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PREPARATION AND APPLICATION OF SOME NITROGENOUS DERIVATIVES OF GUM ARABIC AS PLANT GROWTH PROMOTERS

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Abstract

The study was conducted to determine the effect of adding gum and some of their nitrogenous derivatives to the Winka seeds. Derivatives of arabic acid, arabamide, ammonium arabate, derivative of high protein and derivative of low protein were prepared. The physiochemical parameters for these derivatives were examined and comparisons were carried out. The results showed that there were no significant differences between the derivatives in specific rotation and in emulsifying stability. However, there were significant differences between these derivatives in pH, except between arabamide and aminoarabate with arabic acid. There were significant differences between all derivatives in nitrogen (protein content). These derivatives were added in concentration of either 5% or 10% to pots containing Winka seeds while to other pots only water was added. The experiment duration was 10 weeks. The results showed that there was significant difference in the number of plant leaves which were treated with these derivatives. The highest number of leaves was reported for plants treated with arabic acid (C = 5% and C = 10%). There was significant difference in plant height, the highest plant height was reported for plants to which arabic acid (C= 5% and C = 10%) were added. The shortest height was reported for plant to which only water was added. Concerning the dry matter production, significant differences between these treatments were noticed. The highest value was reported for plants which were treated with arabic acid (C = 5% and C = 10%). The lowest value was reported for plant to which only water was added.

Key Words: *Gum Arabic derivatives**,** Plant growth promoters*

1. INTRODUCTION

Plant gums are organic substances obtained as an exudation from fruit, trunk or branches of trees, spontaneously or after mechanical injury of the plant by incision of the bark, or by the removal of a branch, or after invasion by bacteria or fungi. These gums are classes of high molecular weight polymeric compounds, composed mainly of C, H, and O; they are capable of possessing colloidal properties in an appropriate solvent or swelling agent at low dry weight. Natural gums are those derived from plants and animals, which are of rather indefinite composition. They appear to be complex materials associated with plant file processes.

The term Gum Arabic is used to describe a group of naturally occurring polysaccharides which find wide spread industrial applications. It is probably the oldest food hydrocolloid in current use. Gum Arabic is the dried gummy exudation of Acacia Senegal or closely related species (JECFA – FAO, 1990). It is a complex polysaccharide of high molecular weight which occurs as a mixture of calcium, magnesium and potassium salts of arabic acid and is composed

of polysaccharide moieties namely D – galactose, L – arabinose, L – rahmnose, D – glucuronic acid and its 4 – α methyl ether together with a proteinaceous component (Anderson, et al 1968). The gum is highly soluble in water and solution of up to 60 % w/v gum concentration can be prepared. Gum arabic solutions are slightly acidic with maximum viscosity obtained at the neutral pH (Glicksman and Sand, 1983).

Sudan is the dominant producer of commercial gum arabic and supplies about 85 % of the annual world requirements of gum arabic. Due to its reduced surface tension, extremely high solubility in water and low viscosity, gum Arabic find wide applications in food, pharmaceutical, cosmetics, paints, textile, lithography, and electroplating processes. Derivatives of gum arabic considered in this study are: arabic acid, arabamide, ammonium arabate, derivatives of high protein and derivatives of low protein.

Plants grown in Sudan locally known as (Winka) belong to the genus Catharanthus roseus (L). It is endemic to Madagascar, but cultivated and naturalized all over the tropics and subtropics. Many cultivars have been selected and are for sale as in door and/or garden plants. The traditional medicinal use of the plant is responsible for the wide occurrence of the plant at present. The importance of the plant refers to the anti-cancer alkaloids that are found in leaves. Also it has been found that ethanolic extract of flowers, leaves and stem showed insecticidal activity. The major alkaloids that are found are vinblastines mainly in leaves and ajmalicines mainly in roots (Mohammed, 2004).

In this study several derivatives of gum arabic are prepared, their physicochemical characteristics are analysed, and the effect of these derivatives on Winka plant growth is investigated.

2. MATERIAL AND METHODS

2.1 Materials: Authentic samples of *Acacia Senegal* were taken from Gum Arabic Company. These samples were provided in the form of natural nodular form. The gum samples used in this study were collected specially for research work. Care was taken to ensure that they were relatively free from sand, dust and bark impurities. The samples were ground by electric grinder (mesh size No. 10), and stored in labeled plastic containers till further use.

