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Effect of Cell - Free Cultures Filtrates of Different Bacterial Isolates on Seed Germination and Seedling Growth of *Parkinsomia aculeata* L.

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BACTERIAL species produce a variety of beneficial metabolites for plant growth and survival, as well as defend their hosts from attack of certain pathogens. The present study was carried out to investigate the impact of different bacterial isolates on the seed germination and seedling growth of *Parkinsomia aculeata* L. as a species of arid zones. Both soil samples and tested seeds were collected from El-Madina El-Monawara city, Saudi Arabia in May, 2013. Results evinced the promotion of seed germination by all bacterial isolates used. Isolates 6 and 7 (*Micrococcous* sp. and *Sporosarcia* sp.), respectively isolated from soils of extremely low salinity recorded the highest values of germination percentage (44 and 48). On the same time, the greater seed vigor index (83.04), and energy of germination (1.96) were given by isolate 7 at the 7th day of the experiment. On the other hand, the highest R/S ratio (0.55), on the basis of length, and the highest dry weight of root and shoot were attained by *Bacillus subtilis* isolate.

Keywords: Bacteria – Germination index – Filtrate – Seedling vigor.

Seeds of leguminous plants are characterized by their possession of hard coats. Germination of many seeds is retarded only by impermeable coats which hinder the admission of water. If these coats are not subjected to pretreatment, germination can be erratic and prolonged, sometimes extending over a period of many years (Fordham, 1965). However, very little is known about plant growth stimulates produced by bacterial species in leguminous plants. Naturally, soils are ultimately rich habitats in active microorganisms; including bacteria which interact with their metabolites such as Indole Acetic Acid (IAA) produced by *Bacillus* sp. (Josic *et al.*, 2013), nitrogenase (Das and Kole, 2006), soluble phosphate (Vastakaite and Buzaite, 2011 and Ajilogba *et al.*, 2013) and ammonia (Yadan *et al.*, 2010) that are effective in decaying the seed hard coats and promoting seed germination and seedling growth. Therefore, the objective of this

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study is to investigate the potential of soil bacterial culture filtrate on seedling growth of *Parkinsomia aculeata* L.

Material and Methods

Soil samples were collected from El-Madina El-Monawara city, Saudi Arabia at different localities: Qurban El Nazel, Eastern Hara, Western Hara, Allawy and Ohod Mountain, Soils were air - dried and kept at 25°C. Mechanical analysis of the collected soil samples (texture), pH, and electric conductivity (EC) were estimated.

The bacterial isolates were identified according to Bergey's Manual of Systematic Bacteriology (1984) Sneath *et al*. 1986 and Sheikh, (2010). Bacterial isolates were maintained on nutrient broth medium: Beef extract (0.3 g minerals and carbohydrates), peptone (0.5 g proteins as nitrogen source), 0.5 g NaCl as an electrolyte and 100 ml distilled water. The pH of the medium amounted 7. The cultures were kept at 4°C for further studies. For crude bacterial filtrate preparation, 1 g of soil was inoculated into Erlenmeyer flasks (250 ml) containing 100 ml of the nutrient broth. Culture broth flasks were incubated at 20 ± 2°C for 10 days. Bacterial filtrate was collected by filtration through sterilized bacterial filters (NALGENE 0.45 mm) produced by Nalge Nunc International (Umechuruba and Nwachukwa, 1997) and was stored at 4°C for further use. An experiment was designed to test the biological activities of the cell - free culture filtrate of soil bacteria on the seed germination and growth of *Parkinsomia aculeata*L. (Leguminosae) seeds *in vitro*. Seeds were soaked in each bacterial filtrate for 30 min. for each treatment. For control, seeds were soaked in distilled water. 25 seeds for each treatment were placed in 90 mm diameter Petri dishes on Whatman No.1 filter paper moistened with 5 ml of sterile filtrate, or distilled water for the control. Treated seeds were incubated at 25°C under dark conditions. Germination progress was recorded daily with the addition of 2 ml filtrate. The germination percent (GP) of germinated seeds was recorded after 7 days of planting (ISTA, 1993 and 1999). At harvesting, the seedling height, root and shoot lengths, dry weights of the roots and shoots, seed germination index (SGI), seedling vigor index (SVI) and the energy of germination (GE) were estimated.

