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Performance of some ecofriendly materials on induction of resistance against soil borne diseases and enhancement of roselle productivity in Siwa Oasis

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ABSTRACT

This work was conducted at Khamisa Farm of Desert Research Center (D.R.C.) at Siwa Oasis in North western desert of Egypt during the two seasons (2014-2015) and (2015-2016) to investigate the effect of some natural products (Row-Kaolin, Tracer 24% SC, ALKANZ and Bio-Mix) as seed dressing or foliar application treatments to induce resistance of roselle plants against soil borne diseases and enhancement the productivity of roselle plants. The treatments were installed in split plot design (foliar application treatments arranged in main plot and seed dressing treatment in sub plot). The obtained results indicated that, Bio-Mix product as seed dressing and / or foliar application recorded significant reduction in damping off, root and crown rot incidence and severity as well as increase resistance in roselle plants. Moreover, The interaction effects showed that, using Bio-Mix product as seed dressing and / or foliar application gave the highest values of (fruits number/plant, dry weight of sepals g/plant, dry yield of sepals kg/fed, seed weight g/plant, seed yield kg/ fed, total chlorophyll, anthocyanin content, vitamin A, vitamin C content of sepals and defense enzymes activity (peroxidase and chitenase) compared to other treatments under study.

Key words: *Hibiscus sabdraffia*, productivity, Bio-Mix product, soil borne diseases and defense enzymes activity

Introduction

Hibiscus sabdraffia Linn. is a tropical plant belongs to Malvacae family (Odebunmi *et al.,* 2002). Calices are important part of roselle plant. It was found that when calices are dried under sun light and air, contain organic acids (tartaric, citric, malic, and hibiscic) (Meza-Jimnez *et al.,* 2009). Many biological activity studies were conducted on *H. sabdariffa* and some of the compounds have been shown to have antibacterial, antifungal, antihypertensive, anti-inflammatory, antioxidant, antispasmodic, antitumor and hypoglycaemic (Fidan *et al.,* 2011) activities. Medical uses of calices are wide. Infusion of calices are regarded as diuretic, choleretic, febrifugal, hypotensive, decreasing the viscosity of blood and stimulating intestinal peristalsis (Morton , 1987).

Roselle is cultivated in Egypt and producing 94% from total production of medicinal plants in Upper Egypt (Hassan *et al.,* 2014). However, biotic constraints such soil borne diseases have led to a steep decline in roselle yield.One of the most serious obstacles that limit roselle production in many growing areas is the damping-off, wilt and root rot diseases in Egypt (Abu El-Ela, 1975).The most frequent pathogenic soil-borne fungi associated with rotted roots were *Fusarium oxysporum, Fusarium solani, Macrophomina phaseolina, Rhizoctonia solani, and Fusarium equiseti* (Ottai and Abd-El-Khair, 2004) and (Hassan *et al.,* 2014).*Fusarium* sp. could induce vascular wilt and root and crown rot symptoms on *Hibiscus moscheutos* (Lupien and Dugan, 2017). However, the use of chemical pesticides is still a prevalent approach to contain many plant pathogens and pests. The excessive usage of chemical pesticides posses a major health and environmental hazard and also pose a risk of resistance development in phytopathogens and pests against them; therefore, other eco-friendly alternatives must be explored to sustainable management of plant diseases and pests(Subash *et al.,* 2013).

Biocontrol using natural antagonistic microorganisms, i.e. biocontrol agents (BCA), is an environmentally safe method of pest and disease management (Singh and Nautiyal, 2012). Furthermore,

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besides preventing plant pests and diseases, BCA also promote the growth of the plants (Azarmi *et al*., 2011). They also enhance stress tolerance, aid in nutrient acquisition and induce disease resistance in plants,based on the mechanisms and effects of products from BCA can be used as biofertilizers, as plant strengtheners and as biopesticides (Meena *et al*., 2017).

Kaolin is a naturally occurring, chemically inert clay mineral. Kaolin particle film applications have been used to reduce negative impacts of environmental stresses on crop plants, to suppress diseases, and to protect crops from insect pests (Kahn and Damicone, 2008).

Induction of systemic resistance to elicit a defense response in the host plant, called induced systemic resistance (IRS) by using microorganisms or environmentally safe chemicals became a good target for minimizing disease severity with low cost and without environmental hazards (Kuc, 2006and Salomon*8 et al*., 2017). Various beneficial rhizospheric *Pseudomonas* species induce systemic resistance mechanism in plants, which provides protection against wide range of phytopathogenic organisms including fungi, bacteria and viruses. Seed or seedlings treatment with rhizobacterial strains has resulted in enhanced resistance in the treated plants (Kloepper *et al*., 2004).The microbial isolates and silicon were tested for their ability to induce the production of defense-related enzymes in treated plants (Konappa *et al.,*2016 and Mburuab *et al*.,2016).

