

Full Length Research Paper

Effect of methyl jasmonate on *Acacia senegal* (Hashab trees) production and characteristics of gum

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Received 24 June, 2020; Accepted 15 October, 2020

The study aimed to investigate the effect of the application of different concentrations of Methyl Jasmonate (JA-Me) on yield and quality of gum in Hashab (*Acacia senegal*) trees. Moreover, also studied the anatomy of the treated trees, and characteristics of gums produced. The study was carried out in two locations, Gedarif State (high rainfall area-clay soil) and North Kordofan State (low rainfall area- sand soil) in eastern and western Sudan respectively. JA-Me was applied at three different concentrations (50, 100 and 150 mg/L) as foliar spray and covered with plastic bags for 4-16hours to allow the JA-Me to enter into the tissues. The results showed that a suitable concentration of JA-Me for maximum gum yield per tree was 100 mg/L. The gum ducts in *A. senegal* trees treated by JA-Me was wider compared to those of untreated (control) trees and finally one large duct was formed, where gum appeared as drops on the bark of the tree. There was no effect of JA-Me (100 mg/L) application on gum shape and colour when compared to the untreated control. The gum produced was red coloured spherical nodules with high solubility in cold water. The significance of the study and its outcomes is very important to treat the trees in areas of gum Arabic trees which have less capability to produce gums due to climatic changes and drought occurred in the areas of Acacia belt in Sudan. The study focused on areas of high production affected by environmental changes.

Key words: Sudan, gum Arabic, gum yield, natural hormones, gum duct, gummosis, Jasmonate.

INTRODUCTION

Jasmonates are a new group of additives plant used to enhance the biological activities and to regulate the growth of some fruit crops like, peach, cherry and apricot, and also promote the gum production. Jasmonates are involved in plant wound responses and defence against insects and fungal elicitors (Mabood et al., 2005). The gummosis in trees in response to the stresses regulated

by jasmonates is similar to the mode of action of ethylene. Also, it stimulates anthocyanin accumulation in peach shoots (Saniewski et al., 1998). The effect of JA-Me on the induction of gum was studied in relation to peach (*Prunus persica* Batsch), and the studies have shown the induction of various potential defense-related proteins in white spruce by jasmonate treatment (Richard et al., 1999,

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Figure 1. Sudan Map , Site I in Gadaref State and Um Rawaba Area Site II in North of Kordofan State.

2000; Lapointe et al., 2001).

This study was carried out based on the areas of less production, and the effect of climate change and other unknown reasons. It is important to find a way to increase the production due to the dependence of local communities on this tree and the use of the product for income generation and is considered as an economic tree in Sudan. The main objectives of this study; were to assess the effect of JA-Me on gum production of Hashab; (*Acacia senegal*) trees, characteristics of gums produces, and to study the anatomy of the trees.

MATERIALS AND METHODS

Study area

Site I (Gadaref State)

This study was carried out in El Rawashda and Wad Kabo Reserved Forest which is situated at approximately latitude 14° 15'N and longitude 35° 45'E. These forests occur in southern central clay plains at 650 m altitude to the Atbara River. The elevation of El Rawashda Forest is about 540-550 m. The site is characterized by

low rain fall savannah woodland on claysoil where *A. senegal*, *Acacia mellifera* and *Acacia seyal* are the dominant tree species.

Site II (Kordofan State)

Kordofan State located at Western of Sudan which is known as drought areas, situated at approximately latitude 14° 22'N and longitude 29° 32'E. The state is surrounded by White Nile State and Darfur States. The site occurred in semi desert zone and characterized by sandy soil, locally called *qoz*, and the communities depend on rainfed agriculture and gum production from the trees of *A. senegal*. The study area located within the gum belt which is crossed Sudan from East to West (Figure 1).

Treatment of trees by JA-Me

The forest was planted and divided by compartments, each compartments were arranged in a randomized complete block design. Ten trees with more or less the same age, height, diameter and morphological shape in each block were selected randomly and tapped with a tool called "sonky" and labeled. The JA-Me solution was applied as a foliar spray, and the solution was prepared by dissolving JA-Me in the wetting agent water + alcohol. Two experiments were conducted.



Figure 2. Black Polythene bags used for the protection of the inoculated branches of *A. senegal* trees.

Experiment I

The method described by Vincent et al. (2001) was followed for the application of JA-Me solution. The solution was applied on both sides of the trunk surface of the tree. Two squares (10×12 cm) were made by cutting 0.5 m of the bark with sonky (local tapping device) in two parts. The two parts were coated with prepared 100 mg/L JA-Me in water with 0.1% Tween 20, using a cotton pad for wetting the bark for 5 min.

