



EFFECT OF PLANT GROWTH PROMOTING RHIZOBACTERIA AND SOME PLANT EXTRACTS ON ROOTABILITY OF AERIAL HAYANY DATE PALM OFFSHOOTS

A- ROOTING PARAMETERS AND SURVIVAL (%)

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ABSTRACT

This experiment was carried out at the nursery of Faculty of Environmental and Agricultural Sciences, El-Arish, North Sinai, Egypt, to study the effect of inoculation with the plant growth promoting rhizobacteria (PGPR), *i.e.* Rhizobacterien (75g/l) or Nitrobien (65g/l) and the plant extracts namely : Gifert (Algae aqueous extracts), Ginger + Cinnamon extract and Roselle aqueous extract at 10% alone and in combination on the rootability and survival percentage of small high (unrooted) "Hayany" date palm (*Phoenix dactylifera* L.) offshoots. Results showed that, the highest rooting and survival percentages were observed with Rhizobacterien inoculated offshoots than Nitrobien inoculated ones. Moreover, treating them offshoots with Gifert (Algae extract) gave the highest values of rooting and survival percentages of small offshoots, followed by Roselle plant extract and Ginger combined with Cinnamon aqueous extract, respectively. The highest values of rooting and survival percentages were observed with Gifert (Algae extract) treated offshoots and inoculated with Rhizobacterien, while small offshoots treated with Nitrobien X Ginger and Cinnamon had the least values in this respect. The other interactions came in between them in this concern.

Key words: Date palm, rooting, plant growth promoting rhizobacteria (PGPR), Rhizobacterien, Nitrobien, plant extracts (antioxidants), Gifert (Algae extract), Roselle and Ginger + Cinnamon extract mixture.

INTRODUCTION

There is always an increased demand for date palm offshoots, especially superior cultivars. Using offshoots for propagating date palms is up till now the main method in order to insure true to type trees (Al-Bakr, 1972).

Problems of breeding and propagating date palm have been arisen from the fact that the palm has a long life cycle (Ammar and Badeis, 1983), and that the number of offshoots produced by the mother palm is linked to a certain period in the life date palm (Barret, 1973). During recent years there is an extreme demand for date palm offshoots for cultivation. The use of small

sized and aerial high offshoots is not practiced due the absence of roots and low survival percentage which lead to shy rootability. The rooting capacity (riders) has been correlated with their endogenous promoters and carbohydrate contents (Reuveni and Adato, 1974). There are several other rooting co-factors that occur naturally in cuttings or offshoots of several plant species.

These co-factors appear to act synergistically with auxin in rooting initiation (James and Thurbon, 1981). The action of these co-factors in rooting promotion could be, at least, protecting the root induction. The rooting medium is an

important factor in determining the extent of root formation on the offshoots (Al-Mana *et al.*, 1996).

Different strains of rhizosphere bacteria, called plant growth promoting rhizobacteria (PGPR) are a heterogeneous group of bacteria that can be found in the rhizosphere at root surfaces and associate with roots to improve the extent or quality of plant growth directly and or indirectly (Gilick, 1995). The use of plant extracts that contain a lot of active compounds could be a successful alternative to chemical improving root formation on small ridder date palm offshoots (El-Deeb *et al.*, 2008).

Thus, this study was carried out to evaluate the effect of plant growth promoting rhizobacteria and some plant extracts on rootability and survival percentage of small ridder Hayany date palm offshoots of the treated offshoots.

MATERIALS AND METHODS

In early April of 2010/2011 seasons, 120 small ridder "Hayany" date palm offshoots (*Phoenix dactylifera* L.), weighing about 2.15-3 Kg with 10-17 cm in stem diameter were devoted for this study. The selected offshoots were healthy and approximately similar in size and growth vigour.

Then, the lower layer of the leaves was removed and the base of the small ridder offshoots were cleaned and submerged for ten minutes in disinfection solution containing 1% cupper sulfate (CuSO_4) at 10 g.l^{-1} . Each offshoot was planted in a black plastic pot 30 cm in diameter filled with 4 kg of mixture by volume of (3 sand : 2 peatmoss : 1vermiculite), Each pot was inoculated with 75 g Rhizobacterein or 65 g Nitrobein, except those of the control treatment. Rhizobacterein is a mixture of *Azotobacter chroococcum* and *Azospirillum barasilense*, while Nitrobein contain *Azospirillum* spp and *Azotobacter*

chroococcum. Such products are produced by the Ministry of Agric., Egypt.

