

## **Stimulatory Effects of Tryptophan or Indole Acetic Acid and *Arbuscular mycorrhizae* on Growth, Yield, Some Chemical Composition and Nutritional Quality of Quinoa Plants Grown in Sandy Soil**

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**Abstract:** This study was implemented at the Station of Agricultural Production and Research, National Research Centre (NRC), Nubaria district El-Behira Governorate, Egypt during two successive winter seasons 2019/2020 and 2020/2021. The effects of *arbuscular mycorrhizae* (AM) application with different concentrations of L-tryptophan and indole acetic acid (IAA) on some morphological parameters, biochemical estimations and seed yield quantity and quality of quinoa plant grown under sandy soil conditions were studied. In general, results proved that all growth criteria (shoot and root lengths, shoot fresh and dry weights and root fresh & dry weights) increased due to the foliar applications of tryptophan (50, 75 & 100 mg/l) treatments and (100, 200 & 300 mg/l) of IAA. Seed yield and its attributes associated with improve in the levels of studied physiological parameters (photosynthetic pigments, total soluble sugar, total carbohydrates, IAA, phenol and free amino acids). In addition, tryptophan and IAA at all studied levels increased significantly the nutritional values of the yielded seed (carbohydrates %, proteins %, oil % macro and micronutrients, total flavonoids, total phenols and antioxidant activity). Application of AM to the soil generally increased significantly all the above mentioned estimations of quinoa plant. The combination between tryptophan or IAA and AM was more valuable and induced pronounced promotions in all studied estimations as compared to control plants. So, using tryptophan or IAA in the presence of mycorrhizae stimulated the nutritional values of the produced seeds of quinoa grown under unfavorable sandy soil conditions.

**Key words:** *Arbuscular mycorrhizae* • Nutritional values • Tryptophan • IAA • Quinoa • Yield

### **INTRODUCTION**

In the least development countries, the over population and their requirements leads to the call for consummate agricultural production system to confront their requirements of food. Since agriculture take into accounts the principle pivotal activity of the economy of such countries. However, alterations occurred in the environmental conditions instigate a challenge to the agriculture yield, so that evolving any agricultural system of the country is a must to confront its population requirements.

Quinoa is a very riveting food because it's inveterate nutritional quality. The seeds are starchy dicotyledonous, so it's not a cereal and known as a pseudo cereal [1]. Quinoa seeds have been arousing recognition due to the quality and nutritional value of its proteins [2]. The seed is rich in lysine as essential amino acid, that's inventing it excess of protein than many other vegetables [1]. Also, it does not contain gluten, so it can be safe to celiac disease people and people who are allergic to wheat. Moreover, the oil fraction of the seeds has high quality and highly nutritious. Their oil is intense in Fe, Mg, fiber, vitamin E, Cu, P, some B vitamins, K and Zn. The seeds

have an outer layer contains saponins, which are toxic compounds and give the bitter tasting for Quinoa. The seeds also contain high nutritional quality, carbohydrates (77.6%), protein (12.9%), a balanced amino acid spectrum with high lysine and methionine contents and lipids (6.5%). In addition seeds are rich in dietary fibers and minerals, so, it attract the attention all over the world [3].

AM (*Arbuscular mycorrhizae*) are important fungi in sustainable agriculture due to their beneficial role in improving plant water relations consequently increase the resistance of host plant to drought stress. They also promote disease defenses and promote mineral uptake via increasing amassing of phosphorus and other low mobile mineral nutrients, that's parallel to minimize the application of fertilizers. Mycorrhizae can crash down some complex minerals and organic compounds in the soil to be obtainable to their hosts [4]. The fungi stimulate the addition of water and nutrients, such as N and P to the host plant via extra radical and intra radical hyphae.

MF have been shown to promote plant growth and stress through several mechanisms, such as promoting nutrient acquisition [5] synthesis of plant growth hormones, stimulating rhizospheric and soil conditions [6] ameliorating the physiological and biochemical attributes of the host and standing up roots against soil-borne pathogens [7].

Moreover, AMF can promote the physiological processes of the host plants as water absorption capacity of plants via stimulating root hydraulic conductivity and controlling the osmotic equilibrium and carbohydrate contents [8]. In this regard, [9] demonstrated that *Arbuscular mycorrhizal* fungi mechanisms employ to enhance the nutrient equilibrium (Ca, P, N and Mg), preservation of the  $K^+ : Na^+$  ratio, biochemical variations (assembling of antioxidants, betaines, carbohydrates, polyamines and proline), physiological modifications (abscisic acid accumulation, photosynthetic efficiency, relative permeability, nodulation, nitrogen fixation and water status).

Auxins hormone in plants is found mostly as indole-3-acetic acid (IAA) which manufactured regularly in the shoot apex, buds and young leaves of plants. Also, the meristematic tissues, flowers, fruits and young seeds are the sites of this hormone production. IAA has several biovital roles on most physiological processes (apical dominance, cell division, cell elongation, differentiation of vascular tissues, flowering, fruit setting, geotropism, photosynthetic activities, root initiation, ripening, senescence and translocation of [10, 11].

L-tryptophan is an amino acid and considered the main precursor of IAA. It is naturally occurring in the plant roots, synthesized by hydrolysis of proteins of dead cells and converted by the rhizo bacteria into IAA, [12]; [13]. External application of L-tryptophan to plant improved growth of many crops like chickpea [14] and wheat [15]. It also promotes the synthesis of auxins, consequently improved plant growth, division and development [16]. Moreover, tryptophan retards the prematurity flower and fruit falls. So, it is necessary in the production of catalyses' synthesis enzyme that involved in reaction of auxin production [17].

Therefore, the target of this study is to investigate the impact of foliar treatment of IAA and its precursor tryptophan with different concentrations in absence and presence of mycorrhizae on vegetative growth, some metabolic activities and yield, of quinoa plants grown under the circumstance of sandy soil.

## MATERIALS AND METHODS

A field experiment was dispatched at the Experimental Station of National Research Centre, Nubaria district, Beheira Governorate, Egypt, during two winter season of 2019/2020 and 2020/2021. The mechanical and chemical analysis of soil at the experimental site at newly reclaimed sandy soil where is reported in Table 1 according to [18].

