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Screening and increase of exopolysaccharide production by rhizobial strains, stress tolerances and its efficiency with *"in Vivo"* peanut plants

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ABSTRACT

Five rhizobial strains were screened to explore exopolysaccharide (EPS) production. Maximum EPS production was shown by *R. leguminosarum* bv. *viciae* (Bani sweef) at 5.48 g L-1, followed by *Bradyrhizobium* sp. (strain ARC 617) at 5.2 g L-1. EPS production was enhanced by 7.52 and 7.2 g L-1 by castor oil followed by olive oil, which gave 7.08 and 6.56 g L-1 in *R. leguminosarum* bv. *viciae* (Bani sweef) and Bradyrhizobium sp. (strain ARC 617), respectively, as compared to YEM media. The maximum level of EPS production was reached at pH 6, followed by pH 7. The optimum inoculum amount for maximum EPS production was discovered to be 2% inoculum. Exopolyssacharides-producing bacteria were tested in vitro for their ability to withstand various biotic and abiotic stresses. Selected rhizobia and bradyrhizobia grown on modified YEM media resistance salinity and had higher cell log counts than those grown on YEM media. Moreover, the EPSs of rhizobia and bradyrhizobia grown on modified YEM media may have a preventive role against the impacts of heavy metals where they are more tolerant to heavy metals than those of YEM media. Concerning antibiotics, *R. leguminosarum* bv. *viciae* based on YEM modified media resistance to the seven antibiotics, while *Bradyrhizobium* sp. resistance all antibiotics except oxytetracycline and streptomycin. The data from the pot experiment suggested that inoculation with EPS producing bacteria based on modified media increased plant height, the shoots' dry weight, nitrogen content, chlorophyll a, b, and total chlorophyll as compared to other treatments.

Keywords: Exopolysaccharide, rhizobia, cultural optimization, antibiotics, heavy metals.

INTRODUCTION

Bacterial exopolysaccharides are one of the metabolites that have recently piqued the interest of modern microbiologists and biotechnologists. (Sengupta *et al*., 2018). They are polymers of high molecular weight that are composed of sugar residues and their maximal production occurs generally at the end of the growth phase (Staudt, *et al*., 2004). Bacterial exopolysaccharides are polysaccharides produced and released into the cellular external environment, which may remain loosely attached to the surface (capsule) or be entirely detached (Nwodo *et al.*, 2012). Culture conditions such as pH and inoculation size can impact EPS production (Ozlem, 2015). Moreover, Peacock *et al.* (2003) confirmed that plant oil plays a role to increase the production of bioactive metabolites. Olive, castor, and peppermint oil are examples of plant oils that could be useful as EPS additives (Krishna *et al.*, 2008). Under typical conditions, the composition and amount of EPS produced by rhizobial strains are highly varied (Bomfeti, 2011).

Several biotic and abiotic stress factors have a significant impact on plant growth, productivity, and yield (Zhang *et al.,* 2018; Singh *et al.*, 2018). Damages or infections produced by various pathogens are examples of biotic stress. Salinity, heavy metals, and other organic pollutants are examples of abiotic stresses. Soil salinization is the most harmful of all abiotic stresses (Daliakopoulos *et al.,* 2016) and is one of the most major limiting factors of agricultural output and food security. Exopolysaccharide (EPS) can help to reduce the amount of Na^+ available for plant absorption, which can help to alleviate salt stress (Upadhyay *et al.*, 2011). Rhizobial populations should be able to compete to infect host plants. The rhizosphere has enormous populations of various microorganisms. Some of them produce antibiotics and become harmful to susceptible rhizobial populations in the soil. Thus, the resistance to antibiotics is a desirable characteristic for the rhizobial population. It increases the rhizobia's chances of growth, multiplication and persistence in the soil (Naamala *et al*., 2016). Soil pollution by heavy metals is a major environmental issue that has a severe influence on human health and agriculture (Ledin, 2000). Bacterial polysaccharides, which are attached to the cell surface and discharged into the medium, are among the compounds capable of binding heavy metals (González *et al.,* 2010). The production of EPS by soil bacteria surrounding roots also improves plant water potential and nutrient uptake (Naseem and Bano, 2014).

The purpose of this study is to screen the EPS producing rhizobia, study effects of pH, inoculation amount and the additives of plant oils in the media and study the role of bacterial EPS in tolerance to salinity, heavy metals and antibiotics "*in vitro*" and the efficiency of rhizobial inoculants *"in Vivo".*

MATERIALS AND METHODS

Strains of bacteria:

Five strains of *Rhizobium leguminosarum bv. viciae (Bani sweef and El khatatba) specific to Faba bean, Mesorhizobium loti* strain (ARC 408) specific to lupin and *Bradyrhizobium japonicum* strain (ARC 517) specific to soybean *and Bradyrhizobium* sp. strain (ARC 617) specific to peanut were used in this investigation. The strains were generously donated by the Biofertilizers Production Unit, Agriculture Microbiology-Soil, Water and Environment Research Institute Agriculture Research Center, Giza, Egypt.