2.2 Methods

2.2.1. Preparation of derivatives of gum Arabic: Derivatives of an *Acacia Senegal* gum were prepared; these were arabic acid, ammonium arabate, arabamide, fraction of high protein and fraction of low protein.

2.2.2. Arabic acid: Preparation of arabic acid was carried out by modification of the method described by Glicksman and Sand (1973). A suitable glass column was packed with about 275g of an Amberlite Resin IR120H. The column was thoroughly washed with a solution of 1:5 HCl: water. Aqueous gum solution (7%) was slowly passed through the column, followed by distilled water. All eluents and washings were acidic, and were collected. The collected solution (arabic acid) was put in a suitable glass container, transferred to an oven at 55-60 ^{o}C until it was dry and transferred to a desiccator for 2-3 days. At that time it was in the form of small glassy fragments. The fragments were powdered using a pestle and mortar, packed in labeled plastic containers for further use in analysis.

2.2.3. Preparation of ammonium arabate and arabamide: Ammonium arabate and arabamide, were prepared by direct neutralization of arabic acid with the solution of 0.1N ammonia

(aqueous), and ammonium carbonate, respectively. These solutions were placed in a suitable glass container, transferred to an oven at 55-60 ^{o}C until it was dry then transferred to a desiccator for 2-3 days. At that time it was in the form of glassy fragments, which were powdered by using a pestle and mortar, packed in labeled plastic containers till further use for analysis.

2.2.4. Preparation of fraction of high protein and fraction of low protein: The method described by Karamalla (1998) was followed to prepare high protein fraction of gum arabic and low protein fraction of gum arabic. The method used 10% concentration of gum solution in flask, then the solution was exposed to air current through rubber pipe until air bubbles were accumulated at the solution surface, then these bubbles were collected and analysed. The bubbles were found to contain high protein level compared to that of the solution.

2.3. Analytical Methods: Moisture Content: Moisture content was determined as described by AOAC (1984). Ash Content: Ash content was determined according to FAO paper No. 49 (1990), as follows: A crucible was heated at 550^{o} C, cooled in a desiccator and weighed (W1). Two grams of sample were accurately weighed in the crucible (W2), which was then ignited at 550^{o} C in electronic muffle furnace for 5 hours.

The sample was then cooled in a desiccator and weighed again (W3).

$$\frac{W3 - W1}{W2 - W1} \times 100$$

Where,

W1 = weight of the empty crucible.

W2 = weight of the crucible + sample.

W3 = weight of the crucible + Ash.

2.3.3. pH Value: pH value was determined in 1% aqueous solution using Beckman aromatic IV pH meter at room temperature.

2.3.4. Viscosity measurements: Viscosity was measured using capillary viscometer (SHOTT GERATE, Type 50/20) using 2% gum solution. The viscosity was measured in cps (centipoise). Then the viscosity was calculated as follows:

$$\text{Reduced viscosity} = \frac{V - V0}{V0 \times C`}$$

$$\text{Intrinsic viscosity} = \frac{V - V0}{V0 \times C}^{C-0}$$

Whereas,

V = viscosity of the solvent.

V0 = viscosity of the gum solution.

C = concentration of the gum solution.

C – 0 = Zero concentration.

The intrinsic viscosity was obtained by extrapolation of reduced viscosity against concentration back to zero concentration.

Specific optical rotation[α] D $^{-T}$: The specific optical rotation was determined for 1% solution on dry weight basis. The samples were filtered using 42 filter papers before carrying out the measurements at room temperature.

These measurements were made using a polarimeter (Perkin-Elmner 353), and distilled water was used as reference. The angle of rotation (α) was displayed on the instrument panel. Then the specific optical rotation was calculated as follows:

$$\text{Specific optical rotation } [\alpha]\, D^{-T} = \frac{\alpha \times 100}{I \times C}$$

Where,

α = Observed angle of rotation.
I = The length of the sample hold (m).
C = Concentration of the sample in Kg^{-3}
T = Temperature.
D = Sodium light.

Nitrogen Content: The micro-Kjeldahl method was used to determine the nitrogen content according to AOAC (1984). Protein content of samples was calculated using nitrogen conversion factor (NCF) of 6.6 (Anderson, 1986) as follows:

Protein % = Nitrogen % x 6.6

Determination of uronic acid: Acid alkali titrimetric method was used in this study to determine the equivalent weight and hence uronic acid content according to Osman, (1993).