The experimental design was split plot design with four replications, where *Arbuscular mycorrhizae* (AM) levels of (0.0 and 1.0 kg/feddan) were in main plots, tryptophan at rates of (0.0, 50, 75 & 100 mg/l) and IAA (0.00, 100, 200 & 300 mg/l) treatments allocated in sub plots. Seeds of quinoa (*Chenopodium quinoa* Masr-1.) variety were picked up from Agricultural Research Centre Giza, Egypt. Quinoa seeds were sown on 25 November in both seasons at the rate of 3 kg/feddan (one feddan = 0.42 ha) in rows 3.5 meters long and the distance between rows was 25 cm apart and 15-cm between hills. The area of the plot was 10.5 m<sup>2</sup> (3.0 m in width and 3.5 m in length). The agricultural practices of growing quinoa were applied as recommended. Fertilization occurs as pre-sowing, 150 kg/feddan of calcium super-phosphate (15.5% P<sub>2</sub>O<sub>5</sub>) was added to the soil. Nitrogen was added after emergence in the form of ammonium nitrate 33.5% at a rate of 75 kg/feddan. Potassium sulfate (48.52 % K<sub>2</sub>O) applied in two equal doses of 50 kg/feddan, before the 1<sup>st</sup> and 3<sup>rd</sup> irrigations. Irrigation was conducted out using the new sprinkler irrigation system where water was added every

Table 1: Mechanical, chemical and nutritional analysis of the experimental soil

Season	Constant Depth (cm)	Coarse sand %	Fine sand %	Silt %	Clay %	Texture class
2020	00 – 30	40.7	44.6	10.7	4	sandy
	30 – 60	38.2	43	13.8	5	sandy
2021	00 – 30	38.7	42.6	13.7	5	sandy
	30 – 60	36.5	38.1	17.8	7.6	sandy

Season	Constant Depth (cm)	Electrical conductivity (ds/m)	Saturation percentage	Anions (milliequivalents/ liter)				Cations (milliequivalents/ liter)			Organic Mater %		
				HCO <sub>3</sub> <sup>-</sup>	Cl	SO <sub>4</sub> <sup>=</sup>	Ca <sup>++</sup>	Mg <sup>++</sup>	Na <sup>+</sup>	K <sup>+</sup>		CaCO <sub>3</sub> %	
2019/	00 – 30	7.84	1.17	32	0.50	8.40	1.11	1.80	0.90	7.10	0.20	1.00	0.40
2020	30 – 60	7.89	1.79	27	0.60	8.00	1.40	2.10	1.50	6.20	0.20	1.60	0.07
2020/	00 – 30	7.95	1.59	23	0.32	12.70	1.98	4.00	1.80	9.00	0.20	1.90	0.38
2021	30 – 60	7.85	1.81	25	0.45	15.40	2.15	5.60	2.00	10.20	0.20	1.30	0.32

5 days regularly. Treatments of tryptophan (50, 75 & 100 mg/l) and indole acetic acid (100, 200 & 300 mg/l), were applied twice; sprayed after 30 and 45 days from sowing. Plant samples were collected after 60 days from sowing for estimation of some growth parameters as plant height (cm), fresh & dry weight of shoot/plant (g), root length (cm), fresh & dry weight of root/plant (g), photosynthetic pigments of leaves, endogenous indole acetic acid (IAA), phenolics and total soluble sugars, Oven- dried leaves (for 72 h at 70°C) were ground to a powder and kept in a desiccators to later estimation of proline and free amino acids. Yield and its attributes as plant height (cm), biological yield/ plant (g), seed yield/plant, straw yield/ plant (g), biological yield (ton/ha), seed yield (kg/ha) and oil yield (kg/ha) as well as nutritive value of the seed yield as total carbohydrates%, protein%, flavonoids content, phenolic contents, oil%, phosphorus and potassium contents, in addition to antioxidant activities %.

**Biochemical Analysis:** Photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined according to [19]. Endogenous indole acetic acid contents were determined as expressed by [20]. Total phenolic contents will be estimated as expressed by [21].

Total sugar and total carbohydrate concentrations were determined by using the phenol sulphuric acid method regarding to [22]. Free amino acids contents were estimated with the nin-hydrin reagent method [23]. Total flavonoids were estimated using the method disused by [24]. The antioxidant activity (DPPH radical scavenging) was estimated using the method of [25]. Element contents (N, P and K) contents of the seed yield. Minerals content of P, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup> and N were estimated regarding to the method illustrated by [18]. N and P were examined using Spekol Spectrocolourimeter Carl Zeiss. While, Ca and K contents were examined by the use of flame photometer and Mg<sup>2+</sup> was estimated

using atomic absorption spectrophotometer. Protein content was calculated by multiplying N% x6.25. The oil content of the seeds was determined according to the procedure illustrated by [26].

**Statistical Analysis:** The data were statistically analyzed as split plot design. The analysis of variance of data was calculated according to method illustrated by [27] since the trend was similar in both seasons the homogeneity test Bartlet's equation was applied and the combined analysis of the two seasons was done. Means were compared by using least significant difference (LSD) at 5%.

## RESULTS

**Changes in Morphological Parameters:** Table (2) showed that, cultivation of quinoa plants in soil amended with *Arbuscular mycorrhizae* (AM) increased significantly all the studied morphological criteria (shoot length cm, fresh & dry wt. of shoot, root length, fresh and dry weight of root as compared with plants grown without adding AM. Also, data in Table (2) revealed that, foliar treatment of L- tryptophan or IAA with different concentrations increased significantly all the tested morphological criteria in the absence or presence of AM. The most marked increase in all growth parameters was recorded at 300 mg/l IAA foliar treated plants grown in soil revamped with AM as compared with all other treatments.

**Changes in Photosynthetic Pigments:** The results in Table (3) revealed that cultivation of plants under the application of AM induced significant increases in all photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoid and consequently total pigments contents) at all tested treatments as compared with the corresponding plants grown without AM.