Screening for exopolysaccarides:

Rhizobial strains were inoculated into conical flasks holding 100 ml of YEM broth to estimate EPS production. The inoculated flasks were incubated for 72 hours at 30°C and 200 rpm on a rotator shaker. The culture broth was centrifuged at 3500 rpm after incubation, and the supernatant was combined with two volumes of acetone. Centrifugation at 3500 rpm for 30 minutes was used to collect the crude polysaccharide produced. After washing with distilled water and acetone alternately, the EPS was transferred to filter paper and weighed after drying overnight at 105° C (Damery and Alexander, 1969).

Effect of plant oils on exopolysaccarides production:

Four different plant oils at rate 1% namely castor oil, olive oil, sun flower oil and peppermint oil were applied to each flask of cultural media according to Bolla *et al.* (2011)**.**

The impact of initial pH on the formation of EPS:

The pH of the medium was adjusted from 4 to 9 to see how it affected EPS production. After the incubation period, bacterial samples were removed from the fermentation flask to assess EPS yield (Prakash *et al*., 2016).

The influence of inoculation amount on the generation of EPS

Inoculating the flasks in the range of 0.5 % - 3 % was used to test the effect of inoculation amount on EPS generation. The inoculation flasks were incubated for 48 hours at 30°C and 150 rpm. After the incubation period, the bacterial samples were extracted from the fermentation flasks to calculate the EPS yield (Prakash *et al*., 2016).

Salt tolerance:

The tolerance of selected EPS producing rhizobia to salt concentrations was studied in cultures grown on YEM media according to Elsheikh and Wood (1989). Sodium chloride was added before autoclaving at concentrations of 1, 2, 3, 4, 5 and 6 %. Viable rhizobial colonies were estimated by plate counts on Congo Red YEM agar medium.

Role of EPS producing bacteria in heavy metals resistance:

By using the Plate/Agar-well diffusion method, the selected bacterial strains were evaluated for resistance to heavy metals such as nickel chloride ($NiCl_2$), cobalt chloride ($CoCl_2$), cupric chloride ($CuCl_2$), manganese chloride ($MnCl_2$), and cadmium sulphate ($CdSO_4$). A central well was drilled into the surface of the yeast extract agar plates with a sterile well borer (13mm width). 500 µl of the standard metal salt solution at concentration of 1000µg mL^{-1} was put into the well and was allowed to diffuse evenly across the well. Four treatments of bacterial strains were inoculated in radial streaks on each plate. These plates were incubated for three days at 28°C. The following formula was used to compute the percentage of bacterial growth (Singh *et al.*, 2014):

$$\% \text{ of growth} = \frac{\text{Complete inoculated length of streak - inhibited length Complete}}{\text{inoculated length}} \times 100$$

(Complete length of inoculated streak was = 40 mm).

Antibiotic resistance test:

Seven types of antibiotics were used as shown in Table (1) to estimate the antibiotic resistance of the two selected rhizobial strains using the standard disk diffusion method as described by Bauer *et al*. (1966).

Table 1. Antibiotics resistance standard range of Gram-negative bacteria (Inhibition zone diameter, mm) (Charteris *et al*., 1998).

Chemotherapeutic agents	Conc. (µg)	Diameter of inhibition zone in (mm)		
		Resistant (R)	Intermediate (I)	Sensitive (S)
Ampicillin	10	≤ 12	13-15	≥16
Azithromycin	15	≤13	14-17	≥18
Colistin	10	≤8	9-10	≥11
Gentamicin	10	≤12	13-14	≥15
Kanamycin	30	≤13	14-17	≥18
Oxytetracycline	30	≤14	15-18	≥19
Streptomycin	50	≤11	12-14	≥15

Pot experiment:

A pot experiment was conducted in a greenhouse of Biofertilizer Production Unit (BPU), soils, water and Environ. Res. Instit. ARC, Giza, Egypt during the summer season of 2018 to evaluate the efficiency of using EPS producing rhizobial strains with peanut plants. Seeds of Peanut (*Arachis hypogaea* variety Giza 6) were planted in cleaned pots of 30 cm diameter filled with 5 kg washed and sterilized sandy soil [pH (7.58), E.C.(0.57dSm^{-1}), organic matter (0.4%) and Total nitrogen (0.011%).The seeds of peanut were inoculated with 10 ml (containing 4x10^9cells ml^{-1}) of liquid media of the EPS producing rhizobia for each pot.

The treatments were arranged in a randomized complete block design with three replicates where the treatments are as follow.

1- Control (recommended dose of NPK).

2- *Bradyrhizobium* sp., (strain ARC 617) +15kg N fed^{-1} .

3- Modified *Bradyrhizobium* sp., (strain ARC 617) +15kg N fed^{-1}.

Plant samples were picked up after 45 days from planting to determine chlorophyll a , b and total chlorophyll, plant height (cm), shoot dry weight (g $plant^{-1}$), root dry weight (g $plant^{-1}$) and shoot nitrogen content (mg $plant^{-1}$) .

Determination of plant growth parameters:

Samples of plants were collected after 45 days of planting and those dried at 70°C were then, weighed to obtain their dry matter per plant. Samples of the plant were digested using the methods described by Page *et al.,* (1982). N- contents in shoots were determined according to Cottenie *et al.,* (1982).

