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Full Length Research Paper

Increased gum arabic production after infestation of *Acacia senegal* with *Aspergillus flavus* and *Pseudomonas pseudoalcaligenes* transmitted by *Agrilus nubeculosus*

Sayeda, A. A. Khalil[1], H. A. El Atta[2] and I.M. Aref[2]

[1]Forest National Corporation, Postal code 123543, P.O. Box. 331, Khartoum, Sudan.
[2]Department of Plant Production, King Saud University, Postal code 11451, P.O. Box 2460 Riyadh, Saudi Arabia.

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The aim of this study was to investigate the correlation between the beetle *Agrilus nubeculosus* and gum arabic production by *Acacia senegal*. Some trees were tapped and left open to facilitate infestation by *A. nubeculosus* and others were covered with wire mesh as control. Gum yield, physical and chemical properties of gum were determined for infested and control trees. *A. senegal* infested by *A. nubeculosus* produced significantly more gum than control trees. Infestation also caused significant changes in some physical properties of gum (colour, shape, size, moisture content and optical rotation) and chemical properties (ferrous, calcium, magnesium and nitrogen contents), whereas, no significant difference was recorded in phosphorus and manganese contents. *Aspergillus flavus* Link. and *Pseudomonas pseudoalcaligenes* Monias were isolated from the mouth parts of *A. nubeculosus*. Inoculation of the tapped branches of *A. senegal* with a suspension of *A. flavus* alone or in combination with *P. pseudoalcaligenes*, resulted in highly significant gum yield as compared to the control. It seems that *A. flavus* and *P. pseudoalcaligenes* acted as elicitors that have stimulated the synthesis of gum arabic. *A. nubeculosus* transmitted *A. flavus* and *P. pseudoalcaligenes* to the tapped areas. Hence, the beetle was significantly associated with gum production.

Key words: *Acacia Senegal*, gum arabic, *Aspergillus flavus*, *Pseudomonas pseudoalcaligenes, Agrilus nubeculosus,* inoculation elicitors.

INTRODUCTION

Plant secondary metabolites are important sources of many food ingredients and disease-preventive phyto-chemicals. In recent years, the demand for these products has increased dramatically. The supply of the source plants, however, is often limited because of disease, changes in climate, changes in the economical development, or other problems in the growing regions (Fu, 1998). Gum arabic (INS 414) is the dried gummy exudate from the stems and branches of *Acacia senegal* (L) Willd. or of other related species of Acacia (Family: Leguminosae) (Dondain and Phillips, 1999). It is defined by the FAO/WHO Joint Expert Committee for Food Additives (JECFA) as 'a dried exudation obtained from the stems of *A. senegal* or closely related species of Acacia (family Leguminosae)' (FAO/WHO. Compendium of food additives, 1999). Although, there are many species of Acacia trees botanically, only two species, namely *A. senegal* and *Acacia seyal* are acceptable to the Codex Alimentarius Commission (Al-Assaf et al., 2003; Dondain and Phillips, 1999; FAO/WHO. Compendium of food additives, 1999). Gum arabic has wide Industrial uses as a stabilizer, thickening agent and emulsifier, mainly in the food industry (example, in soft drinks syrup, gummy candies and marshmallows), but also in the textile, pottery, lithography, cosmetics and pharmaceutical industries (Verbeken et al., 2003). It has been approved for use as food additives by the US Food and Drug Administration and is on the list of substances

*Corresponding author. E-mail: sayeda_khalil@yahoo.com.