Experiment II

Trees were originally planted at a spacing of 4 m × 4 m. Three 1 ha blocks of a uniform tree density and stand structure (compartments) at both experimental sites were selected randomly, enumerated, measured and classified according to size. The trees were selected randomly, and two branches per tree were tapped. Forty healthy trees of the same size and age were selected randomly in the two different sites in the same forest for treatment of JA-Me as a foliar spray. The concentration prepared by using amount of JA-Me in mg in one liter of distilled water using the 50, 100 and 150 mg (Molarity calculation). The first ten trees were treated with a concentration of 50 mg/L of JA-Me, and the second was treated with a concentration of 100 mg/L, third was treated with 150 mg/L and the last ten trees were left untreated as a control. After spraying branches with JA-Me, the leaves were covered with black polythene bags immediately, and left for 4-16 h (Figures 1 and 2). The gum was collected after two weeks.

Anatomy of treated branches

Preparation of stem samples for microscopy

Method 1

Samples of Hashab branches were collected and cut in strips of 20 mm length and 4 mm width and dropped in fixative 2%

paraformaldehyde and 1.25% glutaraldehyde buffered in 50 mmol/L L-piperazine-N-N'-bisacid, pH 7.2). The above strips were left for one night at room temperature, then rinsed with the same buffer, the strips were sectioned (1 μm thick), were cut with a diamond knife, dried onto gelatin-coated slides, and stained with Stevenel's blue. A drop of Canada balsam was placed on the sections followed by a cover slip that was sealed to the slide with nail polish. These sections branches were examined and photographed with photomicroscope.

Method 2

Three to six pieces of Hashab logs from branches with size 5-8 cm in length were cut from stems and branches of treated trees. The pieces were transported directly from the field to the laboratory and preserved in the formaldehyde (10 ml of 40%), ethanol (50 ml of 95%) and glacial acetic acid (5 ml) (FAA) (Figure 3). The preservation period was 7-10 days before sectioning. Staining was done by using the safranin-Fast green type. Clove oil mixed with ethanol (1:1. v/v), series of dilutions (50, 70, 90 and 95%) were prepared. After preparation of sections, by using the slide – microtome and staining the permanent section slides of different treatments were prepared, and examined microscopically to study the structure of gum ducts in all treatment (Sass, 1958).

Collection of gum

After two weeks the gum samples from each treatment were collected per tree and weighed.

Gum characteristics

Nitrogen determination

The nitrogen content of the gum from treated and untreated trees was determined according to a microKjeldahl method AOAC (1984).



Figure 3. Samples of *A. senegal* branches used for anatomical study.

Viscosity determination

The viscosity of gum from all treated and untreated trees (control) was determined by using a Brook-field model DV – 1+1 viscometer. One gram of gum was dissolved in 100 ml of distilled water in a conical flask to make a 1% solution, about 4% of NaOH solution. The solutions were filtered through 3 μ m Millipore filter into clean containers and the viscosity determined using a Cannon Ubbelohde (M130) semi micro dilution viscometer size 75. The viscometer was cleaned by washing with distilled water and dried in acetone. Exactly 2ml samples were pipetted into the reservoir and the viscometer was placed into the holder and inserted into a constant temperature water bath set at 25°C. The initial relative viscosity was determined. Three subsequent readings for the flow time were taken. Further dilutions of the samples were made *in-situ* by adding appropriate amounts of the solvent and the flow time for each concentration was determined as described previously. This was replicated three times for all the gum samples using a viscometer and the viscosity was expressed in centipoises (cps). The intrinsic viscosity was determined according to the following equations:

Relative viscosity

$$\text{Reduced Viscosity} = \{V - V_0\} / V_0 \times C$$

$$\text{Intrinsic Viscosity} = \{V - V_0\} C_0 / V_0 \times C$$

Where: V = viscosity of the solvent.

V_0 = viscosity of the gum solution.

C = concentration of the gum solution.

C_0 = concentration of solvent.

Specific optical rotation

The specific optical rotation of gum collected from all treatments and control trees was determined by ADP 220 Polarimeter according to AOAC (1984).

Ash value

The ash value of gum from both control and treated trees was determined according to FAO (1990).