Determination of plant growth parameters:

Samples of plants were collected after 45 days of planting and those dried at 70°C then, weighed to obtain their dry matter per plant. Samples of plant were digested using the methods described by Page *et al.* (1982). N contents in shoots were determined according Cottenie *et al.* (1982).

Determination of pigment content:

According to Arnon, (1949), chlorophyll-a, chlorophyll-b, and total chlorophyll content were measured one month after planting. With a homogenizer, 200 mg fresh leaves were homogenized in 8 ml 80 percent acetone. Homogenates were centrifuged for 15 minutes at 4°C (3000 rpm). Pigment analysis was carried out using supernatants. Absorbances were determined at 645, 652, 663 and 470 nm respectively. Calculations were estimated by using equations of Litchtenthaler and Wellburn (1983).

Analytical statistics:

The obtained results were statistically analyzed using the general linear models procedure of SAS (1999). The differences were statistically tested using Duncan's multiple range tests.

RESULTS

Exopolysaccharide production by rhizobial strains:

Results presented in **figure (1) and Table (2)** showed that five tested strains were found to be positive for EPS production. The maximum amount of exopolysaccharide produced was 5.48 g L^{-1} for *R. leguminosarum bv. viciae* (Bani sweef), followed by *Bradyrhizobium* sp., (strain ARC 617) (5.2 g L^{-1}) and *Mesorhizobium loti* (strain ARC 408) (5 g L^{-1}) after 72h of incubation. Whereas, the corresponding values for *R. leguminosarum bv. viciae* (El khatatba) and *Bradyrhizobium japonicum* (strain ARC 517) were significantly lower than rest of strains and scored 4.8 and 4.0 g L^{-1} respectively. Concerning acid production, after the end of the incubation period, pH was between 5.18 and 5.95 **Table (2).**

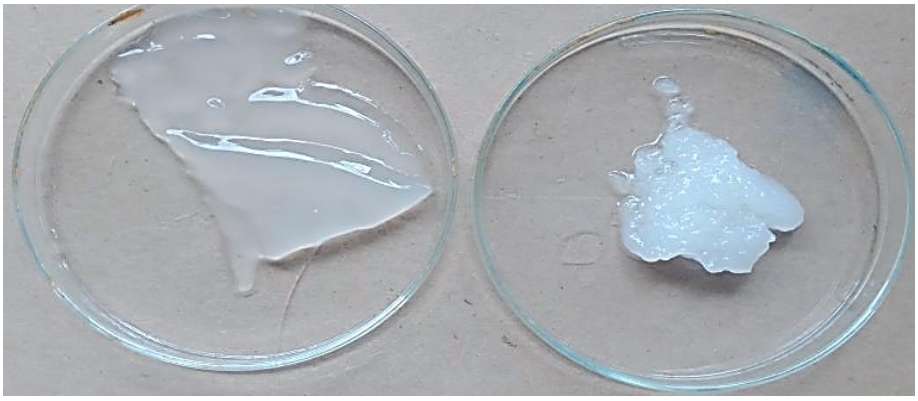


Fig. 1. Exopolysaccharides produced by rhizobial strains

Table 2. Screening of EPS producing bacterial strains and their acid production

Rhizobial strains	pH	EPS (g/L)
R.leguminosarum bv. viciae (Bani sweef)	**5.18**	**5.48^{a*}**
R. leguminosarum bv. viciae (El khatatba)	**5.57**	**4.8^{c}**
Bradyrhizobium sp., (strain ARC 617)	**5.54**	**5.2^{ab}**
Mesorhizobium loti (strain ARC 408)	**5.95**	**5^{bc}**
Bradyrhizobium japonicum (strain ARC 517)	**5.86**	**4^{d}**

*According to Duncan's test, means in the same column followed by the same letters are not significantly different (P=0.05).

Optimization of culture conditions for EPS production

The effect of essential oils on rhizobial EPS production

Results in (Fig. 2) showed that, essential oils stimulate the EPS production. The highest EPS production was obtained in case of using castor oil 7.52 and 7.2 g L^{-1} followed by olive oil that give 7.08 and 6.56 g L^{-1} in *R.leguminosarum bv. viciae* (Bani sweef) and *Bradyrhizobium* sp., (strain ARC 617) respectively.