1. Germination percentage (GP) was calculated using the following formula: Germination percentage (GP) = number of germinated seeds / total number of

$$\text{seeds} \times 100$$

2. Seed germination index (SGI) was calculated according to the following equation (Scoot *et al*., 1984):

$$SGI = \Sigma\, Ti\, Ni/S$$

Where,

Ti = is the number of days after sowing

Ni= is the number of seeds germinated on day i

S= is the total number of seeds planted

3. Energy of germination (GE) was recorded according to Farooq *et al*. (2005) at the 7^{th} day after sowing. It is the percentage of germinating seeds (GP) seven days after sowing relative to the total number of seeds tested (TNST).

$$GE = GP\ (7th\ day) / TNST$$

4. The seedling vigor index was calculated according to equation (Orchard 1977).
Seedling vigor index (SVI) = [seedling length (cm) × germination percentage].

The average means of growth parameters was carried out in four replicates and subjecting to analysis of variance and treatment means according to were computed Duncan's multiple range test (DMRT) at $P = 0.05$.

Results and Discussion

All soils (Table 1) are of the sandy loamy, little alkaline type. The soils from which isolates 1 (*Bacillus subtilis*) and 2 (*Esherichia coli*) were isolated showed the highest value (38,000 µmho cm^{-1}) of electric conductivity. Intermediate values of electric conductivity (21,000 and 10,000 µmho cm-1) were estimated for the soil concerned with the isolation of isolates 5 (*Bacillus subtilis*), 3 (*Bacillus megaterium*) and 4 (*Micrococcous* sp.*).* Lower values of EC (4,000 and 1,800 µmho cm^{-1}) were recorded for the soils concerned with isolates 9, 10 (*Bacillus subtilis*) and 6 (*Micrococcous* sp.), 7 (*Sporosarcia* sp.). All isolates investigated were able to promote the seed germination of the test species (*Parkinsonia aculeata*).

The germination percentages (Table 2) gave the values of 38% for isolate 1 while the percentage of 32 was attained by both 2 and 4 isolates in comparison to the control one (8 %). Higher germination percentages (40, 42, 44 and 48) were attained by isolates 8, 5, 9, 10, 6 and 7, respectively. Highest germination index (10.42) was recorded for isolate 6 (*Micrococcous* sp.). Data elucidated that isolate 7 (*Sporosarcia* sp.) was distinguished by giving the highest values of germination percentage, seed vigor index (83.04), and energy of germination (1.96) at the 7^{th} day of the experiment.

Data indicated that the highest germination percentages (44 and 48) were monitored for the seeds soaked in the filtrates of *Micrococcous* sp. and *Sporosarcia* sp. of isolates 6 and 7 that inhabit the soils with low salt content. In all treatments; including the control, the shoot height was much longer and heavier than the root depth (Fig. 1). The highest value of R/S ratio (0.55) was achieved by isolate 8 (*Bacillus subtilis*) on the basis of length (Fig. 2). On the other hand, the highest dry weight of the root was monitored for the seeds soaked in the filtrate of isolate 8 (from Ohod Mountain) whereas the highest shoot dry weight was given by isolate 10 (*Bacillus subtilis*) isolated from Al-Awaly site.

TABLE 1. Bacterial isolates, soil texture, pH and electric conductivity of the studied soils.

