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Impacts of some biostimulants on guar (*Cyamopsis tetragonoloba* L.) plants

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Abstract

Background: Guar is an economic crop due to guar gum which is extracted from seeds and is used in several industries such as food, ink, plastics, pharmaceutical industry, and cosmetics. It can also be used as a cover crop, animal feed, and green manure. L-Tryptophan (L-β-3-indolylalanine) is a precursor of auxin which regulates plant growth and development. Nicotinamide is known as the amide form of vitamin B3. It is a constituent of the pyridine nucleotide coenzymes involved in many enzymatic oxidation-reduction reactions in cells. L-Tryptophan and nicotinamide are used in this study in order to improve guar growth, yield, and chemical constituents of seeds.

Results: The highest records of plant height and fresh and dry weights of leaves were recorded for plants treated with 300 mg/l nicotinamide followed by foliar treatment with 300 mg/l tryptophan. Fresh and dry weights of stems, number of pods/plant, fresh and dry weights of pods, pods yield, seeds yield, and straw yield followed the same trend. Total protein in guar seeds was significantly increased due to foliar treatment with tryptophan, especially in plants treated with 300 mg/l tryptophan, followed by plants treated with 300 mg/l nicotinamide. Total carbohydrates (mg/g dry wt.), total soluble sugars (mg/g dry wt.), total insoluble sugars (mg/g dry wt.), proline (μmol/g dry wt.), and total phenolic compounds (mg/g dry wt.) in the leaves followed the same trend.

Conclusion: It could be concluded that guar growth and yield are maximized with foliar treatment with nicotinamide (300 mg/l). Also, chemical constituents of seeds improved with nicotinamide and tryptophan treatments (each at 300 mg/l).

Keywords: *Cyamopsis tetragonoloba* L., L-Tryptophan (T), Nicotinamide (NA)

Introduction

Guar (*Cyamopsis tetragonoloba* L.) is a drought and high-temperature–tolerant deep-rooted summer annual legume of high economic and social significance. This crop is of high adaptation toward erratic rainfall. Guar is considered as one of the most significant crops in arid areas in India due to its multiple industrial uses and its importance in cropping system for factors such as soil enrichment properties and low input requirements (Lubbe and Verpoorte 2011). The plant is extremely drought-resistant, being able to absorb efficiently all ground water. It grows easily in those semi-arid regions. Like other legumes, guar is an excellent soil-building crop with respect to availability of nitrogen. Root nodules contain nitrogen-fixing bacteria and crop residues, when ploughed under improve yields of succeeding crops (Siddaraju et al. 2010).

Guar is a multipurpose crop which is used in several industries such as a thickener and emulsifier in the food processing industry. It is also used in cosmetics, printing, pharma, textile, etc. (Mudgil et al. 2011). Guar is the source of a natural hydrocolloid, which is cold water soluble and forms thick solution at low concentrations. Guar gum is used as a thickening agent and emulsifier for hydraulic fracturing ("fracking") of subsurface shale in oil and gas industry (Undersander et al. 1991; Abidi et al. 2015).

L-Tryptophan is one of the 20 L-amino acids incorporated in proteins during the process of mRNA translation. It is the precursor of small bioactive compounds, which can influence a number of cell metabolic pathways and physiological responses. An imbalanced metabolism of L-tryptophan can interfere with the ability of these systems to interact with as well as discriminate, during development,

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stressors and stimuli, exogenous and endogenous antigens, and nutrients (Palego et al. 2016).

Nicotinamide is an amide active form of vitamin B3 or niacin. It is the precursor for the synthesis of nicotinamide adenine dinucleotide (NAD) and the phosphorylated derivative NADPH (Jacob and Swendseid 1996).

The objective of using tryptophan and nicotinamide in this study is to improve growth and yield and maximize the economic value of guar by increasing its nutrient content and active components such as protein, gum, and phenolic compounds, which promote the use of guar in livestock feed, plastics, food, cosmetics, and pharmaceutical industries.

Methods

Two pot experiments were carried out in the screen of the National Research Centre, Dokki, Giza, Egypt, to study the effect of foliar spraying with nicotinamide or tryptophan on guar plants. Seeds were inoculated with specific *Rhizobium* sp. strain before sowing which was on May 21st and 23rd for the first and second seasons (2015, 2016), respectively. The seeds were sown on earthenware pots (30 cm in diameter) filled with air-dried clay soil.