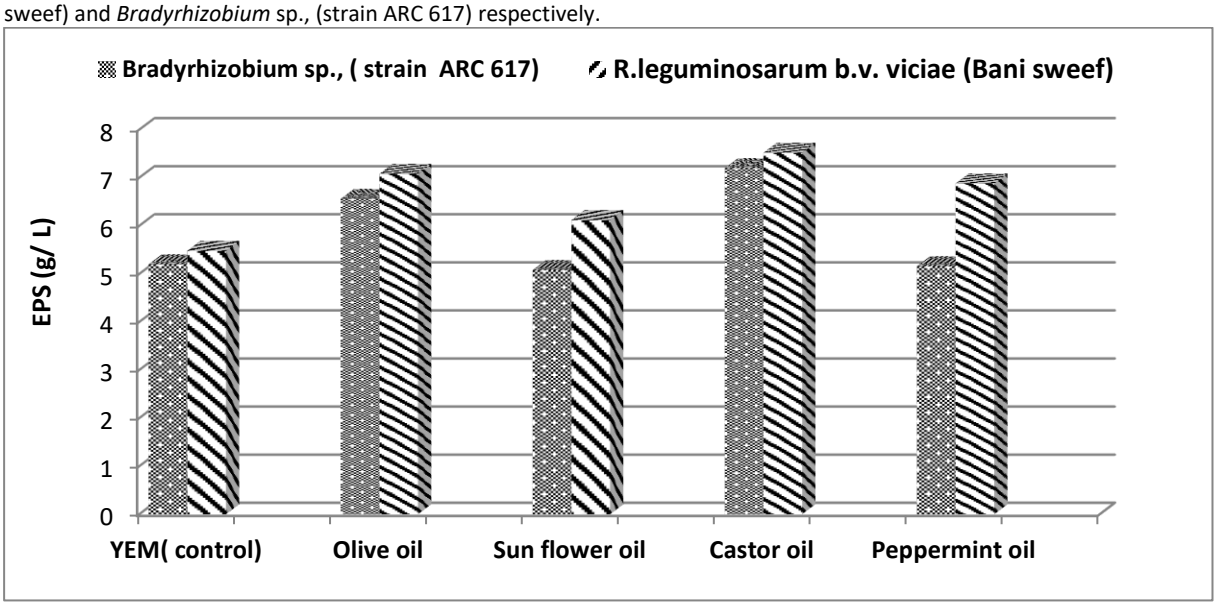


Fig. 2. The effect of plant oils on the production of EPS.

The influence of initial pH on EPS production

The pH of the cultural medium is an important factor in governing the EPS production process. The results showed that, rhizobial strains whether grown on YEM media or modified YEM media appear to be able to grow in a wide pH range of 4.0 to 9.0. The optimal pH for EPS production was PH 6 followed by pH 7 **Fig. (3)**

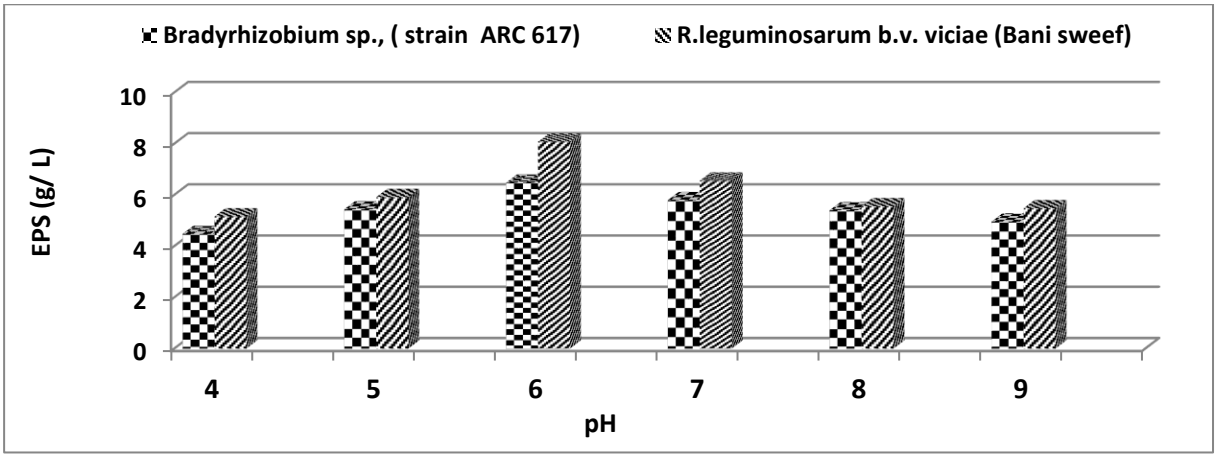


Fig. 3. Effect of different pH values on the amount of EPS.

The impact of inoculation amount on EPS production

The results obtained in **Fig. (4)** demonstrated that, when the inoculation amount was increased from 0.5 percent to 2.0 percent, the amount of EPS production was increased. The optimal inoculation amount for maximum EPS production 8.56 and 6.94 g L^{-1} in *R. leguminosarum bv. viciae* (Bani sweef) and *Bradyrhizobium* sp., (strain ARC 617) respectively were discovered to be 2%. Whereas inoculation amounts greater than 2% result in a lower EPS yield.