The small offshoots were treated with the following plant extracts by submerging them for 24 hours before planting, and after planting they were treated with the final extracts at 100 ml /offshoot twice a week.

1. Gifert (Algae aqueous extract):

At 10% by completing 100 ml of the extract to one liter with distilled water.

2. Ginger plus cinnamon aqueous extract:

This extract was prepared by adding 50 g of powdered ginger +50 g of powdered cinnamon to one litter of distilled water at 50°C for 24 hours, then the extract was filtrated and adjusted to one liter to obtain concentration of 10% (W/V).

3. Roselle extract:

100 grams of powdered Roselle flowers were soaked in distilled water at °C for 24 hours, then the extract was filtered and adjusted to one liter to obtain the concentration of 10% (W/V) extract.

4. Control (tap water):

Small ridder offshoots were watered twice weekly with tap water, the chemical analysis of tap water used in this study is presented in Table (1). The treatments were arranged as a factorial experiment in a completely randomized block design (2 factors) with three replicates for each treatment, each replicate was represented by five offshoots.

Specific and interaction effects of the investigated factors were studied through the determination of the following measurements:

1. Survival percentage (%)

One year after treatment, offshoots growth was observed as survival percentage (%).

Table (1): Chemical analysis of irrigation water used:

Parameters	Value
E.C (dSm ⁻¹)	0.83
Conc. (ppm)	532
pH	8.0
Soluble cations (Meq.l ⁻¹)	
Ca ⁺²	4.0
Mg ⁺²	2.0
Na ⁺	4.5
K ⁺	0.1
Soluble anions (Meq.l ⁻¹)	
Cl ⁻	2.40
CO ₃ ⁻²	-
HCO ₃ ⁻	6.25
SO ₄	1.95
Water quality*	
Total salinity	C3
Sodicity	S1

Where:

Artesian water well from Faculty of Environmental Agricultural Science, El-Arish, North Sinai Governorate, according to Piper (1947)

The survival percentage was calculated using the following equation:

$$\text{Survival percentage} = \frac{\text{Number of survived offshoots after one year}}{\text{Total number of planted offshoots}} \times 100$$

2. Growth measurements

One year after planting, the small offshoots were uprooted from the media and separated. Measurements were taken for each offshoots as survival on root formation and development. After removing survived offshoots from the medium, number and length of main roots, as well as fresh and dry weights were

recorded then all the formed roots were removed and separated according to the diameter, since the secondary roots were 0.1 to 1 mm in diameter, while that of the main roots (above 1.3 mm).

Moreover, the various types of each root were independently dried in an oven at 70°C for forty eight hours to determine the dry weights. The total dry weight of each offshoot root system was calculated.

3. Statistical analysis

The obtained data were subjected to the analysis of variance methods according to Snedecor and Cochran (1980). The means were compared using Dunca's multiple test at (0-05) level (Duncan, 1955). Computations were performed using MSTATC computer programs package (Russell, 1986).

RESULTS AND DISCUSSION

1. Rooting parameters

Tables (2) and (3) show the specific effect and interaction of plant growth promoting rhizobacteria (PGPR) and plant extracts on rooting of small-aerial-Hayany date palm offshoots during the two seasons, of study.

1.1. The total main roots fresh weight small aerial offshoot

Referring to the specific effect of plant growth promoting rhizobacteria (PGPR) on the inoculated offshoots, data in Table (2) clear that the Rhizobacterien inoculated offshoots exhibited a significant higher effect on the total main roots fresh weight (15.40 and 6.80 g) than Nitrobien-inoculated ones (5.95 and 3.64 g) in the first and second seasons, respectively.

Furthermore, Table 2 shows that Gifert (Algae extract) treatment recorded the highest fresh weight of total main roots (36.74 and 13.56 g), followed by Roselle (3.15 and 3.58 g) and Ginger + Cinnamon aqueous extracts (1.67 and 1.97 g) in the first and second seasons, respectively with significant differences between them compared with untreated offshoots (control) which recorded the least values (1.15 and 1.20 g) in the two seasons.