Elemental analysis of the produced gum

The cationic composition of gum produced from both treated and untreated trees was determined according to AOAC (1984). One milliliter extract of each sample of gum was placed in 50 ml of distilled water in a conical flask. Three drops of NaOH, with a small amount of peroxide indicator were added with 0.01N EDTA to the 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 percentage of Fe^{++} , Ca^{++} , P^{3-} , Mg^{++} , and Mn^{++} were calculated as follows:

Fe^{++} , Na^+ , Ca^{++} , P^{3-} and Mg^{++} :

Atomic Absorption = $V \times N \cdot \text{EDTA} \times 1000 / \text{Volume of extract} = \text{mg/L}$

Where:

N = normality of EDTA = 0.01

V = volume of EDTA used

Mg/L \times equivalent wt = mg/L (ppm)

m.wt $\times 100/10^7 \times$ weight of the sample = Ca⁺⁺ or Mg⁺⁺.....

Where: m.wt = molecular weight of element

Statistical analysis

The data checked for homogeneity prior to statistical analysis, and then the statistical analysis was performed with the JMP (3.2.2) statistical software by SAS (JMP 1970s) for gum yield comparison among all the treatment. One way ANOVA and two-way ANOVA were used for all replication in a randomized complete block design. When significant differences were detected, a comparison of all means values was done by Tukey-Kramer HSD all Duncan at Alpha =0.05 combined regression modeling for comparison between the effect of JA-Me and season was also used.

RESULTS AND DISCUSSION

The results presented in Figures 4 and 5 show that the highest amount of gum yield per tree was obtained from trees treated with JA-Me 100 show significant result when compared with JA-Me 50, JA-Me 150 and untreated control. These results are important for determining the appropriate concentration to increase the production of Hashab trees. The effect of 100 mg/L JA-Me on *A. senegal* trees enhanced the flow of gum from the surface bark. Figure 5 shows that when *A. senegal* trees were treated with 100 mg/L JA – Me, gum was exuded from all the surface bark. The produced lumps are pale yellow and relatively large size.

As shown in Figure 6 only JA-Me at 100 mg/L affected significantly the gum production per tree ($P < 0.0001$). There is no significant effect of seasons on gum yield per tree ($P = 0.13$). The survival and growth of the *A. senegal* trees are negatively affected by the frequent drought accentuated with intensive tapping causing a drastic reduction in gum production per area. There is strong belief among local farmers that *A. senegal* trees infested by the insect *Agrilus nubeculosus* (Garaha) produce more gum. The ecology of the insect is yet unknown, but preliminary investigations have shown that there are two microorganisms associated with this insect and might induce gum exudation as a defensive mechanism (Khalil, 2003, Khalil et al., 2011).

This study showed that the JA-Me can induce gum in *A. senegal* trees tapped stems and branches when applied at the concentration of 100 mg/L as a foliar spray, when compared with the concentrations 50, 150 mg/L and control. It induced significant gum yield per tree. This is in agreement with the findings obtained by Saniewski et al. (1998, 2003, 2004, 2006) who stated that JA-Me induced gum formation in plum shoot and fruits, peach, cherry

shoots and apricot, in tulip bulbs and stone-fruit trees and their fruits.

The present study indicated that JA-Me induced gums in *A. Senegal* trees, the gum accumulated into the surface of the bark in tapped and untapped branches and bark. This is in conformity with other researchers such as Abeles (1973) and Boothby (1983), who confirmed that the gummosis promoted by Jasmonate (JA-Me). When JA-Me was applied as a spray onto the surfaces of the leaves and small branches of *A. senegal*, the tree gum yield has increased significantly. This study is in line with different researches; reported that; gummosis in trees occurred in response to stresses was regulated by JA-Me.

The gum ducts in *A. senegal* trees treated by JA-Me compared to those of untreated (control) trees (Figure 7), treatments with JA-Me has widened the gum ducts and finally one large duct was formed, where gum appeared as drops on the bark of the tree (Figure 5). Some results showed that traumatic resin ducts might be induced by JA-Me signaling in a dose-dependent manner (Hudgins et al., 2004; Franceschi et al., 2005) and ethylene may cause the resin ducts (found in the bark) to make more resin (Hudgins et al., 2004; Hudgins et al., 2004; Franceschi et al., 2005). The application of JA-Me to *A. senegal* trees has affected the anatomy of the trees (Figure 7). This is similar to the results obtained by Vincent et al. (2001) who stated that when JA-Me was applied to the surface of the trunk of 30+year-old trees has stimulated the production of a compound which induced anatomical changes similar to those caused by wounding or fungal inoculation. To that end, the application of JA-Me to *A. senegal* trees enhanced the flow of gum from the outer bark in large amount. This result is similar to that of Vincent et al. (2001) who proved that the application of JA-Me treatment led to enhanced resin flow from Norway spruce bark wounds. The present study showed that the gum duct in *A. senegal* tree originated directly from the pith through the wood tissues towards the outer bark in tapped area, through the cross section and longitudinal section in all treated trees with JA-Me and micro-organisms. These results are similar to the findings of Morrison and Polito (1985) who stated that, gum ducts have been found in both woody tissues and fruits in all cultivated *Prunus* species