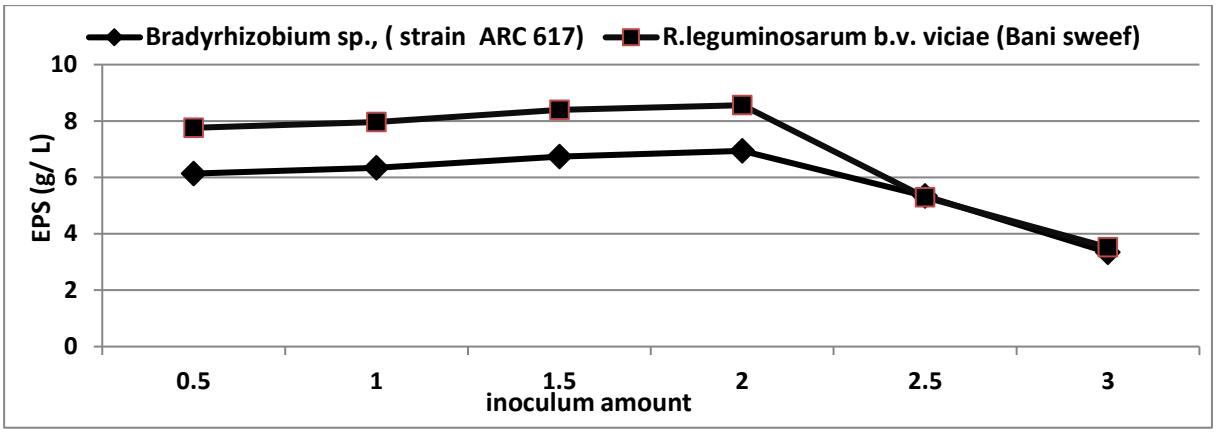
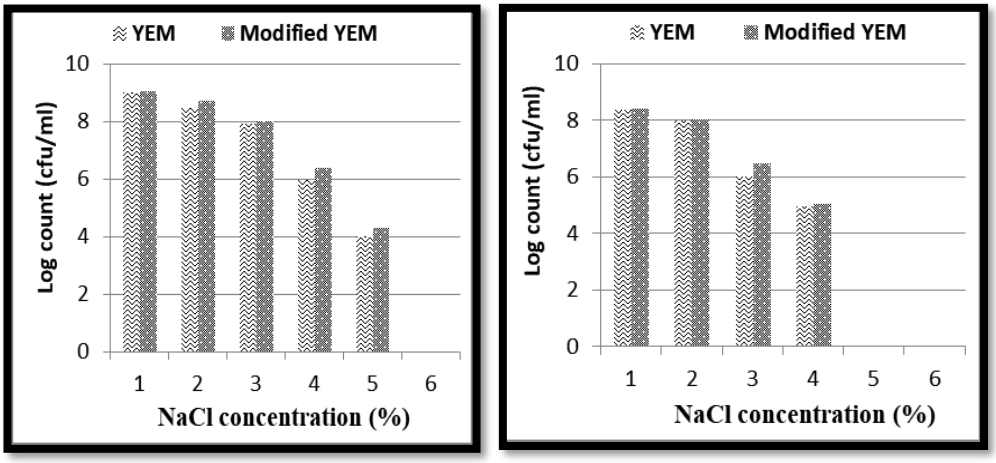


Fig. 4. The impact of inoculation amount on the production of EPS by *R. leguminosarum bv. viciae* and *Bradyrhizobium* sp.

Response of EPS producing rhizobia to salt stress:

In the current study, the selected EPS rhizobial strains grown on YEM media or modified YEM media recorded different responses to salt stress. They showed decreased growth with increasing salt concentration. At 1.0 % NaCl concentration, the two selected rhizobial strains were similar in YEM and modified YEM media, where they have the same cells log count /ml. However, at 4 % salt, *R. leguminosarum bv. viciae* (Bani sweef), and *Bradyrhizobium* sp., (strain ARC 617) showed that modified YEM media have higher cell log counts than YEM media. In addition to *Bradyrhizobium* sp. (strain ARC 617) was unable to grow in presence of 5% salt as compared to *R. leguminosarum bv. viciae* (Bani sweef). On the other hand, both two strains of YEM media and modified YEM media showed no growth in presence of (6%) salt **Fig. (5).**

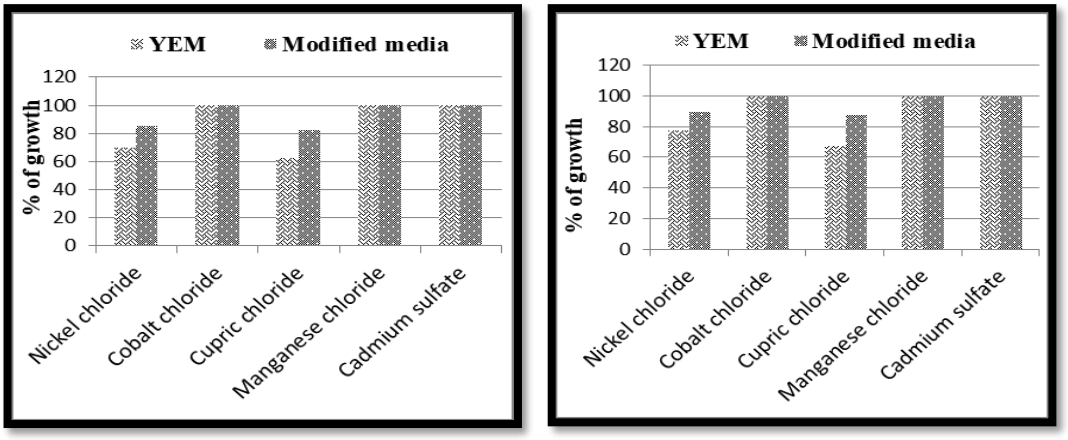


R. leguminosarum bv. *viciae* (Bani sweef) *Bradyrhizobium* sp., (Strain ARC 617)

Fig. 5. Response of selected rhizobial strains to salt stress in YEM and modified YEM media.