Regarding the interaction between plant growth promoting rhizobacteria (PGPR) and the testing plant extracts on rooting of small aerial "Hayany" date palm offshoots. Table (2) reveals that Rhizobacterien-inoculated offshoots and treated with Gifert (Algae extract) had the highest values for total fresh weight of main roots (55.1 and 17.87 g) followed by Rhizobacterien inoculated offshoots while the least values (1.06 and 1.10 g) were recorded for untreated offshoots (control).

Other interactions came among them with significant differences in this respect in both seasons.

1.2. The total main roots dry weight

Data in Table (2) disclose that total main roots dry weight was significantly increased by Rhizobacterien inoculated offshoots than those inoculated with Nitrobien.

Table (2) reveal also that Gifert (Algae extract) gained the highest values of total main roots dry weight per small aerial offshoots (15.22 and 6.74 g), followed by Roselle extract then Ginger + Cinnamon extract treatments, while untreated offshoots (control) showed the least values (1.83 and 0.75 g) in first and second seasons, respectively.

The interaction between plant growth promoting rhizobacteria (PGPR) and plant extracts (antioxidants) on rooting offshoots, showed that the inoculated offshoots with Rhizobacterien combined with Gifert (Algae extract) values induced the highest main roots dry weight, followed by Gifert (Algae extract) Nitrobien inoculated offshoots.

While the untreated offshoots (control) recorded the least main roots dry weight (0.73 and 0.80 g) in both seasons, respectively. These results go in line with those reported by **Janzen *et al.* (1992)**, **Holguin *et al.* (1999)**, **Salamone *et al.* (2001)**, **Zahir *et al.* (2001)**, **Salmeron *et al.* (2003)**, **Vessey (2003)** and **Zahir *et al.* (2003)**.

1.3. Number of main root per small-aerial of fshoot

The Rhizobacterien inoculated offshoots exhibited a significant higher number of main roots (5.28 and 3.50 roots/offshoot) than Nitrobien inoculated ones (3.0 and 2.09 roots/ offshoot) in the first and second seasons, respectively (Table 2).

Furthermore, Table, 2 shows that the most obvious increments in the number of main roots/offshoot observed with Gifert (Algae extract) (12.16 and 6.33) roots/offshoot followed by Roselle (1.83 and 1.78)

Table (2): Effect of plant growth promoting rhizobacteria and some aqueous plant extracts of on No .of main roots/offshoot, fresh and dry weight of main roots of small aerial offshoots of 'Hayany' date palm during 2010/11 and 2011/12 seasons.

PGPR plant extracts	No .of main roots/offshoot			Fresh weight (g)			Dry weight (g)		
	Rhizo.	Nitro.	Mean	Rhizo.	Nitro.	Mean	Rhizo.	Nitro.	Mean
First season (2010/2011)									
Control (tap water)	1.45 c	1.14 c	1.29 c	1.23 d	1.06 d	1.15 c	0.73 e	1.10 e	1.83 c
Gifert (Algae extract)	16.33 a	8.00 b	12.16 a	55.10 a	18.37 b	36.74 a	21.17 a	9.27 b	15.22 a
Roselle (aqueous extract)	2.00 b	1.66 c	1.83 b	3.50 c	2.80 c	3.15 b	5.13 c	2.20 d	3.66 b
Ginger+Cinnamon (aqueous extract)	1.32 d	1.20 c	1.26 c	1.77 d	1.57 d	1.67 c	2.90 d	1.83 d	2.37 bc
Mean	5.28 c	3.00 d		15.40 a	5.95 bc		7.39 b	3.59 c	
Second season (2011/2012)									
Control (tap water)	1.35 c	1.12 c	1.24 c	1.3 d	1.1 d	1.20 d	0.97 e	0.80 e	0.75 d
Gifert (Algae extract)	8.33 a	4.33 b	6.33 a	17.87 a	9.43 b	13.56 a	9.42 a	4.60 b	6.74 a
Roselle (aqueous extract)	2.00 c	1.56 c	1.78 c	5.13 c	2.2 d	3.58 c	2.37 c	1.13 d	1.75 c
Ginger+Cinnamon (aqueous extract)	2.33 bc	1.33 c	1.83 c	2.9 d	1.83 d	1.97 d	1.67 cd	1.40 d	1.85 c
Mean	3.50 b	2.09 b		6.80 b	3.64 c		3.60 b	1.98 c	

Means followed by the same letter(s) within each column are not significantly different at the 0.05 level, according to Duncan's multiple range test.

roots/ offshoot against untreated offshoots (control) (1.29 and 1.24) roots/ offshoot in first and second seasons, respectively. On the other hand, the other treatments came in between in this respect. Disclosed data showed that the highest number of main roots per offshoots was resulted from inoculated offshoots with Rhizobacterien× Gifert (Algae extract) treatment followed by Gifert provided with Nitroben inoculated offshoots. The least values were recorded by untreated offshoots (control).