Isolate no.	Isolates	Site of isolation	Soil physical properties						pH	EC (µmho cm^{-1})
			Texture	Gravel (%)	Coarse sand (%)	Fine sand (%)	Clay (%)	Silt (%)		
1	*Bacillus subtilis*	Qurban El Nazel	Sandy loam soil	0.00	27.61	38.60	20.2	13.54	8.57	38,000
2	*Esherichia coli*	Qurban El Nazel		0.00	27.61	38.60	20.2	13.54	8.57	38,000
3	*Bacillus megaterium*	Eastern Hara		4.37	22.96	46.23	13.04	13.40	8.23	10,000
4	*Micrococcous* sp.	Eastern Hara		4.37	22.96	46.23	13.04	13.40	8.23	10,000
5	*Bacillus subtilis*	Western Hara		5.10	25.9	40.80	14.9	13.30	8.05	21,000
6	*Micrococcous* sp.	Al-Awaly		8.30	32.89	36.70	11.47	10.64	8.54	1,800
7	*Sporosarcia* sp.	Al-Awaly		8.30	32.89	36.70	11.47	10.64	8.54	1,800
8	*Bacillus subtilis*	Al-Awaly		8.30	32.89	36.70	11.47	10.64	8.54	1,800
9	*Bacillus subtilis*	Ohod mountain		8.50	25.95	32.00	16.05	17.50	8.40	4,000
10	*subtilis Bacillus*	Ohod mountain		8.50	25.95	32.00	16.05	17.50	8.40	4,000

TABLE 2. Germination percentage, index, vigor, and energy of germination for *Parkinsonia aculeata* seeds after soaking in bacterial filtrates. In columns, means followed by common letters are not significantly different at 5% level by LSD. GP, Germination percentage; SGI, seed germination Index; GE, Energy of Germination; SVI, Seedling Vigor Index.

Bacterial isolate filtrate	Seed Germination Parameters			
	GP	SGI	SVI	GE (at 7^{th} d)
Control (s.d.w.)	8^{d} ±3.27	1.97^{c} ±0.77	13.6^{e} ±5.55	0.32^{d} ±0.13
1	38^{bc} ±5.16	9.07^{ab} ±1.14	66.7^{bcd} ±11.25	1.52^{bc} ±0.21
2	32^{c} ±3.27	7.63^{b} ±0.71	54.4^{d} ±7.28	1.28^{c} ±0.13
3	44^{ab} ±9.79	10.35^{a} ±2.26	75.8^{ab} ±17.99	1.76^{ab} ±0.39
4	32^{c} ±3.27	7.33^{b} ±0.57	58.7^{cd} ±5.27	1.28^{c} ± 0.13
5	42^{ab} ±5.16	10.14^{a} ±1.36	73.8^{abc} ±9.72	1.69^{ab} ±0.24
6	44^{ab} ±3.27	10.42^{a} ±0.81	78.4^{ab} ±5.82	1.78^{ab} ±0.16
7	48^{a} ±3.27	10.12^{a} ±1.64	83.04^{a} ±5.65	1.96^{a} ±0.14
8	40^{abc} ±7.30	8.87^{ab} ±1.40	71.2^{abc} ±12.99	1.62^{abc} ±0.32
9	42^{ab} ±5.16	9.14^{ab} ±1.34	75.2^{ab} ±9.24	1.69^{ab} ±0.24
10	42^{ab} ±5.16	8.37^{ab} ±1.67	67.2^{abcd} ±8.26	1.69^{ab} ±0.24

(s.d.w. = sterile dist. Water)

