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Effect of Foliar Application of Amino Acid and NAA on the Growth, Yield and Some Phytoconstituents of Melon *Citrullus colocynthis* L.

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ABSTRACT

A Filed experiment was conducted during the summer season of 2014 to study the effect of foliar application Amino acid (AA) at concentrations 0, 0.75 and 1.5 ml.L^{-1} and Naphthalene acetic acid (NAA) at concentrations 0, 50, 100 and 150 ppm and their interactions on growth parameters (plant length, branches number, dry weight of herb and days to 50% flowering), yield parameters (fruits number, fruit fresh weight, fruits dry weight, seed weight of fruit, weight of 100 seeds, plant seed yield and seed yield) and some phytoconstituents (cucurbitacins, flavonoids, alkaloids, saponins, phenols and fixed oil) of Melon (*Citrullus colocynthis* L.). The AA and NAA improved growth and yield parameters. AA was more effective than NAA except the plant length and branches number, and it was increased all phytoconstituents except cucurbitacins compounds which increasing with increased concentration of NAA. the concentrations of AA at 1.5 ml.L^{-1} and NAA at 100 ppm were strongly effects on growth and yield parameters. Consequently, the phytoconstituents were significantly improved by interaction treatment AA at 1.5 ml.L^{-1} × NAA at 100 ppm.

Keywords: Melon, Phytoconstituents, Amino acids, NAA.

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INTRODUCTION

Melon is perennial herbs belong to family Cucurbitaceae, commonly found wild in the sandy lands, the fruits are very bitter but uses on treating many diseases such as rheumatism, hypertension, dermatological problems, gynecological, gastrointestinal, pulmonary infections, ulcers, anthelmintic, antipyretic, carminative, asthma, bronchitis, enlargement of spleen, dyspepsia, anemia, elephantiasis [1], antimicrobial activity [2, 3], cures tumors [4], breast cancer [5], hypoglycemic [6, 7], hyperlipidemia [8], hair loss [9], immunostimulant [10].

Amino acids (AA) can act as growth factors for plants since they are the build blocks of protein synthesis, which could be enzymes important for metabolic activities [11]. It is a well-known biostimulant which has positive effects on plant growth, yield and significantly mitigates the injuries caused by abiotic stresses, Photosynthesis, Phytohormones, Pollination and fruit formation [12]. [13] on soybean found that treatments of amino acids significantly improved growth parameters of shoots and fresh weight as well as pod yield. [14] on potato found that spraying of amino acids at 0.25 $ml.L^{-1}$ significantly increased vegetative growth expressed as plant height and dry weight of plant.

Plant growth regulators are synthesized indigenously by plants, however, several studies demonstrated that plants can respond to exogenous application of these chemicals. An exogenous application of plant growth regulators affects the endogenous hormonal pattern of the plant, either by supplementation of suboptimal levels or by interaction with their synthesis, translocation or inactivation of existing hormone levels [15]. Plant growth regulators are one of the most important factors for increasing higher yield in leafy vegetables. Application of growth regulators has good management effect on growth and yield of field crops. Hormones regulate physiological process and synthetic growth regulators may enhance growth and development of field crops thereby increased total dry mass of a field crop [16, 17, 18]. Naphthalene Acetic Acid (NAA) belongs to synthetic forms of Auxins. Auxins play key role in cell elongation, cell division, vascular tissue, differentiation, root initiation, apical dominance, leaf senescence, leaf and fruit abscission, fruit setting and flowering [19]. NAA had a significant effect on plant height, number of fruiting, branches, volume of boll and yield in cotton [20], NAA have been used for the enhancement of growth and yield of seeds [21]. Rice spraying with 10 and 100 ppm NAA at tillering stage significantly increased root dry weight [22]. NAA can increase fruit setting ratio, prevent fruit dropping, promote flower sex ratio.

This study aimed to examine the influence of exogenously applied AA and NAA on yield of fruits, seeds, fixed oil and phytoconstituents contents of fruits. And we investigated to find out if the combinations of these substances have beneficial effects or if they interfere with each other.