The gum ducts were found to differ in width in treatments with JA-Me, in case of untreated tree the gum was found to exude from the tapped area in the bark while trees treated with 100 mg/L of JA-Me gum exuded from both tapped and untapped surface of the bark. These results are similar to those obtained by Christiansen et al. (1999a) Franceschi et al. (2000) Nagy et al. (2000) whose findings showed that the exogenous application of JA-Me to mature or young spruce tree bark led to enhanced resin flow from Norway spruce bark wounds.

All treatments: untreated trees (control), JA-Me (100 mg/L) produced red coloured gum, spherical nodules with high solubility in cold water. Table 1 show the physical and

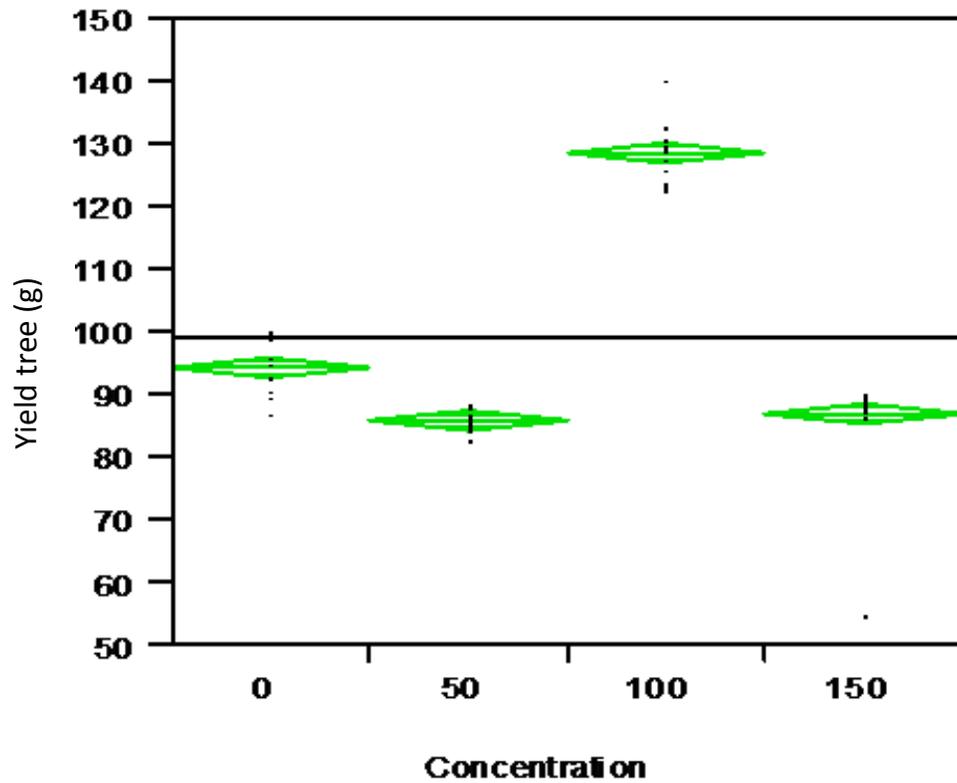
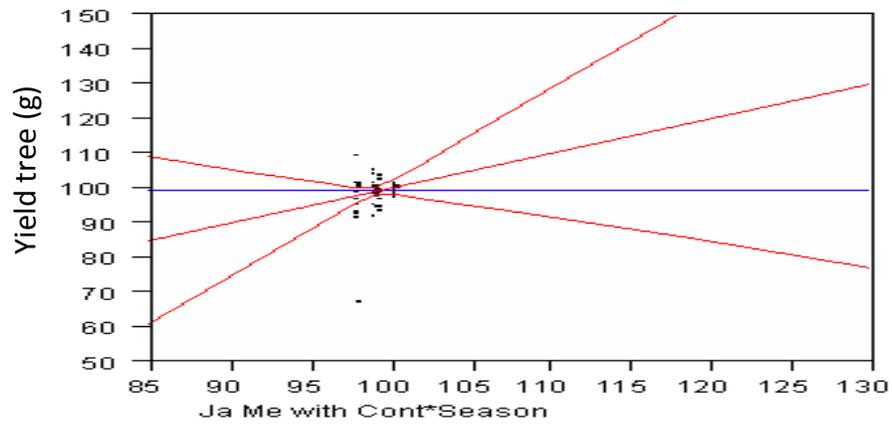