The present investigation clarified the importance of soil bacteria inhabiting the arid zones; especially *Micrococcous* sp., *Sporosarcia* sp. and *Bacillus subtilis* in seed germination promotion through the dissolution of the seed coat. On the contrary, the effect of extracts of *Bacillus subtilis* in the inhibition of seed germination of *Sorghum halepense* and *Amaranthus hybridis* was documented (Mendoza *et al.*, 2012). Maximum seed germination and maximum shoot and root length of *Pennisetum americanum* was recorded in the combination of *Bacillus subtilis* and *Aspergillus flavus* in a pot experiment (Bhushan *et al.*, 2013). Authors added that *Bacillus subtilis* is able to reduce the seed - borne mycoflora of *P. americanum*. Ajilogba *et al.* (2013) found that all *Bacillus* spp. produce Indole Acetic Acid which supported growth of tomato. Production of IAA helps to increase the root dry weight and thereby increases the plant's ability to uptake N, P, K, Ca and Mg compared to the control (Etesami *et al.*, 2009). Lamasal *et al*., (2012) investigated that *B. licheniformis* and *B. subtilis* both produce *B*–glucanose, siderophene and auxins, they were also involved in phosphate solubilization, which led up to 20% increase in leaf, stem and root growth of red pepper and tomato. In a similar trial that agrees with ours, the effect of *Bacillus licheniformis* in promoting the seed germination and the seedling growth of the arid zone species; *Acacia senegal* was proved (Singh *et al.*, 2011).

In an experiment using the plant growth - promoting rhizobacteria, Gholami *et al.* (2009) concluded that the seed inoculation with bacteria significantly enhanced the seed germination, the seedling vigor, leaf and shoot dry weight, and the leaf area of maize. Also, *Bacillus* sp. and *Mycobacterium* sp. greatly increased the stem length of tomato (Tabli *et al.*, 2014). Pathak *et al.* 2013 reported that the seed germination and plant growth of guava recorded positive response when inoculated with plant growth promoting bacteria (PGPB) combined with *Azotobacter*, *Chroococcum and* farmyard manure (FYM). The possible reason for better plant growth and germination can be attributed to maximum and early bacterization near the root zone which induces germination by inducing root inducing substances (Wani *et al.*, 1988). Similar reports have been made by Nath and Koria (2000) in ginger (*Zingiber officinale* Rosc.).

The present investigation showed that greater germination of the tested seeds ouccrhed in soils with high salinity. This observaian can tradicts the results of Bojovic *et al.* (2010) who reported that seeds of all species belonging to Brassicaceae and Solanaceae germinate only in the low NaCl concentration. They added that all examined seeds germinate in great numbers after rinsing in distilled water. Naturally, the seeds of arid zones, such as *Parkinsonia aculeata* inhabiting the soils with high salinity always germinate in the rainy season and after the dilution of salts in soil. Under laboratory conditions, seed treatment with the test strain improved seed germination and seedling emergence over the control. Bacteria are known to produce different metabolites like IAA, GA and cytokinin - like substances, which can exert positive effect on seed germination and radicle length (Minax, 2012). Significant increase in seedling vigor could be due to better synthesis of auxins (Bharathi *et al*., 2004).