2.4 Methods for determination of functionality of gum: Water holding capacity (WHC): One gram of gum sample was accurately weighed in a petri-dish, and then transferred to desiccator (half filled with distilled water) and incubated for a certain length of time viz: 24, 72, 96, 120 and 144 hours. The petri-dish with sample was then reweighed. The increase in weight was expressed as percentage to indicate the water holding capacity of the sample.

Emulsifying Stability (ES): Gum solution of 20% w/v was prepared, and then it was mixed with oil in the ratio of 80:20 w/w, respectively. Then they were mixed using a blender for 1 minute at 18 000 rpm. Then the mixture was diluted in the ratio of 1:1000 and was read at 520 nm. The second reading was taken after one hour. The reading represents emulsifying index. Emulsifying stability was calculated using the following formula:

$$\text{Emulsifying stability (E.S)} = \frac{\text{First Reading}}{\text{Reading after one hour}}$$

2.5 Experiment for determination of the effect of addition of gum *Senegal* and their nitrogenous derivatives on Winka plant growth: An experiment took place in the nursery of the Faculty of Agriculture, University of Khartoum to observe and investigate the effect of addition of gum Senegal and some of their nitrogenous derivatives on Winka plant growth in terms of number of leaves, plant height and dry matter production. Gum Senegal, arabic acid, ammonium arabate, arabamide, fractions of A. Senegal of high protein, fraction of A. Senegal of low protein were added to plastic pots to which Winka seeds were planted.

The compounds were added in concentrations of 5% and 10% aqueous solutions. Each pot received only one kind of these compounds. Each treatment was done in triplicate. Three pots of each to which only water was added were used as control.

Readings of plant height and number of leaves were taken every week for 10 weeks. Ultimately plants were dried at room temperature and weighed for determination of dry matter production.

3. Results and discussion

3.1. Physiochemical characteristics of *Acacia Senegal* gum: Table1 illustrates sample codes used in the study. The results of the physiochemical characteristics of *Acacia Senegal* gum collected in season 1998/99 from El Obeid are shown in Table 2.

Table 1: Sample codes used in this study

Sample No.	Sample code	Name
1-	X1	gum Arabic
2-	X2	arabic acid
3-	X3	ammonium arabate
4-	X4	arabmide
5-	X5	fraction of high protein
6-	X6	fraction of low protein

Table 2: Chemical analysis of *Acacia Senegal* gum and their derivatives

Sample Code	Moisture %	Ash %	Nitrogen %	Protein %	pH	Specific Viscosity %
X1	10.2	3.4	0.3	1.87	4.80	0.45
X2	11.0	0.1	0.27	1.68	2.31	0.82
X3	1.07	0.1	0.81	5.06	5.72	0.74
X4	10.9	0.0	1.23	7.68	6.13	0.76
X5	4.3	3.5	1.52	9.88	4.50	0.79
X6	6.1	3.5	0.08	0.05	4.00	0.03
Max	11.0	3.5	1.52	9.88	6.13	0.82
Min	1.07	0.0	0.08	0.05	2.31	0.03
Mean	7.26	1.767	0.702	4.37	4.58	0.598
StDev	4.11	1.86	0.58	3.85	1.36	0.306

3.1.1. Moisture Content: As shown in Table 2 the value of moisture content is (10.2%) for Gum arabic (X1) which is in close agreement with the value (11.1%) given by Awad Elkarim (1994), and in agreement with the value (10.9) reported by Karamalla et al (1998) and the value (10.1) reported by Ali (2001).

3.1.2. Ash Content: As shown in Table 2 the value of the ash content is 3.4% for Gum arabic (X1) . The ash content is in close agreement with the value (3.1) reported by Anderson (1999), and the value 3.5% reported by Karamalla et al (1998). The value is close to 3.2% reported by Mohammed (2004), and in agreement with the value 3.4% reported by Ali (2001).

3.1.3. pH Value: As seen from Table 2 the pH value 4.80 for Gum Arabic is in close agreement with the value 4.70 reported by Anderson et al (1991) and the value 4.70 given by Mohammed (2004), and in agreement with the value 4.80 obtained by Karamalla et al (1998).

3.1.4. Specific Viscosity: Table 2 shows that the value of the specific viscosity is 0.45 for Gum Arabic which is in close agreement with the value 0.42 given by Mohammed (2004).