that is a generally recognized as safe (GRAS) with specific limitations (FDA Proposed affirmation of GRAS status for gum arabic, 1974). In folk medicine, gum arabic has been reported to be used internally for the treatment of inflammation of the intestinal mucosa, and externally to cover inflamed surfaces (Gamal el-din et al., 2003). It is an edible, dried, gummy exudate that is rich in non-viscous soluble fiber (Williams and Phillips, 2000). Clinically, it has been tried in patients with chronic renal failure, and it was claimed that it helps reduce urea and creatinine plasma concentrations and reduces the need for dialysis from 3 to 2 times per week (Suliman et al., 2000). Despite the fact that gum arabic is widely used as a vehicle for drugs in experimental physiological and pharmacological experiments, and is assumed to be an ‘‘inert” substance, some recent reports have claimed that it possesses anti-oxidant, nephroprotectant and other effects (Ali et al., 2008; Gamal el-din et al., 2003). Pharmacologically, gum arabic has been claimed to act as an anti-oxidant, and to protect against experimental hepatic, renal and cardiac toxicities in rats (Ali et al., 2009). Analysis of gum arabic has indicated that it consists of three distinct components. Fraction 1, which represents 88.4% of the total, is an arabinogalactan with molecular mass 2.79×10^5 and is deficient in protein. Fraction 2, which represents 10.4% of the total, is an arabinogalactan protein complex with a molecular mass of 1.45×10^6, containing ~50% of the total protein. It is envisaged that on average each molecule of fraction 2 consists of five carbohydrate blocks of molecular mass $\sim 2.8 \times 10^5$ covalently linked through a chain of amino acid residues. Fraction 3 represents only 1.24% of the total gum but contains ~25% of the total protein and has been shown to consist of one or more glycoproteins. Whereas the proteinaceous components of fractions 1 and 2 contain predominantly hydroxyproline and serine, this is not the case for fraction 3 (Randall et al., 1989). Gum arabic is a branched-chain, complex polysaccharide, either neutral or slightly acidic, found as mixed calcium, magnesium and potassium salt of a polysaccharidic acid. The backbone is composed of 1,3-linked b-D-galactopyranosyl units. The side chains are composed of two to five 1,3-linked b-D-galactopyranosyl units, joined to the main chain by 1,6-linkages. Only a few plant species are cultivated at present to obtain gums used in the food industry as additives; most of them belong to the Leguminosae family. Some examples are: *A. senegal*, source of acacia or arabic gum; *Astragalus* spp., source of tragacanth; *Cyamopsis tetragonolobus*, source of guar gum; *Ceratonia siliqua*, source of locust bean gum (Ibañez and Ferrero, 2003). The most commonly recognized is arabic gum, but a wide range of other tree exudates is used for variety of uses in their countries of origin, such as mesquite gum (Anderson and Farquhar, 1982; Anderson, 1990; Vernon-Carter, et al., 2000; Williams and Phillips, 2000). *A. senegal* trees grow widely across in Sahelian countries of Africa, especially in Sudan, and gum arabic, as a food additive, has been an important item of commerce since ancient times (Glicksman, 1969). The gum belt in Sudan provides a natural buffer zone between the desert in the North and the more fertile agricultural lands in the South. Deforestation within the gum belt has lead to an increase in desert encroachment and threatens agricultural production (IEED and IES, 1990; Keddeman, 1994; Olsson and Ardö, 2002). Following the Sahel drought of the 1970s and 1980s a southward shifts in the tapping of gum has been reported (IEED and IES, 1990) as people moved from the more fragile environment in the northern parts of the gum belt to the less fragile and better environment of the south. Over the last three to four decades, the land use practices have moved from a rotation with long fallow periods (15 to 20 years) of gum cultivation interspersed with short period of cultivation (4 to 6 years) towards a more or less continuous cultivation (Barbier, 2000). Gum arabic agriculture plays an important role as a cash crop produced in the traditional rain-fed areas of North Kordofan in western Sudan (El-Dukheri, 1997). *A. senegal* trees are managed in the Sudan in an agroforestry system known as the bush-fallow system (Obeid and Seif El Din, 1970). However, the recent disruption of this traditional agroforestry system due to the misuse of land, drought and desertification is considered to be among the main factors that have led to fluctuations in gum arabic yield and the consequent instability of supply (Awouda, 2000; Seif El Din, 1995). It has been reported that rainfall and temperature have an effect on the time of tapping the tree and consequently on gum yield (Abdel, 1978; Awouda, 1973; Muthana, 1988). Apart from drought, desertification and mismanagement, gum arabic production also varies as a result of complex factors in the physical, biological and socio-economic environments. The impact of all or some of these factors on gum arabic production has been reported (Abdel Rahman, 2001). There is still an information gap regarding the factors that control gum arabic yield. The International Institute for Environment and Development and the Institute of Environmental Studies (IIED and IES, 1989) reported that rainfall and its distribution pattern together with the minimum temperature during tapping and gum picking, and the relative humidity, are the main factors affecting gum arabic yield. However, the relationship between gum arabic yield and rainfall is complex and the available information is sparse and imprecise (IIED and IES, 1989). Large-scale planting programs with the help of local communities have been implemented since the early 1980s to restock the gum arabic belt in order to curb desertification and to improve the gum arabic yield and production in western Sudan (Afaf et al., 2007). Gummosis is widespread in plant kingdom and is known to be produced by stress conditions such as heat, drought and wounding. Gums form a barrier at lesions hindering the invasion of micro-organisms. Fungal and bacterial infections have been