Resistance to heavy metals:

Tolerance of selected rhizobial strains to heavy metals was calculated in terms of percentage of growth as shown in **Fig. (6)** Results obtained clearly suggested that the EPS producing rhizobia and bradyrhizobia grown on YEM and modified YEM media may have a protective effect against heavy metal exposure and % of growth was higher in case of $CoCl_2$, $MnCl_2$ and $CdSO_4$ and lower in case of $CuCl_2$, and $NiCl_2$. Moreover *R. leguminosarum bv. viciae* (Bani sweef) and *Bradyrhizobium* sp., (strain ARC 617) growing on modified YEM media were more tolerant than those of YEM in terms of % of growth. Heavy metal resistance in bacteria can be attributed to a range of detoxifying mechanisms established by resistant strains, such as exopolysaccharide complication. The EPSs producing bacterial strains could sequester the harmful metal, providing the bacteria the time they need to adapt and eliminate the metal's toxic effect. Kazy *et al*. (2002) proved that, bacteria resistant to metals often showed production of high amount of exopolysaccharides at heavy metal stressed growth condition. This confirmed by Foster *et al*. (2000) who proved the ability EPSs of *Rhizobium etli* to bind to metal ions. Slaveykova *et al*. (2010) proved Cd^{2+} complexation by extracellular polymeric substances in *S. meliloti.*



R. leguminosarum bv. *viciae* (Bani sweef) *Bradyrhizobium* sp., (Strain ARC 617)

Fig. 6. Heavy metals tolerance by EPS producing rhizobia on YEM and modified YEM agar media

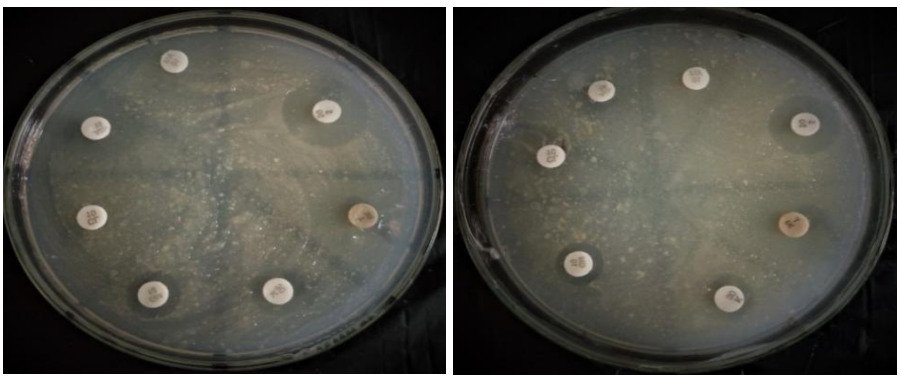
Antibiotic susceptibility:

Results represented in **Table (3) and Figure (7),** showed that oxytetracycline has a larger zone of inhibition than ampicillin and Colistin, which have no zones. Based on the inhibition area values, susceptibility to antibiotics can be expressed in the following order: *Bradyrhizobium sp.* (strain ARC 617 grown on YEM > *R. leguminosarum bv. viciae* (Bani sweef) grown on YEM, > *Bradyrhizobium* sp., (strain ARC 617) grown on modified YEM > *R. leguminosarum bv. viciae* (Bani sweef) grown on modified YEM.

Table 3. Zone of inhibition (in mm) of rhizobia and bradyrhizobia on YEM Plates.

Strains	Diameter of zone inhibition (mm)						
	Ampicillin	**Azithromycin**	**Colistin**	**Gentamicin**	**Kanamycin**	**Oxytetracycline**	**Streptomycin**
R.leguminosarum b.v. viciae (YEM)	0 R	10 R	0 R	12 R	15 I	18 I	0 R
R.leguminosarum b.v. viciae (modified YEM media)	0 R	0 R	0 R	10 R	10 R	10 R	0 R
Bradyrhizobium sp., (strain ARC 617) (YEM)	0 R	20 S	0 R	14 I	15 I	25 S	22 S
Bradyrhizobium sp.,(strain ARC 617) (modified YEM media)	0 R	0 R	0 R	10 R	10 R	15 I	17 I

R: Resistant I: Intermediate S: Sensetive



R. leguminosarum bv. viciae (YEM) *R. leguminosarum bv. viciae* (modified media)

Fig. 7. Antibiotic susceptibility shown by inhibition zone of rhizobia grown on YEM and modified YEM media.

The impact of EPS-producing rhizobia on growth of peanut plant:

Results in **Table (4)** showed that modified treatment of *Bradyrhizobium* sp., (strain ARC 617) was significantly scored the highest values in terms of plant height (25.1 cm), Shoot dry weight (7.92 g/plant), root dry weight (2.14g/plant) and Shoot N- content (219 mg/plant).

Table 4. Effect of EPS producing rhizobia on plant growth parameters of peanut plants after 45 days of planting

Treatments	Plant height (cm)	Shoot dry weight (g/plant)	Root dry weight (g/plant)	Shoot N- content (mg/plant)
Control	23.4^{ab}	7.68^{b}	2.01^{c}	98^{c*}
***Bradyrhizobium* sp., (strain ARC 617)**	22.9^{b}	6.28^{c}	2.08^{b}	210^{b}
Modified *Bradyrhizobium* sp., (strain ARC 617)	25.1^{a}	7.92^{a}	2.14^{a}	219^{a}

*Values having different superscripts within the same column are significantly different (P<0.05).