The other interactions came in between with significant difference among them. This increment of number of main roots

numbers may be due to the promotion effect of (PGPR) and plant extracts, which greatly affect root development. Similar results were reported earlier by **Rizk and El-Sayed (2004)**.

1.4. Fresh weight of Total roots

Data in Table (3) show that total fresh weight of root system of the inoculated offshoots with Rhizobacterien was greater than that with inoculated Nitroben ones in both seasons.

In reference to the effect of plant extracts, results showed that the untreated offshoots (control), significantly gained the

Table (3): Effect of plant growth promoting rhizobacteria and aqueous plant extracts on total root system and the length of the longest main root of small aerial of 'Hayany' date palm offshoots during 2010/2011 and 2011/2012 seasons.

PGRP plant extracts	Fresh weight (g)			Dry weight (g)			Length(mm)		
	Rhizo.	Nitro.	Mean	Rhizo.	Nitro.	Mean	Rhizo.	Nitro.	Mean
First season (2010/2011)									
Control (tap water)	3.10 e	1.90 e	2.50 e	1.86 e	1.50 e	1.68 d	2.57 d	1.57 c	2.07 d
Gifert (Algae extract)	72.17 a	29.63 b	50.90 a	32.0 a	16.13 b	24.11 a	92.50 a	20.17 b	56.34 a
Roselle (aqueous extract)	12.93 c	8.93 d	10.93 c	7.77 c	4.93 d	6.35 c	22.17 b	12.03 bc	17.10 b
Ginger+Cinnamon (aqueous extract)	7.73 d	6.50 d	7.12 d	3.97 d	3.03 d	3.50 cd	7.98 c	9.83 cd	8.90 c
Mean	23.98 b	11.74 c		11.40 b	6.41 c		31.31 bc	10.9 c	
Second season (2011/2012)									
Control (tap water)	3.90 e	1.76 e	10.75 c	1.97 d	1.2 d	1.62 d	2.89 d	1.97 d	2.43 d
Gifert (Algae extract)	29.63 a	19.2 b	24.42 a	14.97 a	8.34 b	11.71 a	21.57 a	12.77 b	17.17 a
Roselle (aqueous extract)	13.47 c	8.74 d	8.71 d	6.23 b	4.90 c	5.61 b	13.20 b	7.77 c	10.49 b
Ginger+Cinnamon (aqueous extract)	8.03 d	6.50 d	7.31 d	4.63 c	3.47 c	2.03 c	2.16 c	3.64 d	2.90 d
Mean	13.76 b	9.05 d		6.95c	4.47d		9.95 b	6.54 c	

Means followed by the same letter(s) within each column are not significantly different at the 0.05 level, according to Duncan's multiple range test.

Rhizo= Rhizobacteria and Nitro= Nitroben

lowest total root system fresh weight in both seasons, while Gifert (Algae extract) recorded the highest total root fresh weight.

The other treatments came in between. Similar observations were reported by **Al-Obeed (2005)**.

1.5. Dry weight of total roots and the length of the longest main root

Data in Table (3) clear that the Rhizobacterien inoculated offshoots, significantly increased total dry weight of root system and produced the longest main root than Nitroben inoculated offshoots. The same results were found by **Shaban and Mohsen (2009)**.

As for the effect of plant extracts, data in the same Table show that Gifert (Algae

extract) caused a significant increase in total dry weight of root system and produced the longest main root followed by Roselle aqueous extract then Ginger + Cinnamon aqueous extract. The least values in this respect were recorded for the untreated offshoots.

Regarding the interaction between plant growth promoting rhizobacteria (PGPR) and aqueous plant extracts, data in Table (3) indicated that the heaviest root system dry weight and the longest main roots were gained by the inoculated offshoots with Rhizobacterien combine with Gifert (Algae extract) (Fig. 1). While the least values were recorded by untreated offshoots (control). The other interactions came in between with significant differences between them. These results go in line with

those reported by El Hodairi, *et al.* (1992) Wange and Ranawade (1998) and Rizk and El Sayed (2002).