Physical and chemical properties of a representative soil sample were analyzed according to the method described by Chapman and Pratt 1978. Results illustrated in Table 1 revealed that sand, silt, and clay percentages were 18.50, 21.30, and 60.20 respectively, indicating that soil type was clay. Other findings were field capacity 47.60%, wilting point 23.60%, electrical conductivity (EC) 2.70 dS/m, pH 7.30, soluble cations of K^+, Na^+, Mg^{++}, and Ca^{++} recorded 4, 3.20, 8, and 18 mg/100 g soil, respectively, and anions of HCO_3^- and Cl^- recorded 4.20 and 5.10 mg/100 g soil, respectively.

Table 1 Physical and chemical analyses of the experimental soil

Variables	Soil type (clay)
Field capacity (%)	47.60
Wilting point (%)	23.60
Sand %	18.50
Silt %	21.30
Clay %	60.20
pH	7.30
EC dS /m	2.70
$CaCO_3$%	2.83
Soluble ions mg/100 g soil	
Ca^{++}	18.00
Mg^{++}	8.00
Na^+	3.20
K^+	4.00
CO_3^-	–
HCO_3^-	4.20
Cl^-	5.10

The pots were arranged in complete randomized block design of seven treatments. One month later, plants were sprayed with tryptophan (100, 200, and 300 mg/l), nicotinamide (100, 200, and 300 mg/l) and control (untreated).

Each treatment has five replicates, and each pot received equal and adequate amount of tap water and fertilizers. Thinning was performed to leave three seedlings in each pot.

Two plant samples were drawn to represent two stages of growth and development for guar plants, the first sample after 60 days from sowing (vegetative growth) and the second sample at maturity to determine yield.

The data of vegetative growth were recorded as follows: plant height (cm) and fresh and dry weights of leaves and stems (g/plant). Data included at the second sample were number of pods/plant, weight of pods (g/plant), weight of seeds (g/plant), and straw weight (g/plant).

Chemical analysis

The dried parts of plants, leaves and seeds, were finely ground and reset for chemical determination of the following constituents:

1- Determination of total carbohydrates, total soluble sugars, and total insoluble sugars were carried out according to Dubois et al. (1956).
2- Seed oil content was determined using Soxhelet apparatus and petroleum ether (40–60 °C) according to AOAC (1990).
3- Total protein concentration was determined by the Bradford method (1976) using bovin serum albumin (BAA) as a standard.
4- Proline was determined in the leaves according to Bates et al. (1973).
5- Guar gum contents, polysaccharides with high molecular weight composed of galactomannans and obtained from the endosperm of guar seed (50 g), were powdered for 5 min in a mechanical blender and soaked in distilled water (500 ml) for 24 h in a round bottom flask. It was boiled for 1 h under reflux with occasional stirring and kept aside for 2 h for the release of mucilage into water. The material was filtered then 100 ml of ethanol was added to the filtrate to precipitate the mucilage and kept inside a refrigerator for one day for effective settling. It was filtered and dried completely in an incubator at 37 °C, then powdered and weighed (Pawar and Mello 2004) and (Malviya et al. 2011).
6- Total phenols of dried leaves were determined by the colorimetric method of folin–ciocalteo reagent according to Singleton et al. (1999).

7- Identification of phenolic acids in the seeds, protocatechuic, P-hydroxybenzoic, vanillic, syringic, caffeic, and coumaric acids were subsequently checked for purity by high-pressure liquid chromatography (HPLC). HPLC grade water and MeOH were used for all analyses. Phosphoric acid buffer was made using HPLC grade $NH_4H_2PO_4$ and H_3PO_4 (Mattila et al. 2005).

Phenolic extraction and hydrolysis

Phenolic compounds in plant were extracted as described by Mattila et al. (2005) with some modifications. Approximately, 15 ml of 4 N NaOH was added to 200 ml of the concentrated water extract in 50-ml Pyrex centrifuge tube purged with nitrogen and shaken for 2 h in dark with a wrist-action shaker.