MATERIALS AND METHODS

The experiment was conducted in a private farm, Babylon governorate (Iraq), during cropping summer season of 2014, the soil texture at the experimental site was sandy loam (65% sand, 23% clay and 12% Silt) with approximately 1.58% organic matter, pH 5.4, EC 1.1 dSm^{-1}, Nitrogen 0.11%, Phosphorus 33.21 ppm, Potassium 2.48 meq/100g. The field was prepared conventionally and add NPK fertilizer (20:20:20) at levels 300 $kg.ha^{-1}$ [23] then dividing into plots, area for each experimental unit (plot) was 4.32 m^2 (2.4×1.8 m). Seeds of Melon plant variety "Local" were obtained from a field in western desert of Karbala (Iraq), Seeds were soaked with running tab water for 12 hours, then planted at 15 April by hand on both sides of row, the distance between rows 1.8 m, between seeds 40 cm and about 10 cm deep, so each experimental unit have 12 plants.

The treatments were consisted of AA at concentrations 0, 0.75 and 1.5 $ml.L^{-1}$ as liquid organic fertilizer content: 12.75% Amino Acid (17 types), 10.75% Nitrogen, 11.69% Calcium, 3.2% Magnesium, 750 ppm Sulphur, 125ppm Boron, 12.5ppm Cobalt, 375ppm Cupper, 1ppm Ferric, 1ppm Manganese, 25ppm Molybdenum and 375ppm Zinc. Another factors were growth regulator NAA at concentrations 0, 50, 100 and 150 ppm, both factors were sprayed at three times: vegetative growth period (2-leaves), flower initiation and fruit initiation [24], the hand-spray was set on both leaf surfaces of plants and totally wet in order to accomplish faster and more effective absorption of AA during late afternoon [25, 26]. The treatments were distributed in Factorial experiment conducted in a Randomized Complete Block Design (RCBD) with three

replicates. Collected data analyzed by using GenStat program and means were compared by Least significant differences Test (LSD) at probability level 0.05 according to [27].

The parameters of growth were measured at final stage of vegetative growth such as: plant length (cm), branches number (plant), dry weight of herb (g) and days to 50% flowering, while fruits number (plant), fruit FW (g), fruits DW ($g.Kg^{-1}$), fruit seeds weight (g), weight of 100 seeds (g), plant seed yield (g), seed yield ($Kg.10\ m^2$) and fixed oil (%) were measured at harvesting stage which was began at October and fixed oil% of seeds was extracted according to the methods described by [28]. However the some phytoconstituents were measured as follow:

Plant Material

We selected 24 fruits for each treatment randomly, fruits were cleaned from the dust, then air-dried separately under room temperature for 15 days and crushed into powder with electrical grinder and finally stored in airtight bottles before analysis.

Cucurbitacins Determination

200 mg was extracted with 5 ml absolute ethanol for 2 h, after centrifugation 2000 rpm for 3 min, the supernatant was mixed with an equal volume of petroleum ether, the precipitate obtained was filtered and dissolved in 5 ml absolute ethanol, and then reduced to a volume of 2 ml. The reference standard cucurbitacin E was dissolved in ethanol and serial dilutions (0.01-1.0 mg.ml) were prepared. All samples (100µl, in duplicate), together with various concentrations of cucurbitacin E standard, were mixed with 100 µl of a solution of phosphomolybdic acid in absolute ethanol [29, 30] at room temperature. The absorbance was measured at 492 nm after 5 min.

Flavonoids Determination

10 g of the fruit sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight [31].

Alkaloids Determination

5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed [32].

Saponins Determination

20 g of sample was put into a conical flask and 100 cm^3 of 20% aqueous ethanol was added. Then the flask was heated on a hot water bath for 4 h. with constant stirring at about 55°C. The mixture was then filtered and the residue was again extracted with another 200 ml 20% ethanol. The combined extract was reduced to 40 ml on a hot water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel, added 20 ml diethyl ether in it followed by vigorous shaking. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in oven and weighed the saponin.

Phenols Determination

The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 min. 5 ml of the extract was taken into a 50 ml flask, then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 min for colour development [33]. This was measured at 505 nm. The standard curve was prepared using 0, 50, 100, 150, 200 and 250 mg.L^{-1}.

Oil Determination

50 g of dry seeds powder was extracted by using Soxhlet apparatus for 48 h with 300 ml of Petroleum Spirit [34].