Table 2: Effect of inoculation with *Arbuscular mycorrhizae* AM and foliar application with tryptophan and IAA on morphological criteria of quinoa plants grown under sandy soil conditions. (Combined data of two seasons)

Mycorrhizae	Treatments (mg/l)	Shoot length (cm)	Fresh Wt. (g)	Dry Wt. (g)	Root length (cm)	Root Fresh Wt. (g)	Root Dry Wt. (g)	
Without	Control	20.40	21.60	3.57	16.15	1.29	0.29	
	Tryptophan	50	28.87	45.52	9.84	18.05	2.38	0.79
		75	37.03	54.36	13.45	18.53	3.70	1.74
		100	44.34	62.35	16.41	18.60	5.12	1.85
	IAA	100	42.95	60.25	11.03	16.63	3.14	2.39
		200	49.64	69.79	13.17	17.45	3.99	2.72
		300	53.55	75.99	18.39	18.95	5.34	3.00
With	Control	25.43	42.55	4.31	17.00	1.49	0.34	
	Tryptophan	50	42.50	68.16	12.62	19.00	4.18	1.67
		75	45.98	69.37	15.80	19.44	4.71	2.77
		100	54.00	82.24	17.16	19.74	6.76	3.28
	IAA	100	45.98	66.75	14.31	19.00	5.29	2.85
		200	56.43	79.58	15.69	19.25	5.99	3.32
		300	58.18	82.87	19.26	20.12	6.75	3.84
LSD at 5%		0.49	0.65	0.20	0.40	0.19	0.04	

Table 3: Effect of inoculation with *Arbuscular mycorrhizae* AM and foliar application with tryptophan and IAA on photosynthetic pigments (Chlo a, Chlo b, carotenoids and total pigments (mg/100g fresh weight) of quinoa plant grown under sandy soil conditions.(Combined data of two seasons)

Mycorrhizae	Treatments (mg/l)	Chlorophyll a	Chlorophyll b	Carotenoids	Total pigments	
Without	Control	758.30	226.20	193.90	1178.40	
	Tryptophan	50	856.70	271.10	231.80	1359.60
		75	938.80	283.60	275.00	1497.40
		100	992.00	298.70	328.30	1619.00
	IAA	100	953.90	247.70	317.80	1519.40
		200	1063.00	266.40	349.90	1679.30
		300	1160.80	283.30	365.60	1809.70
With	Control	828.80	260.90	292.50	1342.20	
	Tryptophan	50	909.60	285.00	324.30	1518.90
		75	1092.70	294.10	398.20	1785.00
		100	1397.90	308.70	418.10	2124.70
	IAA	100	1092.70	294.20	398.20	1785.10
		200	1290.30	305.60	426.80	2022.70
		300	1498.20	343.00	463.90	2305.10
LSD at 5%		58.65	24.43	19.69	52.42	

Also, Table (3) shows that, foliar spraying of quinoa plants with different concentrations of tryptophan or IAA improved significantly all fractions of photosynthetic pigments in the absence or presence of AM as compared with control plants. The maximum values of the total photosynthetic pigments were obtained by foliar application of 300 mg/l IAA (95.61% over the control treatment) followed by 100mg/l tryptophan by (80.30% )in the presence of AM respectively.

**Changes in Carbohydrates Constituents:** According to carbohydrate contents, Table (4) clearly presents the effect of foliar treatment with different concentrations of tryptophan or IAA on carbohydrates constituents (TSS, total carbohydrates and polysaccharides) of quinoa plant grown under sandy soil condition in the absence and presence of mycorrhizae. Addition of mycorrhizae to sandy soil increased significantly the

studied carbohydrate constituents of quinoa plant grown under sandy soil as compared to those plants grown under sandy soil without mycorrhizae.

Results also revealed the increase in different carbohydrates constituents as a result of treatment with different concentrations of either tryptophan or IAA, without or with application of AM as compared with control plants. These increases were generally gradually with increasing tryptophan or IAA concentrations. The higher values of the TSS were recorded by application of 300 mg/l IAA followed by 100 mg/l tryptophan and in the presence of AM.

**Changes in Endogenous Indole Acetic Acid, Phenolic and Total Free Amino Acids Contents:** The results in Table (5) exhibited the changes in endogenous IAA, phenolic and free amino acids contents in quinoa leaves treated with different concentrations of tryptophan

Table 4: Effect of inoculation with *Arbuscular mycorrhizae* AM and foliar application with tryptophan and IAA on carbohydrates constituents (TSS, polysaccharides and total carbohydrates (mg/g dry wt.), of quinoa leaves under sandy soil conditions. (Combined data of two seasons)

Mycorrhizae	Treatments (mg/l)		TSS	Poly saccharides	Total carbohydrates
Without	Control		13.83	114.69	128.52
	Tryptophan	50	14.00	130.68	130.68
		75	14.17	134.41	134.41
		100	15.25	146.48	146.48
	IAA	100	14.68	132.02	132.02
		200	15.05	139.41	139.42
		300	15.35	147.19	147.19
With	Control		14.09	118.00	132.09
	Tryptophan	50	14.83	145.15	145.15
		75	15.38	154.87	154.87
		100	16.39	163.31	163.31
	IAA	100	15.41	157.99	157.99
		200	16.12	163.28	163.28
		300	17.76	176.57	176.57
LSD at 5%			1.723	16.035	19.082

Table 5: Effect of inoculation with *Arbuscular mycorrhizae* AM and foliar application with tryptophan and IAA on IAA ( $\mu\text{g/g}$  fresh wt.), Phenolics and free amino acids (mg/100 g dry wt.) of leaves of quinoa plant grown under sandy soil conditions. (Combined data of two seasons)

