضحت النتائج التاثير المعنوي لهذه المعاملات علي قيمة مضادات الاكسدة لمستخلص الاوراق للنبات خاصة المعاملة NPK بمعدل ١٢ جم /نبات اعطت اعلى القيم في الموسمين.