After phenolic acids were liberated by alkaline hydrolysis, samples were acidified with ice-cold 6 N HCl to adjust the pH between 1 and 2. Samples were centrifuged at 3000*g*, and the supernatant was decanted into a 25- ml separated funnel. The supernatant was extracted with ethyl acetate (3 × 50 ml) after shaking for 10 s, then the mixture was allowed to settle for 5 min before extraction. Ethyl acetate fractions were collected and pooled. The remaining pellet was diluted with 15 ml of distilled H_2O, vortexed and centrifuged at 3000*g*. The supernatant was re-extracted with ethyl acetate (3 × 50 ml) as before, and all ethyl acetate fractions were pooled. The phenolic acid-rich ethyl acetate fraction was dried by addition of anhydrous sodium sulfate and concentrated using a rotary vacuum evaporator at 35 °C to dryness. The phenolic acid-rich residue was re-solubilized in 2.5 ml of MeOH and stored in a dark prior to separation and quantification by HPLC within 24 h of extraction.

HPLC analysis

Phenolic acids were separated by Shimadzu (Kyoto, Japan) HPLC apparatus (model, LC-4A) equipped with visible/UV detector (model, SPD-2AS) at 280 nm and stainless steel column (25.0 cm × 4.6 mm i.d.) (Phenomenex Co., USA) coated with ODS (RP-18). An aliquot of the sample suspended in Me OH was diluted with 10 mM phosphoric acid buffer (PH 3.5) to the same concentration as the initial mobile phase (15% MeOH). Samples were next filtered through a 0.2-μm poly tetrafluoroethylene (PTFE) filter prior to injection. The two solvent systems consisted of MeOH (A) and 10 mM phosphoric acid buffer, PH 3.5 (B), operated at a flow rate of 1.5 ml/min. The phosphoric acid buffer consisted of 10 mM $NH_4H_2PO_4$ adjusted to PH 3.5 with 10 mM H_3PO_4.

Statistical analysis

The data were statistically analyzed according to Snedecor and Cochran (1980); combined analysis of the two experimental seasons was carried out. Means were compared by using the least significant difference (LSD) at 5% levels of probability.

Results

Data presented in Tables 2 and 3 indicated that foliar application of tryptophan and nicotinamide significantly increased the vegetative growth and yield of cluster bean (*Cyamopsis tetragonoloba* L.) plants.

The highest values of plant height and fresh and dry weights of leaves were recorded for plants treated with 300 mg/l nicotinamide followed by foliar treatment with 300 mg/l tryptophan. Fresh and dry weights of stems, number of pods/plant, fresh and dry weights of pods, pods yield, seeds yield, and straw yield followed the same trend (Tables 2 and 3).

Data presented in Table 4 indicated that protein % in guar seeds was significantly increased as a result of foliar treatment with tryptophan, especially in plants treated with 300 mg/l tryptophan, followed by plants treated with 300 mg/l nicotinamide. The present results emphasized that tryptophan and nicotinamide treatments significantly increased total carbohydrates %, guar gum, and fixed oil % in guar seeds. The highest recorded results were obtained in plants treated with 300 mg/l tryptophan, followed by 300 mg/l nicotinamide.

Table 2 Effect of tryptophan and nicotinamide on growth of guar plants

Treatment (mg/l)	Plant height (cm)	Fresh weight of leaves (g/plant)	Dry weight of leaves (g/plant)	Fresh weight of stems (g/plant)	Dry weight of stems (g/plant)
T 100	67.00	8.09	1.81	7.84	1.44
T 200	75.33	12.42	2.57	10.49	1.53
T 300	77.67	15.36	4.06	15.69	2.68
NA 100	76.67	11.31	1.44	11.94	1.61
NA200	80.67	14.42	3.34	12.66	2.27
NA300	87.00	17.00	3.74	16.96	3.50
Control	58.33	6.04	1.40	7.10	1.05
LSD (5%)	2.69	2.01	0.73	1.21	0.86

Table 3 Effect of tryptophan and nicotinamide on the yield of guar pods, seeds and straw

Treatment	Plant height (cm)	Fresh weight of leaves (g/plant)	Dry weight of leaves (g/plant)	Fresh weight of stems (g/plant)	Dry weight of stems (g/plant)	Number of pods/ plant	Fresh weight of pods (g/plant)	Dry weight of pods (g/plant)	Pods yield (g/plant)	Weight of seeds (g/plant)	Straw yield (g/plant)
T 100	140.50	8.78	3.10	47.52	12.88	17.00	11.70	2.53	148.76	51.54	52.85
T 200	165.75	15.47	4.87	49.44	14.07	22.00	21.51	4.43	158.81	81.42	62.60
T 300	168.00	20.56	6.21	53.52	15.98	28.00	22.54	6.51	163.29	84.48	75.30
NA 100	154.75	13.31	4.22	41.93	12.24	15.00	11.48	3.97	108.62	81.02	60.75
NA 200	156.50	19.11	5.13	50.48	12.27	21.00	15.15	4.03	145.92	81.43	70.59
NA 300	177.00	23.19	5.18	67.68	21.84	36.67	24.42	13.56	169.51	85.66	84.88
Control	138.75	5.33	2.92	32.71	10.17	7.67	8.39	2.27	101.95	44.86	52.70
LSD (5%)	3.08	1.22	1.10	1.94	1.34	1.22	1.35	0.85	2.95	2.65	2.48