The present work was designed to evaluate the ability of the natural materials as seed dressing or as foliar application as single or integration treatments to induce resistance of roselle plants against soil borne diseases and enhancement the productivity of roselle under field conditions.

Materials and Methods

This work was done at Khamisa Farm of Desert Research Center (D.R.C.) at Siwa Oasis, Marsa Matruh Governorate during the two successive seasons of 2014/2015 and 2015/2016.The effect of natural products treatments as seed dressing or foliar application and their interaction on some soil borne diseases and yield as well as their components of roselle plants were investigated under sandy soil with previous history of sever crown and root-rot diseases incidence. In each season, a split plot design with three replicates was used. The main plots assigned to foliar application and the sub plots deviated to seed dressing. The experimental included 15 treatments which were the combination of five foliar applications (control, Row-Kaolin, Tracer 24% SC, ALKANZ and Bio-Mix products) and seed dressing (without, Row-Kaolin at 5% and Bio-Max at 5%).The experimental area contained ten rows at 75 cm apart at the distance between plants was 50 cm. Each treatment contained 60 plants under drip irrigation system. Roselle seeds were dipped in Row-Kaolin or Bio-Mix products for 2 hours before transplanting and were sown on 1st of April in the first and second seasons. Also, the plants were sprayed using hand-held sprayer and the used volumes of the solutions were maintained just to cover the whole plant foliage completely every 15 days from the first spray date and after 30 days from sowing date till the beginning of flowering stage. All treatments were fertilized with 200 kg calcium superphosphate (15.5 % P_2O_5), 50 kg potassium sulphate (50 % K_2O) and 150 kg ammonium nitrate (33 % N) per feddan. Phosphorus fertilizer was applied during soil preparation. Nitrogen and potassium fertilizers were divided into three equal doses and were added to the soil at 30, 60 and 90 days after sowing under drip irrigation system with drippers 4L/hr for an hour twice every week.

The physical and chemical properties of the experimental soil were presented in Table (A).

Table (A): Physical and chemical properties of the experimental soil.

Particle size distribution (%)			Soil texture	O.M.(%)	Ec dsm-1	pH	Soluble cations (meq/l)				Soluble anions (meq/l)			
Sand	Silt	Clay					Na^+	K^+	Ca^{++}	Mg^{++}	$CO3^{--}$	$HCO3^-$	Cl^-	$So4^{--}$
92.91	5.21	1.88	sandy	0.5	4.1	7.5	0.2	24.7	8.6	7.5	-	3.6	31.3	6.1

The chemical analysis of the used irrigation water is shown in Table (B).

Table (B): The chemical analysis of irrigation water.

pH	E.C.	Soluble anions (meq/l)				Soluble cations (meq/l)			
	ppm	$CO3^{--}$	HCO_3^-	Cl^-	$SO4^{--}$	Ca^{++}	Mg^{++}	Na^+	K^+
4.23	2709.60	-	2.17	22.02	15.77	9.47	7.75	21.75	0.99

The components of natural products under this study are as follows:

- Row-Kaolin (aluiminuim silcate), It is an active ingredient of kaolin product. It is produced by Memco Company and applied at rate of 5kg/100L.
- Tracer 24% SC, containing a mixture of spinosyn A and spinosyn D (Ali, 2016).It was formulated by Dow Agro Sciences Limited. 0.5 ml/L from Tracer 24% SC was used as a foliar application in both two seasons.
- ALKANZ 2000 EC is produced by Alkanz Company for oil extractions, its, formulation consists of a number of natural plant extracts like Ricinoleic acid and Elenin. ALKANZ 2000 was applied as a foliar spraying with 2.5 ml/L in both seasons.
- Bio-Mix product: A new modification of EM_1 which was later known as Bio-Mix (BM) was made by Abdel-Ati (under publication) as a new formula for EM_1 through different experiments for series of *Bacillus subtilus* + *Azotobacter chroococcum* + arbuscular mycorrhiza in addition to 20% phosphate as (P_2O_5), Potassium as (K_2O) in addition to 1% of Mg, besides 0.01 of (Fe, Zn and Mn) which were fermented in the classical EM_1 for 45 days before usage. The EM1 was kindly obtained from EM project- EEAA- Ministry State of Environmental Affairs, while the liquid culture of *Bacillus subtilus*, *AzotobacterChrococcum* and arbuscular mycorrhiza were kindly obtained from microbial research center (Cairo- MIRCEN).) The effective microorganisms (EM_1) contains selected species of microorganisms including predominant populations of lactic acid bacteria, yeasts, and smaller numbers of photosynthetic bacteria, actinomycetes (Higa, 1991 and Higa & Parr 1994).