Mycorrhizae	Treatments (mg/l)		IAA	Phenol	Free amino acids
Without	Control		10.25	124.45	266.02
	Tryptophan	50	19.25	139.32	309.92
		75	26.75	151.36	327.67
		100	38.75	163.31	352.42
	IAA	100	24.47	142.69	308.16
		200	31.15	171.02	332.43
		300	39.92	184.34	336.46
With	Control		19.94	139.34	320.25
	Tryptophan	50	30.85	150.31	336.14
		75	41.75	162.35	351.80
		100	54.02	178.32	361.85
	IAA	100	33.25	162.35	347.07
		200	41.01	174.23	355.01
		300	60.30	187.02	365.23
LSD at 5%			1.35	4.36	20.02

or IAA grown in sandy soil without or with the addition of mycorrhizae. Addition of mycorrhizae to sandy soil increased significantly the above mentioned contents of quinoa leaves as compared to those grown without mycorrhizae. External treatment of tryptophan or IAA with different concentrations as foliar application on quinoa enhanced and increased significantly the endogenous IAA, phenolic compounds and free amino acids contents as compared with corresponding control plants without or with inoculated AM. The maximum values were recorded at 300 mg/l IAA followed by 100mg/l Tryptophan with increasing percent up to 5 folds and 4 folds when the plants were treated with IAA, 50.28% and 43.29% at phenol & 37.29% and 36.02% at free amino acids in plants treated with AMF, respectively.

**Changes in Seed Yield and its Components:** The results also showed that, addition of mycorrhizae to sandy soil

increased significantly seed yield and its components compared with those grown in soil without mycorrhizae (Table 6).

There were significant gradual increase in all quinoa seed yield parameters and its components with increasing Tryptophan or IAA concentrations as compared to corresponding control in absence and presence of mycorrhizae. The interaction between mycorrhizae and IAA at rate of 300 mg/l gave the highest value of seed, straw and oil yields/ ha<sup>-1</sup>. These increase due to the significant increase in yield components such as (biological, straw and seed yields/ plant), these values recorded percent of increase reached to 134.48% at seed yield/ha<sup>-1</sup>, 173.63% at straw yield/ ha<sup>-1</sup> and 192.56 % at oil yield/ ha<sup>-1</sup> as compared to the control plants.

**Changes in Nutritional Value of the Seed Yield:** Data in (Table 7, Figs. 1 a and b) show that carbohydrates, protein

Table 6: Effect of inoculation with *Arbuscular mycorrhizae* AM and foliar application with tryptophan and IAA on seed yield, yield components and total carbohydrates of seeds of quinoa plant grown under sandy soil conditions.(Combined data of two seasons)

Mycorrhizae	Treatments (mg/l)	Plant height (cm)	Biological yield/Plant (g)	Seed yield /plant (g)	Straw yield/ plant (g)	Seed yield (kg/ ha)	Straw yield (ton/ha)	Oil yield (kg/ha)	
Without	Control	26.00	6.14	2.25	3.68	589.56	0.986	32.13	
	Tryptophan	50	30.67	6.96	2.46	4.31	649.60	1.737	38.39
		75	36.17	8.96	4.64	4.72	1216.40	1.867	76.03
		100	38.67	12.61	6.85	5.76	1473.40	2.262	87.98
	IAA	100	41.34	6.67	3.19	3.98	839.40	1.887	49.36
		200	44.50	10.04	5.03	5.01	1258.60	1.981	76.85
		300	46.00	10.22	5.24	5.98	1364.35	2.130	89.86
With	Control	33.50	7.77	2.60	3.87	676.41	1.839	38.83	
	Tryptophan	50	44.34	8.59	3.80	4.79	989.53	1.992	58.58
		75	51.17	10.99	5.61	6.38	1298.60	2.374	81.03
		100	52.67	17.17	7.59	8.58	1588.45	2.968	103.41
	IAA	100	40.00	8.85	3.66	5.19	967.60	2.090	61.06
		200	44.17	11.88	5.71	7.17	1337.80	2.475	86.56
		300	59.50	12.59	5.85	6.74	1382.40	2.698	94.00
LSD at 5%	1.11	0.19	0.14	0.21	5.35	0.131	1.45		

Table 7: Effect of inoculation with *Arbuscular mycorrhizae* AM and foliar application with tryptophan and IAA on nutritional values (total carbohydrates, protein and oil%) of seeds of quinoa plant grown under sandy soil conditions. (Combined data of two seasons)

Mycorrhizae	Treatments (mg/l)	Carbohydrates %	Protein %	Oil %	
Without	Control	69.75	13.89	5.45	
	Tryptophan	50	71.85	15.06	5.91
		75	72.97	15.82	6.25
		100	73.92	16.11	6.65
	IAA	100	70.05	14.46	5.88
		200	71.97	15.31	6.09
		300	72.85	16.24	6.44
With	Control	70.80	14.64	5.74	
	Tryptophan	50	71.94	16.88	6.12
		75	73.35	17.31	6.44
		100	74.42	17.78	6.71
	IAA	100	72.25	16.15	6.31
		200	72.92	17.47	6.47
		300	73.69	17.97	6.89
LSD at 5%		0.95	0.62	0.20	

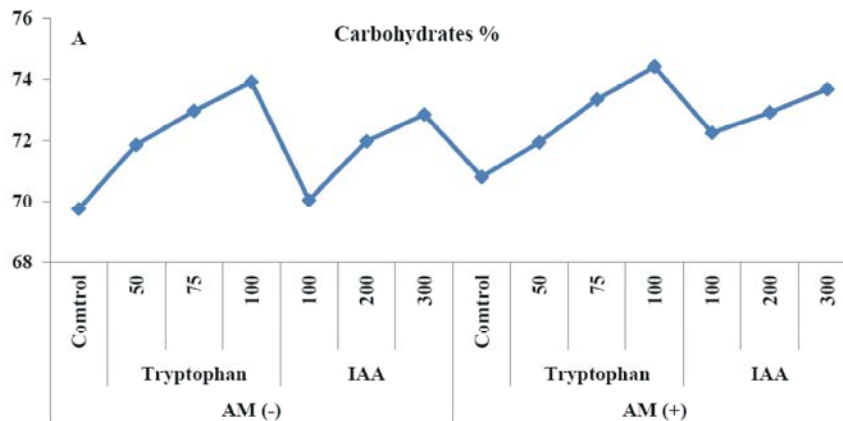


Fig. 1(A): Effect of different concentrations of tryptophan or IAA in absence (-) or presence (+) of AM on Carbohydrates % of quinoa seed under sandy soil conditions