Generally, the root formation will proceed according to a genetically determined pattern as modified by each of

chemical, physical and bio-environmental factors. Among physical factors the plant growth promoting rhizobacteria (PGPR) and plant extracts which greatly affect both root development and distribution patterns.



Fig. (1): Gifert (Algae extract) × Rhizobacterien inoculation treatment induced the longest main roots.



(2)

(3)

(4)

(5)

Fig . (2): Unrooted in control treatment.

Fig. (3): Offshoots roots in Ginger + Cinnamon (aqueous extract) × Rhizobacterien treatment,

Fig . (4): Offshoots roots in Roselle (aqueous extract) × Rhizobacterien treatment

Fig . (5): Offshoots roots in Gifert (Algae extract) × Rhizobacterien treatment.

All treatments induced drastic modifications in the hormonal patterns at the different stages of root formation. On the other hand, the cut surface of the offshoots (the attachment point with the mother palm) is accompanied by secretion of some substances into the medium, such as phenols, may have profound physiological effect on the cultured offshoots. These results are in line with those reported by **El-Hamady *et al.* (1992)** and **Hodel and Pittenger (2003)**.

On the other hand, browning of the tissue and adjacent medium is assumed to be due to the ordination of polyphenoles and formation of quinines which are aerial reactive and toxic to the tissues.

Aside from, several enzymes which are widely distributed in plant oxides phenols to quinines *e.g.*, monophenol oxidase (tyrosinase and polyphynol oxidase (catchall oxidase). Furthermore, adding Gifert (Algae extract), Roselle and Ginger + Cinnamon aqueous extracts plus the plant growth promoting rhizobacteria (PGPR) (Rhizobacterien or/and Nitrobien) for curtailing the oxidations of the phenolic compounds.

In other words, using plant growth promoting rhizobacteria (PGPR) may prevent or minimize releasing and oxidation of endogenous phenolic compounds, which included either through antioxidant or absorbent substance. **Al-Mana *et al.* (1996)**, **Sourour (2001)**, **Okawara *et al.* (2003)**, **Qaddoury and Amssa (2003)**, **Vezaei, *et al.* (2003)**, **Rizk and El sayed (2004)** **Qaddoury and Amssa (2004)**, **El-Assar *et al.* (2004)** and **Al-Obeed (2005)**.

2. Survival percentage (%)

Concerning the effect of plant growth promoting rhizobacteria (PGPR) Fig. (6) show that the Rhizobacterien inoculated

offshoots gave the highest surviving percentage than the Nitrobien inoculated ones in the first season. But, in the second one the survival percentages were similar for both Rhizobacterien and Nitrobien inoculated offshoots.

As for the effect of plant extracts, Fig. (7) clear that all tested plant extracts caused a significant increase in the survival percentage of small aerial offshoots than untreated ones (control). The highest survival percentage was recorded for Gifert (Algae extract) treatment, followed by Roselle aqueous extract, Ginger + Cinnamon aqueous extract treatments, respectively.

In relation to the interaction between plant growth promoting rhizobacteria (PGPR) and plant extracts, Fig. (8) clear that inoculated offshoots with Rhizobacterien and those inoculated with Nitrobien which treated by Gifert (Algae extract) induced the highest simulative effect in survival percentage, while untreated offshoots (control) had the least values in this respect. The other interactions came in between with significant differences among them. Similar observations were reported by **Al Ghamdi (1988)**, **Nagarajan *et al.* (1989)** **Kloepper and Beauchamp (1992)** **Al Mana *et al.* (1996)** **Haggag and Azzazy (1996)**. **El Bahr *et al.* (2003)** and **Hodel and Pittenger (2003)** and Generally, it was concluded that the application of synergists of antioxidant to the medium may be enable to promote the establishment of planted offshoots and increased the percentage of survival. This results are similar with **Gupta and Godara (1984)**, **Sourour (2001)**, **El Deeb *et al.* (2008)**, **Bakr *et al.* (2010)**, **Shaban and Mohsen (2009)** and **El Kosary (2009)**.