linked with the synthesis process, although, this has by no means been proved (Greenwood and Morey, 1979; Ghosh and Purkayastha, 1962; Luckner, 1990).

The community inhabiting the gum producing areas in the Sudan, believe that high gum yield by *A. senegal* is directly correlated with the abundance of certain beetles locally known as *Al Garraha* (Injector) that is in years when the occurrence of these beetles was high, gum production was maximum (Osman, 1993). Several gum producers interviewed in Kordofan State (western Sudan), firmly believe that *Al Garraha* (Injector) pierces holes into the tapped branches of *A. senegal* (El Khalifa et al., 1989). This study aimed at testing the hypothesis that gum arabic production is correlated with certain beetles that infest the freshly tapped branches of *A. senegal*. The study is the first attempt in this direction.

MATERIALS AND METHODS

The study area

The study was carried out in El Himaiyra forest plantation (13°19´05 N- 13°09´58 E, 545 m above sea level) 12 km north to El Obeid (Capital of Northern Kordofan State). Rainfall varies between 250 to 450 mm from May to October; the soil is uniform deep reddish sand with little textural differentiation in the profile. The climate is long hot season, rainy season with scattered irregular rains and short winter.

Identification of *Al Garraha* (Injector)

Samples of the adult *Al Garraha* (Injector) were collected from the tapped branches of *A. senegal* and sent to the British Museum of Natural History for identification. The insect was identified as *Agrilus nubeculosus* Fairm (Coleoptera, Buprestisae).

The effect of *A. nubeculosus* on gum arabic yield

A total of 120 healthy *A. senegal* trees (5-9, 10-14 and 15-20 years old) with mean diameters of 24.75, 33.2 and 39.9 cm, respectively and 2.7, 3.4 and 4.3 m mean height, respectively were selected randomly from El Himaiyra forest. Each tree class comprised 40 trees. In each tree, two branches were selected randomly and tapped with a sharp knife locally known as *sonky* (20 cm long and 5 cm wide with a 3 m long wooden handle). Tapping removes only the outer bark without injuring the cambium. Tapping was carried out at two intervals, the first tapping immediately after the rainy season (November) and the second tapping in summer (February). Half of the tapped trees in each class were left open (Group 1), whereas the remainder (Group 2) were covered with a wire mesh to prevent insect infestation and served as control. The produced gum arabic was collected from trees after 40 days from tapping, weighed and kept in a refrigerator at 10°C for further analysis.

Physical properties of gum arabic

Physical properties of gum arabic produced by both groups of trees were determined as follows:

Viscosity: Viscosity of gum collected from all trees was determined using a viscometer (Brook-Field model DV-1+1). One gram of gum was dissolved in 100 ml distilled water in a 500 ml conical flask to make 1% solution in reference to 4% NaOH. This was replicated three times for each group of trees (1 and 2). Viscosity of gum was measured in cps (centipoises) and calculated as follows:

$$\text{Reduced Viscosity} = V\text{-}V_o / V_o * C$$
$$\text{Intrinsic Viscosity} = (V\text{-}V_o) * C_o / V_o * C$$

Where, V is the viscosity of solvent; V_o is the viscosity of gum solution; C is the concentration of the gum solution and C_o is the Concentration of solvent.