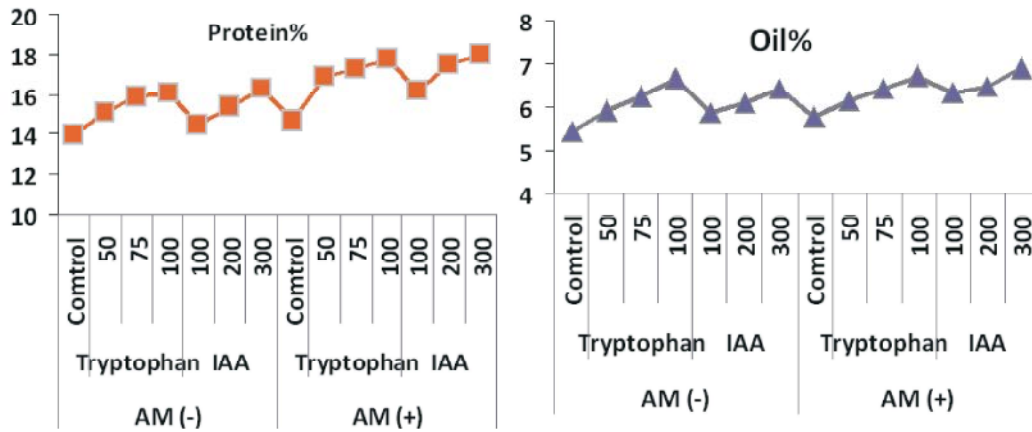


Fig. 1 (B & C): Effect of different concentrations of tryptophan or IAA in absence (-) or presence (+) of AM on protein and oil % of quinoa seeds under sandy soil conditions

Table 8: Effect of inoculation with *Arbuscular mycorrhizae* AM and foliar application with tryptophan and IAA on element contents (mg/100g dry wt.) of seeds of quinoa plant grown under sandy soil conditions.(Combined data of two seasons)

Mycorrhizae	Treatments (mg/l)	N	P	K	Ca	Mg	
Without	Control	2267.00	253.50	376.30	206.50	276.70	
	Tryptophan	50	2357.00	263.40	382.40	222.80	291.50
		75	2462.00	285.40	399.30	247.40	311.40
		100	2609.00	345.00	401.10	255.90	323.70
	IAA	100	2671.00	261.20	382.70	229.50	288.80
		200	2755.00	284.90	402.50	242.40	316.10
		300	2963.00	301.70	420.50	255.80	331.80
With	Control	2420.00	279.50	388.50	237.30	295.20	
	Tryptophan	50	2499.00	291.80	406.40	253.80	319.40
		75	2630.00	304.90	413.60	264.10	345.00
		100	2685.00	363.90	421.20	268.10	348.50
	IAA	100	2694.00	287.30	391.70	248.70	321.90
		200	2783.00	301.90	422.70	263.80	335.80
		300	3001.00	313.10	431.60	270.60	351.90
LSD at 5%		20.92	3.27	2.59	1.37	2.53	

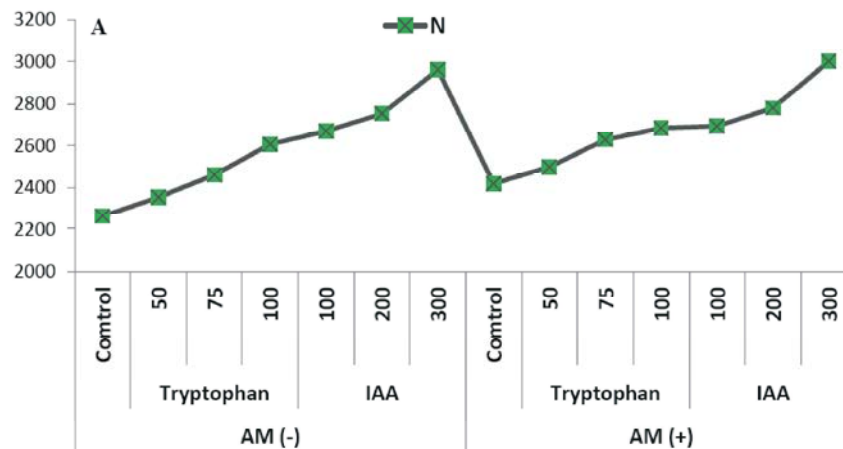


Fig. 2A: Effect of different concentrations of tryptophan or IAA in absence (-) or presence (+) of AM on Nitrogen (N) (mg/100g dry wt.) Macronutrient contents of quinoa seeds under sandy soil conditions

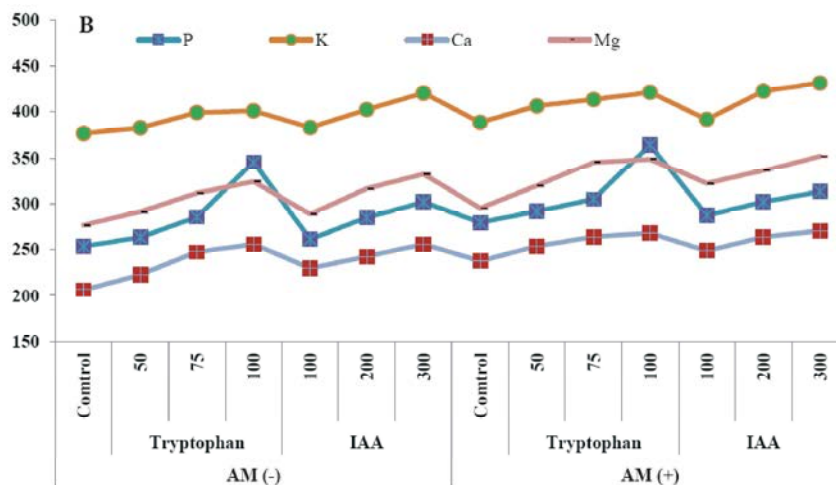


Fig. 2B: Effect of different concentrations of tryptophan or IAA in absence (-) or presence (+) of AM on Macronutrients (P, K, Ca and Mg) (mg/100g dry wt.) Contents of quinoa seeds under sandy soil conditions