Chlorophyll content:

Data in **Figure (8)** showed that, the treatment of *Bradyrhizobium* sp.,(strain ARC 617) grown on modified YEM media scored the highest values of chlorophyll-a (0.735µg ml^{-1}), chlorophyll- b (0.626µg ml^{-1}) and total chlorophyll (1.58 µg ml^{-1}) as compared to other treatments (control or *Bradyrhizobium* sp., (strain ARC 617) grown on YEM media).

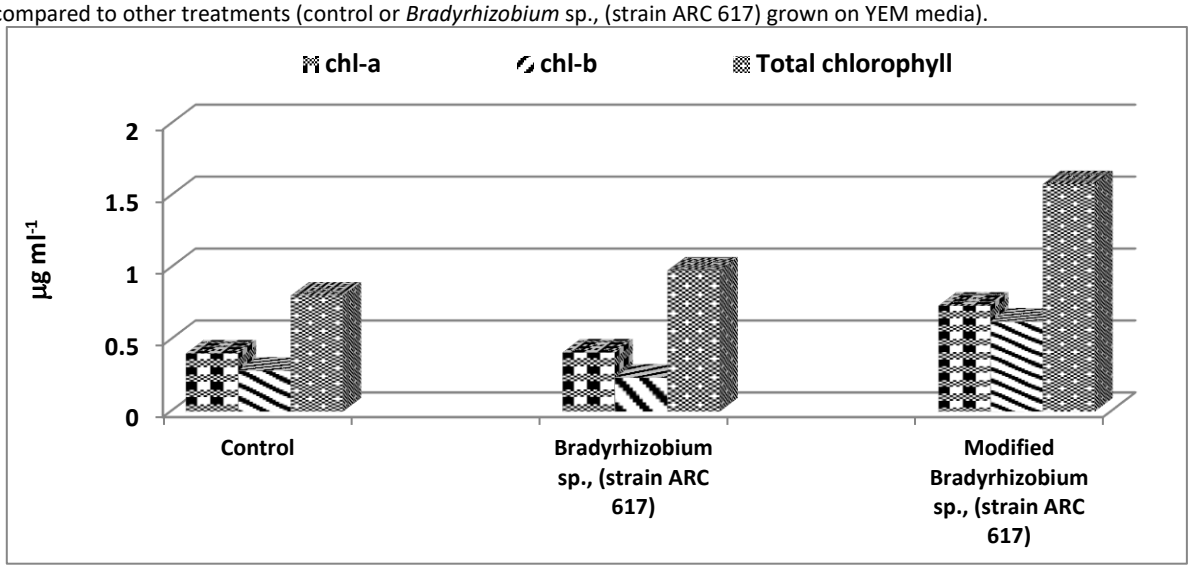


Fig. 8. Chlorophyll contents as affected by EPS producing rhizobia on YEM and modified YEM agar media

DISCUSSION

Bacterial growth is often accompanied by the production of exopolysaccharides (EPS). This study is concerned with the production of the extracellular polysaccharides from some rhizobial strains. According to numerous researches, EPS quantities and properties are highly dependent on microorganisms, their culture conditions, media composition, as well as the genus and species of bacteria (Baharuddin *et al.,* 2015). Concerning acid production after the end of the incubation period, Tavernier *et al*. (1997) indicated that growth was accompanied by the acidity of the culture medium, which led to log phase in EPS production, this may be due to an initial extracellular conversion of the carbohydrate into acid.

Previous researches have shown that, oil addition to cultural media increases EPS production (Yang *et al.*, 2000; Park *et al.* (2002), where the low pH of broth media resulted from oil addition may favor the production of EPS continuously (Hsieh *et al.,* 2006). Rabha *et al.* (2012) confirmed that, the yield of EPS is highly dependent on the substrate composition and environmental conditions. The pH of the cultural medium is an important factor in governing the EPS production process. These findings are consistent with Raza *et al*. (2011) who confirmed that initial medium pH was a factor of major impact on EPS production. Mahapatra and Banerjee (2013) reported that maximum production of EPS was observed in the range of pH 5 - 7 in many organisms. Haroun *et al*., (2013) confirmed that *Lactobacillus plantarum* produced the highest amount of EPS when the pH was 6.2. Sharma *et al*., (2017) discovered that *Pediococcus acidilactici* produced maximum EPS at pH 6.0 after a 24-hour incubation period at 35 °C. **R**esults of the inoculation amount effect have been shown by many researchers (Sivakumar *et al.,* 2012). Most other studies used an inoculation amount of 2% (Bragadeeswaran *et al.,* 2011) or 3% (Borgio *et al.,* 2009).

Thrall *et al.* (2008) revealed that increasing salt concentrations may harm rhizobial populations through direct toxicity as well as osmotic stress. On the other hand, exopolysaccharides production by rhizobial strains can be helpful against osmotic stress, whereas many previous studies showed that, the beneficial bacteria have some strategies for dealing with salt stress, including maintaining an optimal Na^{+}/K^{+} ratio through the production of extracellular polymeric molecules known

as exopolysaccharide (EPS), which ensures their survivability under unfavorable soil conditions (Vurukonda *et al.*, 2016). *Rhizobium* strains (fast growers) are more salt-tolerant than strains of *Bradyrhizobium* (slow growers) as proved by (Elsheikh, 1998).