Moisture content: Two gram samples of gum from each group of trees (1 and 2) were placed on crucibles and oven dried in an oven at 105°C for 5 h until constant weights were obtained, and by subtraction the moisture content percent was calculated (FAO, 1990). Samples from each group of trees were tri-replicated.

Ash value: A crucible was heated at 55°C, cooled in a dissector and weighed. Two grams of each gum sample were placed in a crucible and ignited at 550°C in a Heraeus electronic muffle until free from carbon, cooled in a dissector and weighed. This was replicated three times for gum from each group of trees. The ash value was then calculated as follows (FAO, 1990):

$$(W_3 - W_1 / W_2 - W_1) * 100\%$$

Where, W_1 is the weight of the empty crucible; W_2 is the weight of crucible + sample and W_3 is the weight of crucible + ash

Specific optical rotation: Optical rotation of gum from each group of trees was determined using ADP 220 polarimeter. The specific optical rotation (SOR) was determined for 1% solution (w/v) of gum in distilled water on dry weight basis using an optical activity polarimeter fitted with a sodium lamp and a cell path length of 20 cm. The solutions were passed through a No. 42 filter paper before carrying out the measurements at room temperature (30.2 to 33.4°C). Readings were taken 5 times. Tri-replicates of gum samples were used. The specific optical rotation was calculated as follows:

$$SOR = \alpha * 100 / (C \times L)$$

Where, α is the observed optical rotation; C is the concentration of the solution and L is the length of the Polarimeter tube.

Chemical properties of gum arabic

Gum arabic produced by trees of each group was chemically analyzed.

Nitrogen content: The nitrogen content of gum was determined according to the Micro-Kjeldahl method (A.O.A.C. Official Methods of Analysis, 1984). Samples of gum weighing 0.2 g were transferred to a digestion flask of a Micro-Kjeldahl apparatus. One gram of a powdery mixture of potassium sulphate and cupric sulphate (10:1) and 3.5 ml of concentrated nitrogen-free sulphuric acid were added. The flask with the contents was then heated over an electric heater until the solution attained a clear blue colour and the wall of the flask was free from carbonized materials. The contents of the flask were then transferred to a steam distillation unit and 20 ml of 40% sodium hydroxide solution were added. The distillate was collected in 10 ml of 2% boric acid to which 3 drops of methyl red/methyl blue indicators were added and titrated against 0.01 N HCl (A.O.A.C. Official Methods of Analysis, 1984). Each sample of gum from both tree groups (1 and 2) was tri-replicated. The gum nitrogen content was calculated as follows:

$$N\ \% = \{(M_1 - M_2) * N * 14 / S * 1000\} * 100$$

where, M_1 is Thevolume (ml) of HCl that neutralized the sample distillate; M_2 is the volume (ml) of HCl that neutralized the blank distillate; N is the normality of HCl titrant and S is the sample weight

Cationic composition: The cationic composition of gum obtained from group 1 and 2 trees was determined (FAO, 1990). One milliliter extract of each sample of gum was placed in 50 ml distilled water in a conical flask. Three drops of NaOH, with a small amount of peroxide indicator, were added with 0.01 N EDTA to violet end point. The contents of the flask were titrated. Calcium, magnesium, manganese, iron and phosphorus, in the diluted extracts were determined volumetrically by titration against EDTA. The percentages of Fe^{3+}, Ca^{2+}, P^{3-}, Mg^{2+} and Mn^{2+} were calculated as follows:

Atomic Absorption = V*N EDTA*1000/Volume of extract (mg/l).

Where, V is the volume of EDTA; N is the m normality of EDTA = 0.01; Mg/l * equivalent weight = mg/l (ppm); Molecular weight * 100/1000000 * weight of sample = Ca^{2+} or Mg^{2+} etc and mwt = molecular weight of element.