Total carbohydrates (mg/g dry wt.), soluble sugar compounds and insoluble sugar compounds (mg/g dry wt.) in the leaves, proline (μmol/g dry wt.), and total phenolic compounds (mg/g dry wt.) in the leaves followed the same trend (Table 4).

All treatments showed P-hydroxybenzoic acid and protocatechuic acid as the two major phenolic compounds followed by vanillic acid (Table 5). P-Hydroxybenzoic is the main phenolic acid in guar seeds, and its ratio ranged from 21.77% in control plants to 39.19% in plants treated with 300 mg/l nicotinamide. Foliar application of 300 mg/l tryptophan also recorded 34.00% P-hydroxybenzoic acid. On the other hand, protocatechuic acid ranged from 0.95% in plants treated with 200 mg/l nicotinamide to 8.45% in plants treated with 300 mg/l tryptophan and vanillic acid ranged from 0.57 to 4.77%. Data presented in Table 5 also showed that coumaric acid was not detected in plants treated with 100 mg/l tryptophan, while syringic acid and caffeic acid were not detected in most treatments.

Discussion

From the foregoing presentation of results, it appeared that foliar application of tryptophan significantly increased the vegetative growth and yield of Cluster bean (*Cyamopsis tetragonoloba* L.) plants. Amino acids are important for promoting cell growth and function as buffers. They maintain the plant cell pH value. They also play an important role in the synthesis of protein, amines, purines, pyrimidines, etc. (Abd El-Aziz and Balbaa 2007). In this concern, Russell (1982) reported that the increase in the herb fresh weight in plants treated with tryptophan may be due to its conversion into indole-3-acetic acid (IAA) which promotes stem elongation, especially at low rates. Koriesh (1984) sprayed *Catharanthus roseus* plants with tryptophan at the rate of 25, 50, and 100 ppm and found that tryptophan at 25 ppm was the most effective on plant height. Harridy (1986) also reported that 50 and 100 ppm tryptophan increased plant height and herb fresh and dry weights of *Catharanthus roseus* G. Don, especially at the rate of 100 ppm. Similar results were obtained by Attoa et al. (2000) who observed that treatment of *Iberis amara* L. plants with 75 ppm tryptophan increased the plant growth parameters. It could be stated that tryptophan treatments at 25, 50, and 100 ppm increase chlorophylls a and b in the leaves as found by Milad (1998) who recorded an increase in chlorophylls a and b content in *Mentha longifolia*, *M. viridis*, and *Ocimum canum*. Meanwhile, Shoala (2000) found that plants sprayed with tryptophan at 25 ppm increased chlorophylls a and b in *Lavendula multifida*. In addition, spraying *Iberis amara* L.

Table 4 Effect of tryptophan and nicotinamide on chemical constituents of guar plants

Treatment (mg/l)	Total Carbohydrate in the leaves (mg/g dry wt.)	Soluble sugar in the leaves (mg/g dry wt.)	Insoluble sugars in the leaves (mg/g dry wt.)	Proline in the leaves (μmol/g dry wt.)	Phenolic compounds in the leaves (mg/g dry wt.)	Protein % in guar seeds	Total Carbohydrate % in guar seeds	Gum % in guar seeds	Fixed oil % in guar seeds
T 100	64.88	24.77	40.11	5.65	0.68	20.06	42.46	34.41	2.86
T 200	72.46	28.39	44.07	9.69	0.81	22.27	47.68	37.79	3.77
T 300	78.51	34.00	44.51	11.22	1.18	23.34	48.40	38.61	4.86
NA 100	63.50	27.78	35.72	5.74	0.61	20.09	40.36	33.11	2.76
NA 200	66.21	30.38	35.83	7.39	0.89	20.62	43.49	36.53	3.49
NA 300	74.65	33.94	40.71	10.31	1.04	22.62	48.37	37.89	4.39
control	62.38	23.59	38.79	6.26	0.61	19.66	37.46	29.78	2.57
LSD (5%)	0.24	0.26	0.25	0.09	0.06	0.16	0.32	0.21	0.12