RESULTS AND DISCUSSION

Growth Parameters

Table 1: Effect of AA, NAA and their interactions on the growth parameters of Melon

Treatments	Plant length (cm)	Branches No. (plant)	DW (g.plant)	Days 50% flowering
AA^0	223.66	30.35	80.94	26.30
$AA^{0.75}$	230.90	34.34	105.85	27.01
$AA^{1.5}$	238.79	36.19	121.69	27.08
LSD (0.05)	2.13	0.11	0.62	0.09
NAA^0	207.01	29.43	85.86	27.10
NAA^{50}	227.55	33.55	101.77	26.67
NAA^{100}	238.55	34.79	115.58	26.69
NAA^{150}	251.35	36.73	108.10	26.72
LSD (0.05)	2.68	0.14	0.78	0.11
$AA^0 \times NAA^0$	193.81	26.30	66.85	26.76
$AA^0 \times NAA^{50}$	221.75	30.10	79.83	26.13
$AA^0 \times NAA^{100}$	232.44	31.54	91.37	26.14
$AA^0 \times NAA^{150}$	246.63	33.48	85.71	26.17
$AA^{0.75} \times NAA^0$	209.71	28.48	91.77	27.49
$AA^{0.75} \times NAA^{50}$	227.29	34.86	102.38	26.83
$AA^{0.75} \times NAA^{100}$	236.86	36.14	118.49	26.85
$AA^{0.75} \times NAA^{150}$	249.73	37.89	110.76	26.87
$AA^{1.5} \times NAA^0$	217.52	33.52	98.95	27.06
$AA^{1.5} \times NAA^{50}$	233.61	35.69	123.11	27.06
$AA^{1.5} \times NAA^{100}$	246.35	36.71	136.88	27.10
$AA^{1.5} \times NAA^{150}$	257.69	38.84	127.82	27.12
LSD (0.05)	4.51	0.23	1.32	0.19

Results in Table 1 indicate that spraying of AA or NAA gave significant effects ($P \leq 0.05$) of Melon plants during vegetative stages compared to the controls. Treatments of AA were gave more impact when the concentrations were increased, the values of plant length (cm), branches number, dry weight of herb (g.plant) and days 50% flowering which resulted to be 238.79 cm, 36.19 branches, 121.69 (g.plant) and 27.08 days respectively, compared with control which gave 223.66 cm, 30.35 branches, 80.94 (g.plant) and 26.30 days respectively. The regulatory effect of AA on growth parameters could be explained by the notion that some amino acids for example phenylalanine, tryptophan and ornithine can affect plant growth and development through their influence on gibberellins biosynthesis [35]. While NAA at concentration 150 ppm resulted in highest values on parameters above which resulted to be 251.35 cm, 36.73 branches and 26.72 days respectively, compared with control which gave 207.01 cm, 29.43 branches and 27.10 days respectively, except dry weight (g.plant) was achieved highest value at concentration 100 ppm which resulted to be 108.10

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compared with control which gave 85.86 (g.plant), NAA belongs to synthetic forms of Auxins, Auxins play key role in vascular tissue, differentiation, root initiation, apical dominance, leaf senescence and leaf abscission [19]. The interaction treatment between 3 ml.L^{-1} AA×150 ppm NAA superiority significant (P≤0.05) for all treatments on growth parameters: plant length, branches number and days 50% flowering except of dry weight of herb were given highest values at interaction treatment between 3 ml.L^{-1} HA×100 ppm NAA, the properties above were evaluated to be 257.69 cm, 38.84 branches, 27.12 days and 127.82 (g.plant) respectively, compared with treatment control which gave 193.81 cm, 26.30 branches, 26.76 days and 66.85 (g.plant) respectively.