Extraction of material from *A. nubeculosus*

Twenty (20) adults of *A. nubeculosus* were randomly selected, rinsed thrice in sterile distilled water. A small loop was inserted into the mouth of *A. nubeculosus* to collect some of the liquid contents. The mouth contents were then streaked on Petri dishes containing sterile PDA and Nutrient Agar and incubated at 30 ± 2°C in an attempt to isolate any microorganism (s). Gram staining and oxidase test were performed to assist identification of bacteria (Awouda, 2000).

Identification of cultures

On PDA, a fungus was isolated, purified and identified. As for the bacterium isolated on Nutrient Agar, the following tests for identification were carried out (Collins et al., 1995).

Gram staining: A loop-full of bacterial colony from the slanted stock culture was mixed with a drop of sterile distilled water at the centre of a clean glass slide. A thin film was obtained and left to dry. It was then fixed by passing over a flame 5 times. The dry smear was then flooded with crystal violet-ammonium oxalate prepared by dissolving 2 g of crystal violet in 20 ml of 95% ethanol and 0.8 g ammonium oxalate in 80 ml of distilled water. The excess dye was washed off with gently running tap water. The smear was decolourized with 95% ethanol. It was then stained with safranin (0.25 g safranin + 10 ml ethanol and completed to 100 ml by the addition of water) for 30 s. Excess safranin was washed off with running tap water, and the smears were blotted dry and finally examined under the microscope. Bacterial cells which were violet coloured were recorded as Gram-positive and those which were red are Gram-negative.

Oxidase test: Two milliliter of 10% of aqueous methyl phenyl-enediamine dihydrochloride (w/v) was added to a filter paper in a sterile Petri dish. Using a platinum loop, a heavy amount of growth from a 24 h old culture on Nutrient Agar was added to the filter paper and quickly spread. Development of a purple colour within 10 s was recorded as a positive reaction indicating the presence of cytochrome oxidase, otherwise the reaction was considered negative (Collins et al., 1995).

Description and identification of microorganisms

The cultures prepared were dominated by a fungus and a bacterium and were identified. As for the fungus: The colony diameter was 4.0 to 4.5 cm, yellowish-green becoming green with age on obverse view, and creamy-yellow on reverse view. The head: Radiating, becoming loosely columnar with age. The stipe: Long, verrucose and hyaline. The vesicle: Dome-shaped. Medullae: Present and small. Phialidae: small ampullifprm. Conidia: Globose to sub-globose, usually rough yellowish green. Accordingly, the fungus was identified as *Aspergillus flavus* Link. The bacterium colony was negative for Gram stain and oxidase test and identified as *Pseudomonas pseudoalcaligenes* Monias.

The effect of *A. flavus* and *P. pseudoalcaligenes* on gum production

Stock cultures of *A. flavus* on PDB (potato dextrose broth) and *P. pseudoalcaligenes* on NB (nutrient broth) were prepared and incubated at 30 ± 1°C for 3 weeks. A total of fifty healthy 5 to10 years old *A. senegal* trees in the study area were selected randomly and 2 branches/tree were tapped and immediately treated as follows:

(a) Inoculated with *A. flavus* (A).
(b) Inoculated with *P. pseudoalcaligenes* (P).
(c) Inoculated with both *A. flavus* and *P. pseudoalcaligenes* (AP).
(d) Not inoculated and left open (N).
(e) Not inoculated and covered with a wire mesh and served as control trees (group C).

Inoculation was carried out by taking 10 ml of each liquid culture by using a syringe and sprayed on the surface of the freshly tapped branch. Ten trees were used for each treatment. Statistical analysis was carried out using ANOVA, Tukey's test for mean separation and T-test of the SAS computer software. Significance tests of means were determined at the 5% level.

RESULTS

Activity of *A. nubeculosus*

Two to eight hours after tapping, the insect pierces holes into the tapped branches of *A. senegal* by pushing a sharp posterior organ into the freshly tapped surface. The beetle secrets a dirty greenish substance over the wounded area. Usually 1 to 3 insects were found on the tapped branch. The insect took flight from the tapped area of the branch after 1 to 4 h leaving behind a more or less rounded dirty green spots.