Heavy metal resistance in bacteria can be attributed to a range of detoxifying mechanisms established by resistant strains, such as exopolysaccharide complication. The EPSs producing bacterial strains could sequester the harmful metal, providing the bacteria the time they need to adapt and eliminate the metal's toxic effect. Kazy *et al*. (2002) proved that bacteria resistant to metals often showed production of a high amount of exopolysaccharides at heavy metal stressed growth conditions. This was confirmed by Foster *et al*. (2000) who proved the ability EPSs of *Rhizobium etli* to bind to metal ions. Slaveykova *et al*. (2010) proved the Cd^{2+} complexation by extracellular polymeric substances in *S. meliloti.*

Resistance to antibiotics may be due to the ability of these selected strains to produce exopolysaccarides as indicated by Suresh Kumar *et al*. (2007) who showed that antibiotics susceptibility may be physically protected by EPS. Pinto *et al.* (2020**)** confirmed that bacteria are protected from antibiotics by the extracellular polymeric substances (EPS) matrix. Inoculation of maize seeds with EPS-producing bacteria improved root and shoot length and plant biomass (Naseem and Bano, 2014). Khan and Bano, (2019) explained that the exopolysaccharides produced by PGPR have a significant impact on plant growth by the formation of a rhizosheath around the roots, and thus protect the plant roots from desiccation. Han and Lee, (2005) found that inoculation increased the chlorophyll content in lettuce. Danish *et al.* (2020) confirmed that, EPS producing PGPR inoculated plants showed higher chlorophyll contents as compared to un-inoculated plants. This could be due to the increased photosynthetic leaf area of the plant caused by EPS producing bacterial inoculation compared to the control as indicated by (Marcelis and Van Hooijdonk, 1999).

CONCLUSION

The provided findings shed light on the synthesis of exopolysaccharides from rhizobial strains and the factors that influence their production such as essential oils, initial pH and inoculation amount that could be used to stimulate EPS production. There was a link between exopolyssacharide production and tolerance to biotic, abiotic stress and plant growth.

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انتقاء وزيادة انتاج السكريات العديدة بواسطة سلالات الريزوبيا والتحمل للاجهاد وكفاءتها مع نباتات الفول السوداني

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الملخص
تم اختيار خمس سلالات من الريزوبيا واختبار مقدرتها على انتاج السكريات العديدة.أعلي انتاجية للسكريات العديدة تم الحصول عليها بواسطة (Bani sweef) *R.leguminosarum b.v. viciae* (5.48 جم لتر $^{-1}$) تليها (strain *Bradyrhizobium* sp., (ARC 617) (5.2 جم لتر $^{-1}$). تم زيادة انتاجية السكريات العديدة الى (7.52 جم لتر$^{-1}$) و(7.2 جم لتر $^{-1}$)باستخدام زيت الخروع يليه استخدام زيت الزيتون الذى اعطى انتاجية (7.08 لتر$^{-1}$) و (6.56 جم لتر$^{-1}$) لكلا من سلالة *R. leguminosarum bv. viciae* و(ARC 617) *Bradyrhizobium* sp., على الترتيب مقارنة ببيئة YEM.تم الوصول الى اقصى انتاجية من البولى سكريدعند استخدام درجة حموضة pH6 يليها فى الانتاجية pH7 وافضل كمية لقاح مستخدمة للحصول على أعلى انتاجية من البولى سكريد هو 2% .وتم تقدير مقاومة البكتريا المنتجة للسكريات العديدة لمختلف صور الاجهاد الحيوى واللاأحيائي تحت الظروف المعملية .أظهرت سلالات الريزوبيا والبراديرزوبيا المنتخبة لانتاج البولى سكريد والنامية على بيئة YEMالمعدلة مقاومة للملوحة واعطت اعلى معدلات من لوغاريتم اعداد الخلايا اذا ما قورنت بمثيلاتها النامية على بيئة YEMالاصلية . علاوة على ذلك اظهرت السلالات مقاومة للعناصر الثقيلة بدرجة اكبر من مثيلاتها النامية على بيئةYEM . وفيما يخص المضادات الحيوية اظهرت سلالة الريزوبيا النامية على بيئة YEMالمعدلة مقاومة للمضادات الحيوية السبعة المستخدمة فى حين قاومت البراديريزوبيوم جميع المضادات الحيوية ماعدا الاوكسى تتراسيكلين والاستربتوميسين .اوضحت نتائج تجربة الصوبة ان التلقيح بالبكتيريا المنتجة للسكريات العديدة النامية على بيئة YEM المعدلة يزيد من ارتفاع النبات والوزن الجاف للمجموع الخضرى والمحتوى النيتروجينى وكلوروفيل a وb والكلوروفيل الكلى لنباتات الفول السودانى اذا ما قورنت بباقى المعاملات.

الكلمات المفتاحية: السكريات العديدة المفرزة خارجيا، الريزوبيا، التحسين المزرعى، المضادات الحيوية، العناصر الثقيلة