Yield Parameters

Data presented in Table 2 show that the AA concentration at 1.5 ml.L^{-1} gave significant effects (P≤0.05) on amounts of fruits number (plant), fruit FW (g), fruits DW (g.Kg^{-1}), fruit seed weight (g), weight of 100 seeds (g), plant seed yield (g) and seed yield (Kg.m^{-2}) which resulted 20.78 plant, 77.16 g, 222.64 g.Kg^{-1}, 16.25 g, 12.69 g, 337.83 g and 9.358 Kg.m^{-2} respectively, compared with control which gave 16.59 plant, 64.14 g, 171.20 g.Kg^{-1}, 15.79 g, 11.66 g, 262.09 g and 7.260 Kg.m^{-2} respectively, AA may be play an important role in plant metabolism and protein assimilation which necessary for cell formation and consequently increase fresh and dry mater [34]. Also table 2 shows that NAA at concentration 100 ppm resulted in highest values on yield parameters above which resulted 19.36 plant, 214.01 g.Kg^{-1}, 16.16 g, 12.53 g, 313.33 g and 8.679 Kg.m^{-2} respectively, compared with control which gave 17.32 plant, 179.05 g.Kg^{-1}, 15.85 g, 11.85 g, 274.83 g and 7.613 Kg.m^{-2} respectively, except fruit FW (g) was achieved highest value at concentration 100 ppm which resulted to be 74.08 (g) compared with control which gave 67.49 (g), NAA play key role in cell elongation, cell division, differentiation, fruit abscission, fruit setting and flowering [19], thus effect on the FW and DW of fruits and seeds. The interaction treatment between 1.5 ml.L^{-1} AA×100 ppm NAA superiority significant (P≤0.05) for all treatments on yield parameters: fruits number (plant), fruits DW (g.Kg^{-1}), fruit seed weight (g), weight of 100 seeds (g), plant seed yield (g) and seed yield (Kg.m^{-2}) which resulted 21.55 plant, 81.26 g, 241.13 g.Kg^{-1}, 16.38 g, 13.09 g, 352.99 g and 9.778 Kg.m^{-2} respectively, compared with control which gave 15.33 plant, 59.68 g, 151.32 g.Kg^{-1}, 15.51 g, 11.23 g, 237.77 g and 6.586 Kg.m^{-2} respectively.

Table 2: Effect of AA, NAA and their interactions on the yield parameters of Melon

Treatments	Fruits No. (plant)	fruit FW (g)	Fruits DW (g.Kg^{-1})	Fruit seeds weight (g)	Weight 100 seeds (g)	Plant seed Yield (g)	Seed Yield (Kg.$10m^2$)
AA^0	16.59	64.14	171.20	15.79	11.66	262.09	7.260
$AA^{0.75}$	18.41	73.41	202.61	16.08	12.50	296.05	8.201
$AA^{1.5}$	20.78	77.16	222.64	16.25	12.69	337.83	9.358
LSD (0.05)	0.02	1.23	1.87	0.14	0.05	1.07	0.013
NAA^0	17.32	67.49	179.05	15.85	11.85	274.83	7.613
NAA^{50}	18.52	70.98	195.25	16.05	12.23	297.56	8.242
NAA^{100}	19.36	73.73	214.01	16.16	12.53	313.33	8.679
NAA^{150}	19.17	74.08	206.94	16.10	12.51	308.90	8.556
LSD (0.05)	0.02	1.55	2.35	0.18	0.06	1.35	0.016
AA^0×NAA^0	15.33	59.68	151.32	15.51	11.23	237.77	6.586
AA^0×NAA^{50}	16.41	64.66	167.77	15.84	11.45	259.89	7.199
AA^0×NAA^{100}	17.03	65.37	186.84	15.93	11.87	271.27	7.514
AA^0×NAA^{150}	17.61	66.86	178.85	15.87	12.09	279.45	7.741
$AA^{0.75}$×NAA^0	17.24	69.58	188.84	15.94	12.25	274.74	7.610
$AA^{0.75}$×NAA^{50}	18.19	72.46	198.99	16.07	12.48	292.29	8.097
$AA^{0.75}$×NAA^{100}	19.51	74.55	214.06	16.18	12.63	315.75	8.746
$AA^{0.75}$×NAA^{150}	18.69	77.04	208.53	16.13	12.62	301.41	8.349
$AA^{1.5}$×NAA^0	19.39	73.22	196.99	16.09	12.08	311.99	8.642
$AA^{1.5}$×NAA^{50}	20.97	75.81	218.98	16.24	12.75	340.49	9.432
$AA^{1.5}$×NAA^{100}	21.55	81.26	241.13	16.38	13.09	352.99	9.778
$AA^{1.5}$×NAA^{150}	21.23	78.33	233.44	16.29	12.83	345.84	9.580
LSD (0.05)	0.04	2.61	3.96	0.31	0.11	2.27	0.028

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Phytoconstituents Parameters