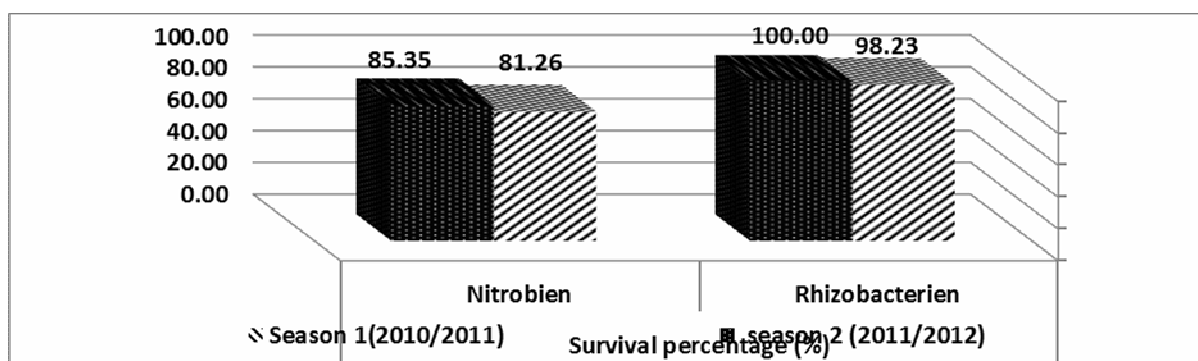
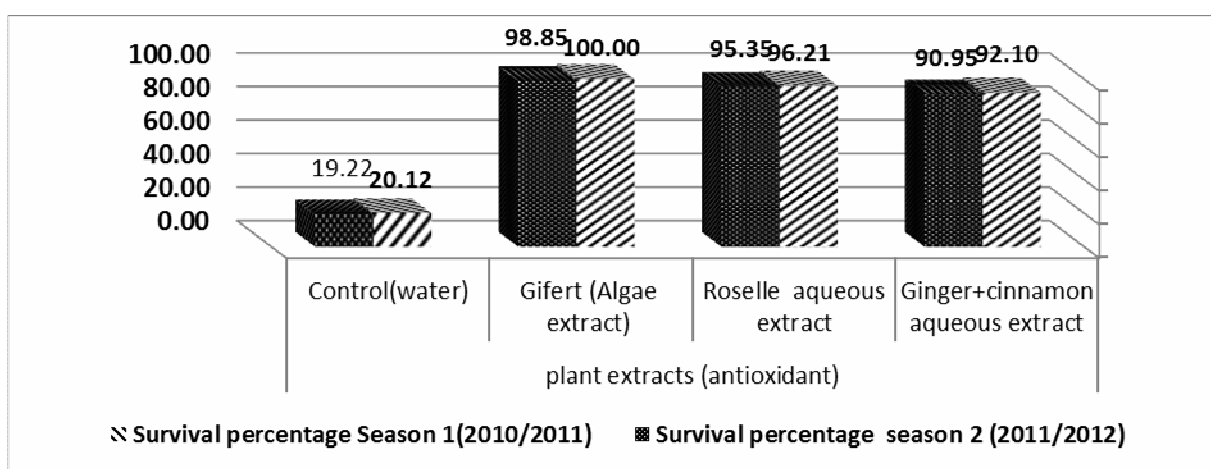


Fig. (6)



Figs. (6&7): Specific effect of plant growth promoting rhizobacteria and (6) plant extracts and (7) on survival percentage of aerial offshoot during 2010/2011 and 2011/2012 seasons.