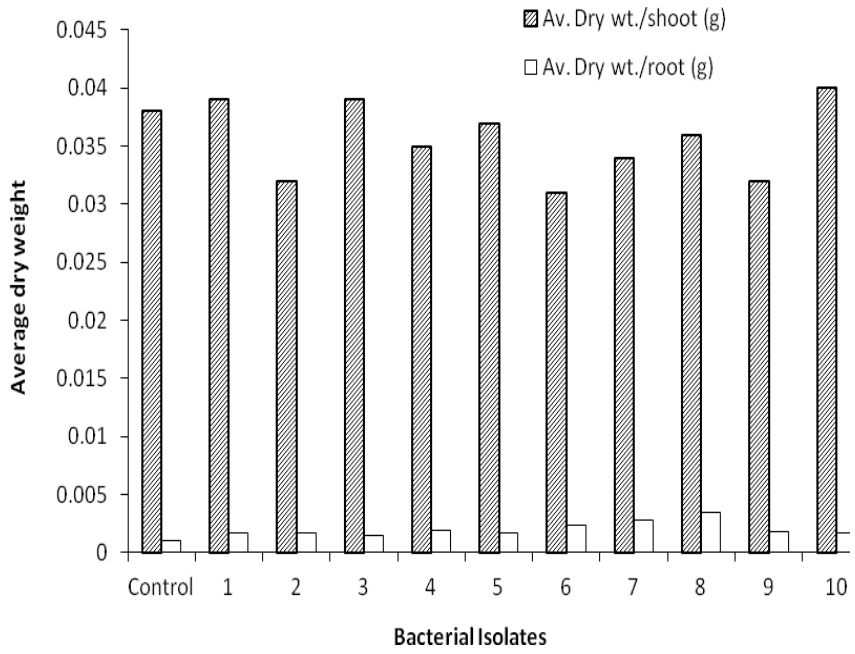

Fig.1. Root and shoot dry weights of 7 days old *Parkinsonia* seedlings after soaking in different bacterial filtrates

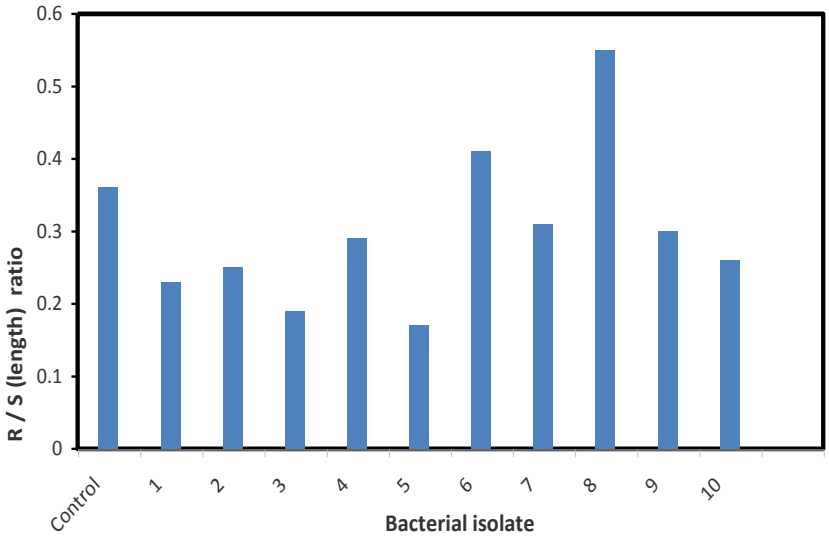

Fig. 2. Root / Shoot ratio (R/S) on basis of length of 7 days old *Parkinsonia* seedlings after soaking in different bacterial filtrates.

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أثر المزارع البكتيرية المختلفة والخالية من الخلايا على انبات بذور ونمو بادرات نبات الباركينسونيا

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و *قسم علم الأحياء -كلية العلوم للبنات – جامعة الملك عبد العزيز – جدة – المملكة العربية السعودية

تنتج الأنواع البكتيرية العديد من النواتج الأيضية المفيدة لنمو النبات وبقائه كما تحمي عوائلها من هجوم أنواع معينة من الكائنات الممرضة. أجريت الدراسة لتقييم أثر عزلات بكتيرية مختلفة على انبات بذور ونمو بادرات نبات الباركينسونيا كواحد من أنواع نباتات المناطق القاحلة. تم جمع كل من عينات التربة وبذو الدراسة من المدينة المنورة بالمملكة العربية السعودية. أكدت الدراسة تحفيز جميع العزلات البكتيرية لانبات البذور، وسجلت العزلات أرقام ٦ (ميكروكوكاس)، ٧ (سبوروسارسيا) المعزولة من ترب منخفضة الملوحة أعلى قيم لنسب الانبات (٤٤، ٤٨). وفي الوقت نفسه سجلت العزلة رقم ٧ (باليوم السابع للتجربة) أعلى قيمة لكل من مؤشر حيوية البذور (٨٣,٠٤) وطاقة الانبات (١,٩٦). ومن جهة أخرى فقد حققت عزلة بكتيريا (باسيللس ساتلس) أعلى قيمة (٠,٥٥) لنسبة الجذر / الساق (على أساس الطول)، وأعلى أوزان جافة للجذر والساق .