3.1.5. Specific Optical Rotation: Table 3 shows values of specific optical rotation of - 32.4 which is in close agreement with the value -29 obtained by Karamalla et al (1998) and the value -33.3 given by Mohammed (2004).

Table 3: Chemical analysis of *Acacia Senegal* gum and their derivatives

Sample Code	Eq. Wt.	Uronic acid %	$[\alpha]^{25}$ SOR*	Tannin content %	WHC	ES
X1	1436.26	13.50	-32.4	0	70.8	1.46
X2	1339.28	14.48	-32.1	0	61.6	1.73
X3	1388.88	13.96	-33.8	0	72.1	0.84
X4	1386.85	13.98	-32.7	0	63.5	0.97
X5	1351.35	14.35	-29.3	0	67.0	1.11
X6	1330.92	14.57	- 30.7	0	71.0	0.93
Max	1436.3	14.57	-29.3	0	72.1	1.73
Min	1330.9	13.5	- 33.8	0	61.6	0.84
Mean	1372.3	14.14	-13.8	0	67.7	1.2
StDev	39.5	0.40	1.6	0	4.4	0.3

* = Specific Optical Rotation

3.1.6. Uronic Acid: As shown in Table 3 the value of uronic acid (13.5) for Gum Arabic is in close agreement with the value 14.4 reported by Ali (2001) and in agreement with the value 13.71 reported by Karamalla et al (1998).

3.1.7. Emulsifying Stability (E.S): As shown in Table 3 the value of emulsifying stability is 16.64 which is not in agreement with the value of 0.91 reported by Mohammed (2004) and the value of 1.01 reported by Ali (2001).

3.1.8. Physiochemical Characteristics of *Acacia Senegal* Gum Derivatives: The results of physiochemical characteristics of gum Senegal derivatives
were shown in Tables 2 and 3. From the Tables it was found that:

-Moisture Content: No significant differences were detected in moisture content between all compounds except for fraction of high protein and fraction of low protein 4.3 and 6.1%, respectively.

- **Ash %:** There was no significant differences in ash% between Gum Arabic and fraction of high protein and fraction of low protein (4.3, 3.5, 3.5, respectively), and also there was no significant difference in ash% between arabic acid and ammonium arabate and arabmide (0.1, 0.1, 0.0)
- **pH Value:** There were significant differences in pH value between Gum Arabic (4.80) and fraction of high protein (4.5) and fraction of low protein (4.00) and also between ammonium arabate (5.72) and arabmide (6.13).

- **Specific Optical Rotation:** There were no significant differences in specific optical rotation between all compounds.
- **Nitrogen and Protein Content:** There were no significant differences between all compounds except between Gum Arabic and arabic acid.

- **Viscosity:** There were significant differences between compounds in viscosity except between arabamide (0.76) and ammonium arabate (0.74), arabic acid (0.82) and derivative of high protein (0.79).
- **Water Holding Capacity:** There were significant differences between the compounds except between Gum Arabic and ammonium arabate and fraction of low protein, and between arabic acid and arabmide.
- **Emulsifying Stability:** There was no significant difference between all compounds in their emulsifying stability.

3.1.9 Effect of addition of gum Senegal and derivatives on growth of Winka plant (number of leaves) The results for the addition of gum Senegal, arabic acid, ammonium arabate, arabmide, fraction of high protein and fraction of low protein in two concentrations (5% and 10%) were presented in Tables 4 and 5.

Table 4: Effect of *Acacia Senegal* gum and derivatives (5%) on Winka plant growth (number of leaves) {the unit of time is week}

Material Added	First	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
Water	…	…	…	2	2	4	6	6	8	8
X1	…	…	…	2	4	4	6	6	8	10
X2	…	…	…	2	4	6	10	14	20	24
X3	…	…	…	…	2	4	4	4	6	8
X4	…	…	…	2	4	4	4	4	6	8
X5	…	…	…	2	4	6	6	6	8	10
X6	…	…	…	2	2	2	2	4	4	6

Table 5: Effect of *Acacia Senegal* gum and derivatives (10%) on Winka plant growth (number of leaves) { the unit of time is week }

Material Added	First	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
Water	…	…	…	2	2	4	6	6	8	8
X1	…	…	…	2	4	4	6	6	8	8
X2	…	…	…	2	4	6	8	12	14	18
X3	…	…	…	2	4	4	4	6	6	8
X4	…	…	…	2	4	4	6	8	8	10
X5	…	…	…	2	4	6	6	8	8	10
X6	…	…	…	2	4	4	6	6	8	8

The highest number of leaves/plant was found to be 24 leaves reported for arabic acid (C=5%) at the end of the 10th week. The lowest number of leaves was found to be 6 reported for fraction of low protein (C=5%) at the end of the 10th week. In addition, there was improvement in the general healthy looking appearance of the plant and leaves of these plants were found to be much green and large in size.