The quantitative determination of secondary constituents substances of Melon are tabulated in table 3. The AA and NAA effects on some active constitutes on dry fruits and fixed oil of seeds of Melon plant, and these effects were significantly variable compared to the control. Furthermore, AA concentration at 1.5 ml.L^{-1} resulted in the highest values of phytoconstituents flavonoids, alkaloids, saponins, phenols and fixed oil, which evaluated to be 13.71, 17.58, 5.291, 12.80 mg.Kg^{-1} DW and 31.38% respectively, compared with control which gave 12.88, 16.53, 4.972, 12.07 mg.Kg^{-1} DW and 24.61% respectively, but control treat suggested the highest values of cucurbitacins which evaluated to be 3.782 mg.Kg^{-1} DW compared with other treatments. The effect of tested amino acids on the phytoconstituents could be through plant protection from ammonia toxicity as they remove amide formation, serving as a source of carbon and energy as well as functioning as buffers and biosynthesis of other organic compounds such as protein, amines, purines, pyrimidines, vitamins, enzyme, terpenoids [36]. Treatment of NAA at concentration 100 ppm resulted in the highest values of phytoconstituents flavonoids, saponins, phenols and fixed oil compounds except of Cucurbitacins and alkaloids were given highest values at concentration 150 ppm NAA, the properties above were evaluated to be 13.40, 5.171, 12.53, 17.34 mg.Kg^{-1} DW and 29.44% respectively, compared with control which gave 12.88, 4.972, 12.07, 16.53 mg.Kg^{-1} DW and 26.54% respectively, NAA was increased the rate of production primary metabolism compounds, It follows that naturally increase the rate of production secondary metabolism compounds such as flavonoids, saponins phenolic and fixed oil compounds, except the alkaloids which increased with AA additive. The interaction treatment 1.5 ml.L^{-1} AA×100 ppm NAA superiority significant for all of phytoconstituents except of cucurbitacins were given highest values at treatment 0.0 ml.L^{-1} AA×150 ppm NAA and alkaloids at 1.5 ml.L^{-1} AA×150 ppm NAA, the properties above were evaluated to be 13.98, 5.397, 13.04, 17.92 mg.Kg^{-1} DW and 33.81% respectively, compared with control which gave 12.77, 4.931, 11.96, 16.40 mg.Kg^{-1} DW and 22.61% respectively.

Table 3: Effect of AA and NAA and their interactions on the some phytoconstituents of Melon

Treatments	Phytoconstituents on fruits (mg.Kg^{-1} DW)					Fixed Oil (%)
	Cucurbitacins	Flavonoids	Alkaloids	Saponins	Phenols	
AA^{0}	3.782	12.88	16.53	4.972	12.07	24.61
$AA^{0.75}$	2.938	13.21	17.10	5.100	12.37	28.57
$AA^{1.5}$	2.637	13.71	17.58	5.291	12.80	31.38
LSD (0.05)	0.07	0.02	0.03	0.011	0.02	0.07
NAA^{0}	2.749	13.09	16.81	5.055	12.26	26.54
NAA^{50}	3.002	13.19	16.98	5.093	12.35	27.71
NAA^{100}	3.217	13.40	17.15	5.171	12.53	29.44
NAA^{150}	3.506	13.38	17.34	5.165	12.52	29.05
LSD (0.05)	0.09	0.03	0.04	0.014	0.03	0.09
AA^{0}×NAA^{0}	3.477	12.77	16.40	4.931	11.96	22.61
AA^{0}×NAA^{50}	3.614	12.82	16.49	4.949	12.02	23.97
AA^{0}×NAA^{100}	3.861	12.95	16.56	4.990	12.13	25.33
AA^{0}×NAA^{150}	4.176	12.99	16.68	5.016	12.18	26.52
$AA^{0.75}$×NAA^{0}	2.633	13.09	16.81	5.054	12.25	27.94
$AA^{0.75}$×NAA^{50}	2.817	13.15	16.97	5.075	12.32	28.49
$AA^{0.75}$×NAA^{100}	3.049	13.28	17.19	5.127	12.43	29.19
$AA^{0.75}$×NAA^{150}	3.252	13.33	17.41	5.146	12.48	28.65
$AA^{1.5}$×NAA^{0}	2.138	13.42	17.21	5.181	12.57	29.08
$AA^{1.5}$×NAA^{50}	2.576	13.61	17.48	5.254	12.71	30.66
$AA^{1.5}$×NAA^{100}	2.741	13.98	17.69	5.397	13.04	33.81
$AA^{1.5}$×NAA^{150}	3.091	13.81	17.92	5.333	12.89	31.98
LSD (0.05)	0.15	0.04	0.06	0.023	0.04	0.15

CONCLUSION

The spraying by nutrient of AA and application with growth regulator treatments by NAA resulted significant increased on growth, yield and photochemistry of the Melon plant. Treatments with 1.5 $ml.L^{-1}$ AA×100 ppm NAA induced the greatest increase in most biochemical constituents content of dry fruits. These treatments could thus be used to enhance the growth and quality of this medicinal plant.

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