The effect of *A. nubeculosus* on gum yield

Gum production was considerably higher in group 1 trees (infested by *A. nubeculosus*) as compared to group 2 trees (control) in all of the three age classes studied (5-9, 10-14 and 15-20 years old), after both first and second tapping. These differences were highly significant (P = 0.0001) (Tables 1 to 3).

Physical properties of gum arabic

Table 4 summarizes description of colour, shape and size

Table 1. Mean gum arabic yield/tree (g) of 5-9 years old *A. senegal* .

Tree group	Mean/tree	T-value	P
First tapping			
1 (infested)	102.4	-60.80	0.0001
2 (control)	3.5		
Second tapping			
1	99.2	-60.80	0.0001
2	2.1		

Means were computed for 20 replicates in each treatment.

Table 2. Mean gum arabic yield/tree (g) of 10-14 years old *A. senegal* .

Tree group	Mean/tree	T-value	P
First tapping			
1 (Infested)	123.3	-24.47	0.0001
2 (Control)	2.5		
Second tapping			
1 (Infested)	110.6	-63.21	0.0001
2 (Control)	2.7		

Means were computed for 20 replicates in each treatment.

of gum arabic obtained from trees in groups 1 and 2. Colour and shape of the produced gum were different between the two groups of trees. Gum produced by infested trees was more or less spherical or rounded and red in colour, whereas that from control trees was irregular in shape and white creamy. Moisture content of gum was significantly (P=0.01) higher in the control trees as compared to those infested. The specific optical rotation of gum was significantly (P=0.001) greater in infested than in control trees. However, there was no significant difference in viscosity and ash contents of gum (Table 4).

Chemical properties of gum arabic

Infestation of *A. senegal* with *A. nubeculosus* had affected the chemical constitution of gum arabic. Ferrous, calcium, magnesium and nitrogen contents were significantly affected, whereas, no significant effect was recorded in phosphorus and manganese contents (Table 5).

Table 3. Mean gum arabic yield/tree (g) of 15-20 years old *A. senegal* .

Tree group	Mean/tree	T-value	P
First tapping			
1 (Infested)	146.2	-13.11	0.0001
2 (Control)	2.3		
Second tapping			
1 (Infested)	111.6	-65.15	0.0001
2 (Control)	1.3		

Means were computed for 20 replicates in each treatment.

Table 4. Physical properties of gum arabic form infested and control *A. Senegal*.

Tree group	Moisture (%)	T-value	P
1	7.66	7.11	0.02
2	8.70		
	Specific optical rotation (º)		
1	-33.23	31.90	0.001
2	-29.40		
	Viscosity (cps)		
1	1.56	- 1.67	0.24*
2	1.30		
	Ash (%)		
1	3.1	- 2.60	0.12*
2	2.8		

1 = Infested, 2 = control, * not significant.

The effect of *A. flavus* and *P. pseudoalcaligenes* on gum production

Inoculation of the tapped branches of *A. senegal* with *A. flavus* and *P. pseudoalcaligenes* alone or in combination significantly (P=0.0001) increased gum production as compared to control trees (Table 6). Gum production was significantly more from trees inoculated with *A. flavus* (249.6 g) than from those inoculated with *P. pseudoalcaligenes* (136.2 g), both *A. flavus* and *P. pseudoalcaligenes* (144.3 g), not inoculated and left open (60.5 g) and not inoculated and covered with a wire mesh (3.4 g). These differences were highly significant (Table 7).

Table 5. Chemical properties of gum arabic from infested and control *A. Senegal*.

Tree group	Ferrous %	T-value	P
1	$1{,}66*10^{-3}$	-5.80	0.02
2	$1.56*10^{-3}$		
	Calcium (%)		
1	0.50	-10.97	0.05
2	0.41		
	Magnesium (%)		
1	$4{,}2*10^{-2}$	37.00	
2	$7.9*10^{-2}$		0.001
	Nitrogen (%)		
1	0.30		
2	0.35	8.66	0.01
	Phosphorus (%)		
1	0.24	-1.99	> 0.05*
2	0.23		
	Manganese (%)		
1	$6.13*10^{-5}$	- 3.63	> 0.05*
2	$8.17*10^{-5}$		

1 = infested, 2 = control, *Not significant.