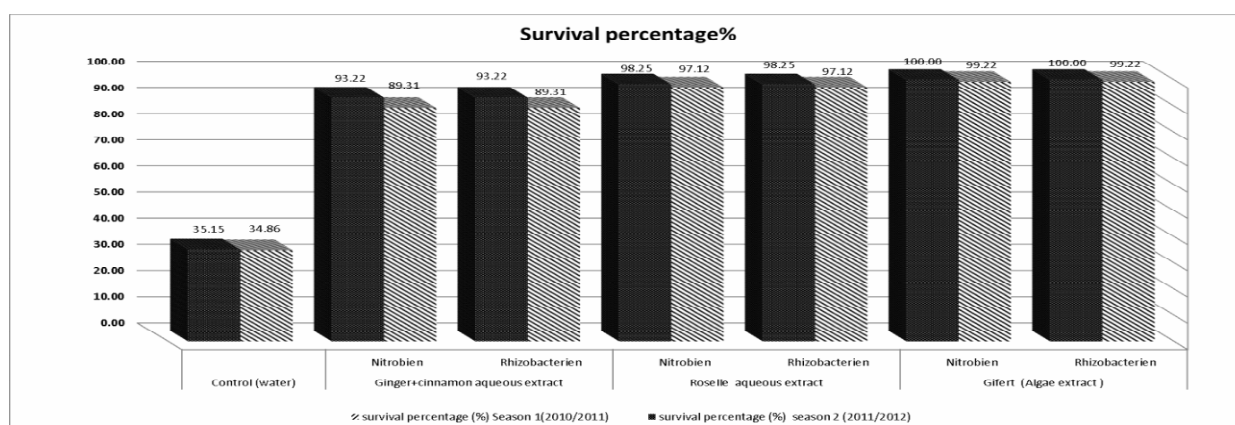


Fig. (8): Interaction effect between plant growth promoting rhizobacteria (PGPR) and plant extracts on survival percentage (%) during 2010/2011 and 2011/2012 seasons.

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الملخص العربي

تأثير بكتريا الريزوسفير المحفزة لنمو النبات وبعض المستخلصات النباتية على المقدرة التجذرية لفسائل نخيل البلح الهوائية (الرواكب) للصنف الحياني

أ- قياسات التجذير ونسبة البقاء

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أجريت هذه التجربة في بداية شهر أبريل خلال موسم ٢٠١٠/٢٠١١، ٢٠١١/٢٠١٢، ٢٠١٢/٢٠١٣ على ١٢٠ فسيلة صغيرة الحجم عديمة الجذور (سرطانات) من نخيل البلح لصنف الحياني خالية من الأمراض، متشابهة في الحجم والوزن إلى حد كبير لدراسة مدي تأثير استخدام بكتريا الريزوسفير المشجعة لنمو النبات (الريزوبكتريين) بمعدل ٧٥ جرام/لتر، النتروبيين بمعدل ٦٥ جرام/لتر وبعض المستخلصات النباتية مثل الجيفرت المستخلص المائي للطحالب البحرية تركيز ١٠% والمستخلص المائي للكردية تركيز ١٠%، المستخلص المائي للجنزيبيل + القرفة تركيز ١٠% حيث لقت الفسائل بأحد النوعين من أنواع بكتريا الريزوسفير المشجعة للنمو تم نعتت الفسائل الصغيرة لمدة ٢٤ ساعة في احدى المستخلصات النباتية السابقة ثم زرعت مباشرة في الاصص وروبت مرتين اسبوعيا بماء الصنبور، تم معاملة الفسائل الصغيرة بإضافة إحدى المستخلصات النباتية سابقة التجهيز بمعدل ١٠٠ مل لكل فسيلة مرتين اسبوعيا ماعدا معاملة المقارنة (اضافة الماء فقط). تم تصميم التجربة بنظام التصميم العشوائي الكامل في ثلاث مكررات ويمثل كل مكررة بخمس فسائل. وتم تقييم التأثير النوعي لكل العوامل المختبرة والتفاعل بينها بعد مرور عام على زراعتها.

أظهرت النتائج خلال موسمي الدراسة التفوق المعنوي الواضح للفسائل الملقحة ببكتريا الريزوبكتريين ممثلا في عدد الجذور الرئيسية، الوزن الطازج والجاف للجذور الرئيسية، الوزن الطازج والجاف للجذور الكلية ونسبة الفسائل الحية المتبقية كما أظهرت النتائج أن معاملات الجيفرت (مستخلص الطحالب البحرية) والمستخلص المائي للكردية بالإضافة إلى مستخلص القرفة والجنزيبيل أحدث زيادة معنوية في عدد الجذور، الوزن الطازج والجاف للجذور الرئيسية، الوزن الطازج والجاف للجذور الكلية ونسبة الفسائل الحية المتبقية، على الترتيب مقارنة بالفسائل غير المعاملة (المقارنة). وكان التفاعل بين عوامل الدراسة معنويا حيث أظهرت الفسائل التي لقت بالريزوبكتريين وعولمت بالجيفرت (مستخلص الطحالب البحرية) تفوقاً وبفروق معنوية في جميع قياسات التجذير وبلغها كل من المستخلص المائي للكردية وكذلك المستخلص المائي لمخلوط القرفة والجنزيبيل مقارنة بالفسائل غير المعاملة (المقارنة).

الكلمات الاسترشادية: نخيل البلح، مرحلة التجذير، بكتريا الريزوسفير، مستخلص الكركدية والقرفة.

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