Figure 4. Effect of various concentrations of JA-Me in mg/L on gum yield per tree. 0= Control. Diamonds are means of 10 replicates \pm S.E =1.0517.



Figure 5. Effect of JA-Me 100 mg/L on *A. senegal* tree gumming.



Season	2.3494	0.1297
Ja Me with Cont	373.4917	<0.0001
Ja Me with Cont*Season	0.9387	0.4266

Figure 6. Effect of JA-Me concentrations, control and seasons and their interaction on gum yield per tree.

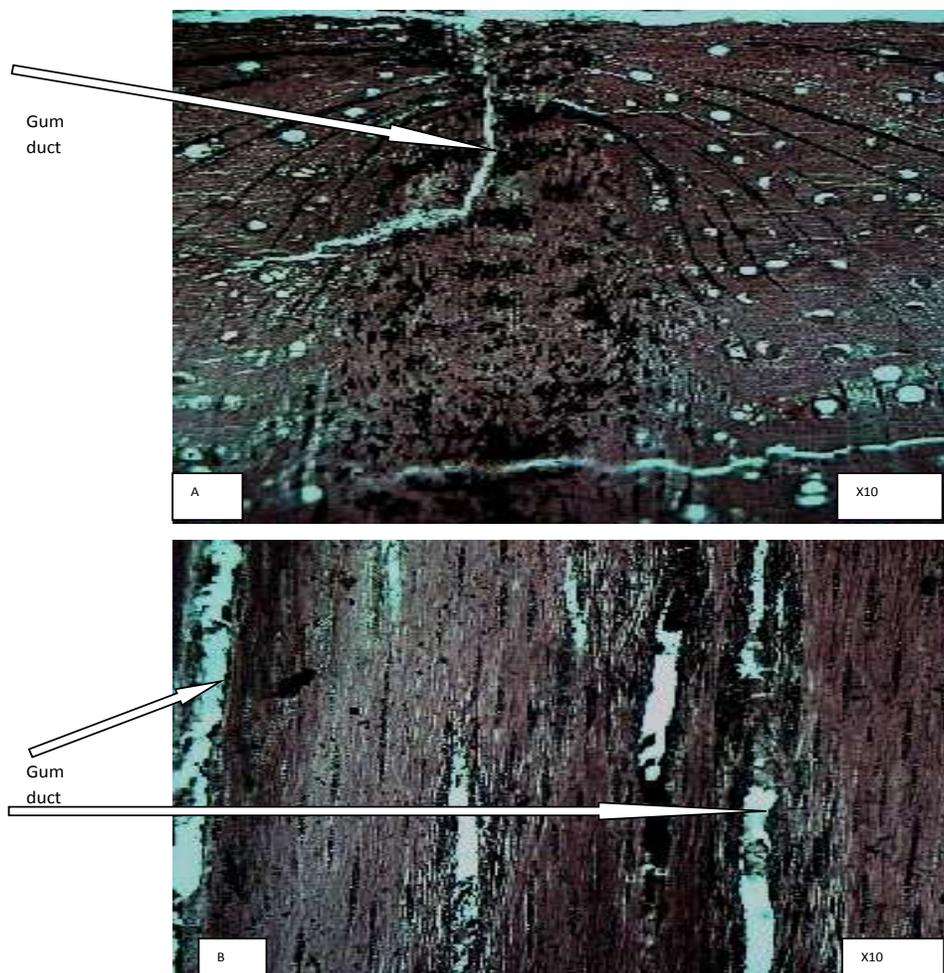


Figure 7. T.S (A) and L.S (B) wood sections of *A. senegal* tree untreated trees showing few and relatively small gum duct.

Table 1. Physical and chemical characteristics of gum.

Specification	Value
Optical rotation*	- 28°*
Viscosity cm ⁻³ /g ⁻³ ***	3.274*
Ash value **	2.629*
Protein%****	2.263*
Nitrogen%*****	0.362*

* Mean values of 3 determinations.

chemical characteristics of gum.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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