Table 9: Effect of inoculation with *Arbuscular mycorrhizae* AM and foliar application with tryptophan and IAA on nutritional value and antioxidant of seeds of quinoa plants grown under sandy soil. (Combined data of two seasons)

Mycorrhizae	Treatments (mg/l)	Phenolic	Flavonoids %	DPPH		
				50 %	100 %	
Without	Control	12.53	63.35	29.35	44.71	
	Tryptophan	50	25.52	66.78	32.46	47.82
		75	37.02	71.45	38.79	54.15
		100	51.02	82.62	42.28	57.64
	IAA	100	23.01	65.68	31.72	47.08
		200	36.02	70.25	37.25	52.61
300		45.23	80.04	40.17	55.53	
With	Control	21.43	65.63	32.25	47.61	
	Tryptophan	50	32.35	73.95	35.77	51.13
		75	46.32	79.35	39.04	57.40
		100	56.36	89.02	49.02	59.38
	IAA	100	29.35	75.35	33.85	49.21
		200	41.30	78.89	45.96	54.32
300		50.63	87.28	47.54	58.90	
LSD at 5%		0.980	0.54	0.58	0.55	

and oil % of quinoa seeds increased significantly when grown in soil amended with AM as compared with those in absence of AM. The results also exhibit marked significant promotion in the above mentioned parameters of quinoa seeds treated with different concentrations of tryptophan or IAA. Applications of 100 mg/l of tryptophan or 300 mg/l IAA are the most effective treatments in absence or presence of AM as compared with the untreated plants and the other treatments.

**Changes in Macronutrients Content of the Quinoa Seeds:** The data in (Table 8, Figs. 2 a and b) represents

the significant effect of foliar treatment of tryptophan (50, 75 & 100 mg/l) or IAA (100, 200 & 300 mg/l) on seeds element contents of quinoa plants grown under sandy soil in absence and presence of mycorrhizae. Application of mycorrhizae to sandy soil improved significantly the elements content of the quinoa seeds (nitrogen, phosphorus, potassium, calcium and magnesium) as compared to those grown under soil without AM application (Table 8). With regard to foliar treatment of tryptophan or IAA, data show that, different concentrations of both of them significantly increased the above mentioned contents (N, P, K, Ca and Mg) as



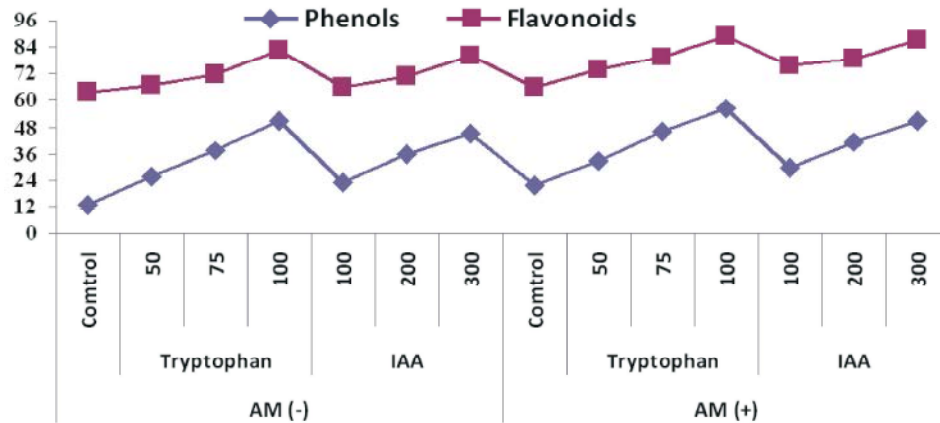


Fig. 3(A): Effect of different concentrations of tryptophan or IAA in absence (-) or presence (+) of AM on total phenols and flavonoids (phenols mg/100g dry wt, flavonoids %) of quinoa seed under sandy soil conditions

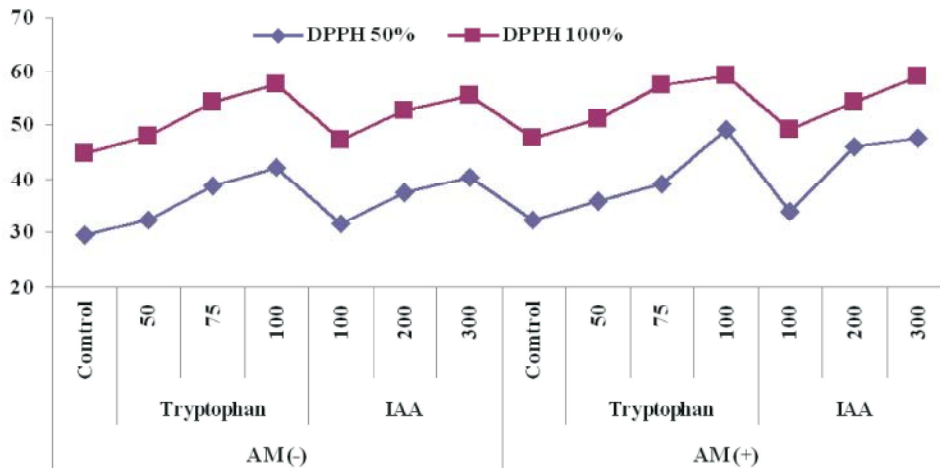


Fig. 3(B): Effect of different concentrations of tryptophan or IAA in absence (-) or presence (+) of AM on (as DPPH-radical scavenging capacity %) of quinoa seed under sandy soil conditions

compared with their corresponding controls (without or with the addition of mycorrhizae).