Table 6. Effect of inoculation on gum production by *A. senegal* (ANOVA).

Source	d.f.	SS	MS	F value	P
Model	4	355867.16	88966.79	7509.87	0.0001
Error	45	533.09	11.84		

$R^2 = 0.99$.

Table 7. Gum produced by inoculated and control *A. Senegal*.

Treatment	Mean gum (g)
A	249.66 a
P	144.33 b
AP	136.19 c
N	60.53 d
C	3.4 e

Means were computed for 10 replicates in each treatment.
Means followed by the same letter are not significantly different according to Tukey's test.
A = Inoculated with *A. flavus*, S = inoculated with *P. pseudoalcaligenes*, AS = inoculated with both *A. flavus* and *P. pseudoalcaligenes*, N = not inoculated and left open, C = not inoculated and covered with a wire mesh and served as control (group C).

DISCUSSION

A. senegal trees infested by *A. nubeculosus*, produced significantly more gum arabic than control trees. Most physical and chemical properties of gum arabic were different when infested trees were compared with the control. Gum from normally tapped *A. senegal* was different from trees infested by wood-borers in two important aspects concerning some of the peripheral end group positions involving rhamnose and glucuronic acid residues (Anderson and Dea, 1967). This was substantiated by the fact that inoculation of the tapped areas of *A. senegal* with *A. flavus* alone or in combination with *P. pseudoalcaligenes*, resulted in a highly significant increase in gum arabic production as compared to the control. Both *A. flavus* and *P. pseudoalcaligenes* were isolated from the mouth of the adult *A. nubeculosus*. Hence, the beetle is basically a vector that mechanically transmitted both microorganisms. These microorganisms might have exerted stress on *A. senegal* trees that probably modified some physiological processes as part of the defensive mechanism of trees against infection. It has been suggested that gum cysts development was preceded by various changes in the xylem and phloem induced by physiological disturbances in the tree due to some pathological conditions caused by bacterial activity (Ghosh and Purkayastha, 1962). In almond plants, gum formation was attributed to the diffusion of fungal toxins (Frisullo and Graniti, 1985). However, non of these studies have reported a particular microorganism which might be directly or indirectly involved in the process of gum production. It is probable that *A. flavus* and *P. pseudoalcaligenes* acted as elicitors. Elicitors are compounds that stimulate the synthesis of phytoalexins and various secondary metabolites in plants. Elicitors such as fungal wall materials, plant and microbial polysaccharides and some chemicals increase secondary metabolite production in various plant cell and tissue culture systems. Several studies have shown that elicitation increased the production of many food ingredients in various types of plant cell and tissue culture (Figuereido, 1990; Johnson et al., 1991). Biotic elicitors are classified as complex or defined depending on their origin and molecular structure. Elicitors with defined composition include carbohydrates, oligosaccharides, peptides, and elicitors with complex composition include yeast cell wall, mycelia cell wall and fungal spores (Radman et al., 2003). The greatest gum production by *Prosopis laevigata* nodal explants cultures occurred when the culture medium was inoculated with the fungal mycelium of *A. nidulans* and bacterial biomass of *P. pseudoalcaligenes* in combination with an incubation temperature of 35°C. These treatments were non-significantly different among themselves, but were significantly different from the rest of the

treatments (Orozco-Villafuerte et al., 2005). This study shed the light on the link between *A. nubeculosus* and gum arabic production. The beetle acted as a vector that transmitted a fungus (*A. flavus*) and a bacterium (*P. pseudoalcaligenes*) which both resulted in a considerable increase in gum production and changes in some physical and chemical properties of gum arabic. The exact process behind quantitative as well as qualitative effect of these microorganisms on gum arabic needs further research. Also, there is a need for this technology to be processed in a suitable form and transferred to the farmers in the gum arabic belt.

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