Table 5 Effect of tryptophan and nicotinamide on phenolic acids of guar seeds

Treatments (mg/l)	Protocatechuic	P-Hydroxybenzoic	Vanillic	Syringic	Caffeic	Coumaric
T 100	3.89	25.92	0.26	ND	0.15	ND
T 200	5.08	28.14	0.74	1.06	1.46	0.41
T 300	8.45	34	2.54	3.24	ND	0.13
NA 100	0.98	28.79	0.52	ND	ND	0.19
NA200	0.95	30.22	0.25	ND	ND	0.21
NA300	2.67	39.19	4.77	ND	ND	0.3
Control	2.04	21.77	0.57	ND	1.05	0.23

ND not detected

with 75 ppm tryptophan increased carbohydrates, nitrogen, and phosphorus contents (Attoa et al. 2000). IAA synthesis exists in plants through several routs which all starting from the amino acid tryptophan (Phillips 1971). Similar results were obtained by Talaat et al. (2005) who reported that the plant growth of *Catharanthus roseus* transplants was considerably promoted at successive developmental stages due to the exogenous application of tryptophan, especially in plants treated with 10^{-3} M tryptophan. In addition, the exogenous application of tryptophan to two sunflower cultivars grown under different saline conditions increased the tolerance of sunflower plants through increasing chlorophylls (a and b), carotenoids, and endogenous hormones, especially indole acetic acid, or increasing the potassium, calcium, and magnesium uptake which in turn increased the plant tolerance to oxidative stress (Abdel-Monem et al. 2010). The biosynthetic pathways of the main phenolic compounds in guar plants might be attributed to that it is independently or simultaneously from shikimic acid or malonic acid pathways. Tryptophan might alter the biosynthetic processes to the advantage of P-hydroxybenzoic acid and protocatechuic acid from shikimic acid or malonic acid (Yao et al. 1995). Nicotinamide may have positive effects on pigments, carbohydrates, nitrogen, RNA, and DNA contents in plants (Sanai and Ota 1977; Bearder 1980; Foda 1987; Sharaf El-Din et al. 1987; Hathout et al. 1993a, 1993b). Foda (1987) and Deyab (1989) found similar results in wheat plants. In addition, treatment of tomato plants with different values of nicotinamide as foliar spray caused positive effects on growth, yield, and endogenous growth promoters (Hathout et al. 1993a, 1993b). Several studies indicated that nicotinamide stimulated growth in many plants (Tarraf et al. 1999; Zhang et al. 2000; El Bassiouny et al. 2005; Hassanein et al. 2009; Sadak et al. 2010). They reported that mitigation of the negative effect of biotic stress on the metabolic activities might be related to the increase of indole acetic acid content due to nicotinamide treatments and to the significantly higher levels of carbohydrates found in the treated plants. Berglund and Ohlsson (1995) reported that nicotinamide induces and regulates the biosynthesis and accumulation of phenolic compounds in plants, especially in association with stress or defense conditions.

Conclusion

It could be concluded that foliar treatment of guar plants with nicotinamide or tryptophan (Each at 300 mg/l) had positive influence for promoting plant growth, yield, and chemical constituents. It is also worth to mention that nicotinamide (300 mg/l) was more effective in influencing the growth and yield of guar plant than tryptophan (300 mg/l).

Abbreviations

BAA: Bovin serum albumin; Dry wt.: Dry weight; EC: *Electrical conductivity*; HPLC: High-performance liquid chromatography; IAA: Indole-3-acetic acid; LSD: Least significant difference; mRNA: Messenger ribonucleic acid; MeOH: Methanol; NA: Nicotinamide; NAD: Nicotinamide adenine dinucleotide; NADPH: Reduced form of nicotinamide adenine dinucleotide phosphate ($NADP^+$); N HCl: Normal hydrochloric acid; ND: Not detected; PTFE: Poly tetrafluoroethylene; T: L-Tryptophan; Vitamin B3: Niacin

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Authors' contributions

All authors have contributed in the design of the study, data collection, paper writing, and data analysis. All authors read and approved the final manuscript.

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The data supporting the current study are included within the article.

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Consent for publication

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Competing interests

The authors declare that they have no competing interests.

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