**Changes in Total Phenol, Total Flavonoid and Antioxidant Activity Contents in Seeds Yield:** Data in Table 9 and Figs. 3a and b exhibited that, application of AM the soil induced significant increases in the total phenols and flavonoids and antioxidant activity (as DPPH- radical scavenging capacity) contents of quinoa seeds as compared with corresponding controls in absence of AM. Results also recorded significant obvious increases in total phenol and total flavonoid and antioxidant activity contents of quinoa seed treated with different concentrations of either tryptophan or IAA. Higher contents of phenols, flavonoids and antioxidant activity were obtained with the higher concentrations

of either tryptophan or IAA application treated with AMF. The maximum percentage of increase reached nearly equal 3.5 folds and 3 folds at phenolic compounds, 40.52% and 37.77% at flavonoids & to 67.02% and 61.98 at DPPH 50%, 32.81% and 31.74% at DPPH 100% in treatment with 100mg/l tryptophan and 300mg/l IAA, respectively.

## DISCUSSION

The results explored that plants inoculated with AMF grow better than non-inoculated plants (Table 1). The same findings are recorded on olive varieties, [28] and on wheat plant [29]. The promotive effects of AM on all growth parameters under this study can be resulted from the beneficial effects of it in improving plant growth

and enhancing plant resistance to biotic and abiotic stresses [30]. In this regard, [31], illustrated that the stimulatory effect of AM may be as a result to their role in absorbing, allocation and transferring of several nutrients as N, P, Ca, K, Cu, Zn and S between stem and root that's leads to significant increase in the dry weight of shoot [29] and [30].

Application of tryptophan clearly affect growth of quinoa plant via promoting photosynthetic pigments (Table 3) and endogenous stimulants especially IAA (Table 5). These results were in accordance with those recorded by [32] on wheat plant; who concluded that, the stimulation in growth of plant in response to tryptophan treatment as compared to untreated plants might be due to improve the levels of endogenous hormones that's leads to stimulation of cell division and/or cell enlargement and subsequently growth. In addition, [33] showed that, tryptophan promoted several physiological processes such as plant growth, differentiation and metabolism of plants and improved the physiological availability of water and nutrients.

Improved growth of plants via fortifies good germination, osmoregulation and nutrient homeostasis. In this connection, [34] described that, exogenous application of IAA elevate growth and development of plants via promoting a several processes, including apical dominance, antioxidative systems, cell elongation and tissue growth, differentiation of vascular tissues, embryogenesis, fruit setting, lateral root initiation, phototropism, ripening and senescence, in potato (*Solanum tuberosum* L.). They also added that, the stimulatory effect of IAA may be ascribed to enlarging leaves and promoting photosynthetic activities.

The results showed that application of AM on plants lead to a marked increase in the photosynthetic pigments as compared to the corresponding treatment of plant grown in the absence of AM (Table 3). In this regard, [35], cleared that *Arbuscular mycorrhizae* promoted chlorophyll activity is restored due to presence of specific enzymes required for its production. With respect to mycorrhizae fungi, similar results were reported by [29] and [30] on wheat plant. Several authors reported that AM symbiosis improved the units of photosynthesis and consequently increased the rates of photosynthetic storage and export at the same time [36]. Significant increases that noticed in all photosynthetic pigment contents in response to application of either IAA or tryptophan, as compared with control plants (Table 3) proved the stimulatory effect of these amino acids on enhancing all photosynthetic pigments. These increments

may be as a result of its role as nitrogen, carbon, energy, enzyme, co-enzymes source and as plant hormones like indole acetic acid (tryptophan), ethylene (methionine) and others as discussed by [17]. In this connection, [37] postulated that tryptophan promotive effects on chloroplast biosynthesis through its role in IAA biosynthesis. Also, it is cleared that, quinoa plants contains high significant carotenoids content after application of IAA or tryptophan. Carotenoids reduce the damage caused by ROS, because it act as a free radical scavenger and enhance their capacity to promote chlorophyll content of such plants [38].

Addition of AM stimulated TSS, polysaccharides and total carbohydrates of quinoa plant as compared with those in absence of AM (Table 4). These results are in harmony with those of [39] who concluded that soybean roots amended with AM had higher contents of soluble sugars than those non mycorrhizal plants. Also, [40] and [29] proved that, addition of AM obviously promoted the contents of TSS on *Helianthus annuus* L and *Triticum aestivum* L. Moreover, improving sugar accumulation may be as a result of starch hydrolysis to sugars in the seedlings treated with mycorrhizae [30].

Foliar application of tryptophan or IAA increased TSS, polysaccharides and total carbohydrates of quinoa plant as compared with those of the control plant. These carbohydrate fractions increases in quinoa plants in response to application of tryptophan treatments may be as a result of its vital role on chlorophyll biosynthesis (Table 3) which parallel to the biosynthesis of carbohydrates and IAA (Table 5). These results are confirmed the results obtained by [41] on sunflower plants, [42] on chickpea plant. Also, [43] concluded that, IAA and its precursor tryptophan improve the sugars translocation during their biosynthesis.

Application of *Arbuscular mycorrhizae* to the soil induced marked increases in the endogenous IAA contents (Table 5). AMF can also benefit plants by stimulating the production of growth regulating substances [44]. These results are in accordance with those obtained by [30] in wheat. Also, [29] found that inoculation of quinoa plants with AM significantly, increased the contents of IAA levels under drought conditions.

Exogenous application of tryptophan or IAA increased endogenous IAA contents in plants as compared to control plants without treatment. The recorded results are in compliance with those obtained by [45] on periwinkle plants and [33] on roselle plants.

The increase in total phenolic compounds in quinoa plants as a result of either tryptophan or IAA treatments (Table 5) may be responsible on reduction or inhibition to the activity of IAA oxidase enzyme consequently increased levels of IAA which improve growth and yield of clover plant as reported by [46].

The results in (Table 5) exhibited that growing plants in the presence of AM lead to a significant increase in phenolic contents. [47] In Cebil (*Anadenanthera colubrina*) a medicinal plant are in accordance with these results. Application of AMF on plants stimulates the production of sets of phenolic compounds [30] in wheat.

The accumulations of free amino acids in mycorrhizal plant were found to be more than those in non-mycorrhizal ones (Table 5). The same trend was observed by [48], in *Zea mays* L.

Application of either tryptophan or IAA cause a significant increase in, free amino acids contents (Table 5). The increment in amino acids may be as a result of their role in improving the efficient of photosynthesis process, via promoting the leaf pigments (chlorophyll a, b and carotenoids) (Table 3), that parallel to induction of the assimilated food, thus the storage of carbohydrate and FAA in the new leaves would be increased. L-Tryptophan may react as an osmolyte, alters the stomatal opening and regulate the ion transport. The same trend of result was reported by [49] and [50] on onion plant and by [42] on different plant species.