3.1.10 Effect of addition of gum Senegal and derivatives on growth of Winka plant (height): The results for the addition of gum Senegal, arabic acid, ammonium arabate, arabmide, fraction of high protein and fraction of low protein in two concentrations (5% and 10%) on Wink a plant height are given in Tables 6 and 7. The highest value was found to be 18.3 cm reported for Arabic acid (C=5%) at the end of the 10th week. The lowest height of plant

was found to be 3.2 cm reported for water and 5.8 cm reported for fraction of low protein at the end of the 10th week.

Table 6: Effect of *Acacia Senegal* gum and derivatives (5%) on Winka plant growth (height in cm) { the unit of time is week }

Material Added	First	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
Water	…	…	…	0.8	1.5	2.1	2.3	2.5	2.8	3.2
X1	…	…	…	0.9	1.2	2.7	3.4	4.6	5.5	6.9
X2	…	…	…	1.8	2.6	3.4	6.3	9.7	13.2	18.3
X3	…	…	…	0.7	0.9	1.1	2.2	4.4	6.6	8.3
X4	…	…	…	0.8	1.8	2.9	4.3	5.2	6.5	7.2
X5	…	…	…	0.9	1.6	2.1	4.9	7.8	10.6	13.2
X6	…	…	…	0.9	1.1	1.7	2.0	2.5	3.9	5.8

Table 7: Effect of *Acacia Senegal* gum and derivatives (10%) on Winka plant growth (height in cm) { the unit of time is week }

Material Added	First	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
Water	…	…	…	0.8	1.5	2.1	2.3	2.5	2.8	3.2
X1	…	…	…	0.9	1.0	2.1	3.1	4.2	5.4	6.6
X2	…	…	…	1.2	1.9	2.4	5.3	8.6	12.8	16.5
X3	…	…	…	…	2.2	4.6	6.5	8.3	9.0	10.2
X4	…	…	…	0.9	1.6	2.8	3.9	8.5	7.4	9.2
X5	…	…	…	0.7	1.8	2.9	5.7	8.8	10.6	11.6
X6	…	…	…	…	0.7	1.6	2.9	4.7	6.1	6.8

3.1.11Effect of addition of gum Senegal and derivatives on Winka plant weight: Senegal gum, arabic acid, ammonium arabate, arabmide, fractions of high protein and fraction of low protein in two concentrations (5% and 10%) and also water were added to the Winka plant pots. Thereafter, only water was added for 10 weeks.

At the end of this period the weight of plant was determined. The highest weight of plant was reported (22.77 g) for plants which were treated with arabic acid (C=10%) at the end of the 10th week and the lowest value was reported to be 3.3 g for plants which were treated with water only (see Table 8).

Table 8: Effect of *Acacia Senegal* gum and derivatives on Winka plant growth (weight of plant)

Material added	Weight (C=5) in g	Weight (C=10) in g
Water	3.33	3.33
X1	5.41	5.66
X2	18.82	22.77
X3	9.21	13.6
X4	7.33	8.55
X5	13.27	16.76
X6	6.3	7.91

4. CONCLUSIONS AND RECOMMENDATIONS

From the results obtained in this study it was found that the addition of *Acacia Senegal* gum and their nitrogenous derivatives had improved Winka plant growth significantly in terms of number of leaves and height and also in dry matter production. Moreover, the general appearance of the plant was improved.

Observation and results obtained also indicated that only small concen-trations of *Acacia Senegal* gum and their derivatives were enough to improve plant growth.

According to these observations the following recommendations could be derived: Further studies should be carried out to test other nitrogenous derivatives. Future studies have to determine the optimum concentrations, type of soil and method of application. Further studies should be carried out to investigate the effect of *A*cacia Senegal gum and their derivatives on other plants. Trials have to be carried out to investigate the effect of other types of gum on plants.

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