Inoculation the soil with AMF induced obvious increases in the yield and yield components in quinoa plants (Table 6). The same trend was recorded in different plant species [40] and [30], they concluded that, AM has promoted role in growth, yield, seeds quality and quantity of several plants grown under drought stress. The positive results of AM on the yield components may be due to their effect on absorbing and transporting several nutrients such as Ca, Cu, K, N, P, S and Zn [31].

Tabulated data in Table 6 illustrated the effect of foliar treatment of quinoa plants grown under sandy soil condition with either tryptophan (50, 75 & 100 mg/l) or IAA (100, 200 & 300 mg/l) in absence or presence of mycorrhizae. The positive effect of these treatments may be a direct result of using amino acids at a suitable levels on the plant growth (Table 2), that's lead to stimulatory effect on photosynthetic pigments (Table 3), respiration rates and leaf carbohydrate (Table 4). These results clearly accompanied with the nutrients uptake and transport, consequently improved plant growth that become with large leaves to store more foods,

consequently reflected on the yield as seed number and weight as illustrated by [51]. The same trends are in harmony with those recorded by [52] and [53, 30] on wheat plant.

Application of AMF markedly induced the total carbohydrate, protein and oil % of harvested quinoa seeds. Additions of it induce good assimilation in the host plant. Similar results were recorded in different plant species [54, 40] in *Acacia saligna* and wheat plants respectively. The same results was recorded under stress condition [29, 30] in sunflower and wheat plants grown under water stress.

Application of tryptophan or IAA and /or AM markedly promoted the total carbohydrate, protein and oil percentages of quinoa seed yield. In this regard, [32] reported that, treatment of wheat plant with tryptophan induced significant increases in the levels of endogenous phytohormones consequently promoted growth and yield. She also added that, plant growth regulators form sink mobilizing to several nutrients, which are involved in building new tissues and/or enhance photosynthesis. Moreover, [55] concluded that application of amino acids induced marked increases in carbohydrates and total protein in produced seeds. In addition, [56] postulated that, proline application as amino acid improved the nutritional values of the produced quinoa seed. Moreover, AM induced obvious increases in the macro element contents (N, P, K, Ca and Mg) of produced seed (Table 8). AM can help in enhance assimilation of (N) in the host plant. Treatment of plants with AM improved nutrients uptake as compared to control (non-inoculated plants). In this connection, AMF have positive effects on the composition of mineral nutrients (certainly immobile nutrients as phosphorus) of plants [57] through increasing and/or selective uptake of nutrients. These increments may be due to the role of AMF through ameliorations of the enzymes associated with N metabolism. Nutrients uptake also promoted through the extended hyphae of the fungus which lets them to explore more soil volume [58]. Also, [59] reported that, AM fungi promoted P and K uptake grown under non-saline and saline conditions of green pepper transplants.

Arbuscular mycorrhizae inoculation in the soil stimulated calcium content in the produced seeds under this study. Also, [60] show that, mycorrhizal fungi promoted Ca contents in yielded seeds grown under water stress. Wheat plants treated with AM keep a higher osmotic potential of cells consequently improved fast growth, N, P, K, Ca, total carbohydrates and chlorophyll contents in leaves [54].

Data also clearly mention that foliar treatment of either tryptophan or IAA were more effective on element contents in plants grown in soil amended with mycorrhizae than those grown without mycorrhizae. Furthermore, tryptophan or IAA foliar treatment promoted the aggregation of mineral contents in quinoa seeds. These results may be as a result of stimulatory roles of either tryptophan or IAA in improving the free amino acid biosynthesis that parallel to their incorporation into protein (Table 6). In this regard, [49] suggested that, amino acids in plants are the main precursor of protein and act as parts of co-enzymes or as initials of certain plant hormones and stimulate the plant growth via promoting photosynthesis, consequently the nitrogen content in the new corms could be increased. The same results was stated by [61] on *Antholyza aethiopica* plant and [52] on garlic plant. Recently, tryptophan or IAA could enhance the contents of ions in the produced seeds of the quinoa via improvement and regulation of various processes as absorption of nutrients from soil solution, mineral homeostasis (Ca<sup>2+</sup>, K<sup>+</sup> and Mg<sup>2+</sup>) and the translocation excessive Na<sup>+</sup> in the roots [62].

The results in Table (9) cleared that, application of AM induced marked increases in the total phenol, total flavonoids and total antioxidant activity (as DPPH- radical scavenging capacity) contents of quinoa seed yield as compared with those in absence of AM. The importance of AM referred to their antioxidant activities that exploit as natural protectants for plants against a biotic stress conditions. In this concern, [63] reported that, flavonoids, as strong radical scavenging activity responsible for the amelioration of oxidative and drought stresses in *Arabidopsis thaliana* plant.

Quinoa seeds treated with different concentrations of tryptophan or IAA and /or AM shown in (Table 9) are in agreement with those obtained by [64] and [29] they concluded that, amended soils with AM enhanced phenolic content on tomato and wheat plants, respectively. Moreover, flavonoids contents in produced seeds may be replace the conventional fungicides in the protection of stored seeds against some fungi [29]. Recently, [65] they recorded that, exogenous application of amino acids play an essential role in the synthesis of proteins, amines, secondary metabolites, enzymes, phenols and flavonoids in plant, these compounds control several plant processes. They also added that, the importance of the flavonoids was known to possess significant antimicrobial activities and was utilized as natural plant protectants.

Higher content of antioxidant activity was obtained with higher concentrations of both tryptophan and IAA

application. Yu *et al.* [66] Stated that, adequate concentrations of antioxidant activities and phenolic components determined in wheat products proved that wheat may utilized as an excellent dietary source of natural antioxidants for disease controller and health promotion. The improvement of the scavenging activity is the main aim of various cures used. This may be reflected to the enhancement in the total phenols and total flavonoids [65].

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