Data Recorded:

1. Pathogens and diseases records:

1.1. Isolation, purification and identification of the causal pathogens:

Samples of roselle plants showed clear symptoms of crown and root-rot, diseases were collected from fields of Siwa oasis in Matrouh Governorate a year before conducting the experiment. The isolated fungi were purified applying hyphal tip isolation technique suggested by Booth, (1985). Identification of the isolated fungi was done in Mycology Center, Assuit University, Assuit, Egypt. The obtained isolates were maintained on PDA slants and kept in a refrigerator at 5°C for further study.

1.2. Preparation of the fungal inocula:

Conical flasks each containing 100 g barely grains and 100 mL of distilled water were autoclaved. These flasks were subsequently inoculated with the fungal isolates using of 1 cm fungal disc, previously grown on PDA for 6 days. The inoculated flasks were incubated at 25°C for 14 days with shake every day.

1.3. Pathogenicity tests:

Seeds of roselle were surface sterilized and dried in sterilized filter paper. The pathogenicity tests were carried out on pots in greenhouse of Plant Protection Department, Desert Research Center. The inocula of the tested fungi were prepared on barely grains as mentioned above to study their pathogenicity. Soil inoculation was performed by mixing 2% of the fungal inoculate with the soil in each pot (100 g/5 kg soil) and then irrigated directly. Non-inoculated sterilized barely grains were used as the control treatment. Ten sterilized surface seeds of roselle were sown in each pot and a set of three replicates was performed. Data of pre- and post-emergence damping-off were recorded after 4 wk. Samples of plants were taken from different treatments for the purpose of re-isolation procedures on PDA.

1.4. Disease assessment:

The disease assessment was done periodically by examining and recording percentages of pre- and post-emergence damping off after 30 and 45 days from sowing date, respectively. At each treatment, approximately 10 roselle plants showing various extents of decline, and also three healthy plants not showing decline symptoms for comparison, were sampled randomly. Sampled plants were carefully washed under running tap water to remove soil. For each individual plant sampled, percentage of root-rot and crown rot diseases incidence was assessed 45 days after sowing. Disease severity (D.S.) of crown and root diseases were separately assessed based on a 0–4 disease severity (D.S) scale described according to (Fang *et al.,* 2011), where: 0 = no crown/root tissue discolored; 1 = <25% crown/root tissue discolored; 2 = ≥25, <50% crown/root tissue discolored; 3 = ≥50, <75% crown/root tissue discolored; 4 = ≥75% crown/root tissue discolored. For each replicate a disease severity index (DSI) was calculated as following:

$$D.S. = \Sigma\,(ab) \times 100\,/AK$$

Where: a = No. of diseased plants having the same degree of infection. b = Degree of infection. A = Total no. of examined plants. K = Highest degree of infection. D.S = Disease severity

2. Yield and its Components:

On 11th November in both seasons, sepals of roselle were harvested then the following data were determined.
2.1. Number of fruits per plant.
2.2. Dry yield of sepals per plant and per feddan.
2.3. Seed yield per plant and per fedaan.

3. Chemical Constituents:

3.1. Total chlorophyll (mg/g as leaves fresh weight) according to Rodriguez and Miller, (2000)
3.2. Determination of the anthocyanin content (mg / 100 g) in dried sepals according to the method described by Fuleki and Francis, (1968) and modified by Francis, (2000) for *Hibiscus sabdariffa*.
3.3. Determination of vitamins A and C in dried sepals during the second season by HPLC according to Wimalasiri and Wills, (2010).
3.4. Determination of defense enzymes activity in leaves during second season:
3.4.1. Peroxidase activity:

Changes in peroxidase activity associated with the different treatments and controls were determined according the procedure described by Sridhar and Ou, (1974). The assay mixture consisted of 0.1 ml of leaf extract, 1.0 ml of 0.001 M pyrogallol in 0.05 M phosphate buffer at pH 6.5, 0.1 ml of 2% hydrogen peroxide and 1.8 ml distilled water. Changes in absorbance using spectrophotometer at 470 nm were recorded at 30 second intervals for 3 min. Peroxidase activity was expressed as change in absorbance per min per gram fresh weight.

3.4.2. Chitinase activity:

Changes in chitinase activity associated with the different treatments and controls were determined according to the colourimetric method suggested by Monreal and Reese, (1969) using 1% colloidal chitin. Chitinase activity was measured by the release of N-acetyl-D-glucosamine (NAG) from colloidal chitin, and expressed as acetylglucosamine released/ gram fresh weight tissue/60 min.

Results

1. **Pathological studies:**

1.1. Isolation of the damping off and root-rot causal organisms:

Isolated and identified fungi from sampling of the roselle plants showed clear symptoms of crown and root-rot, diseases were recorded according to their morphological characteristics. Whereas, their percentage of occurrence were as follow: *F. oxysporum* was the dominant pathogen with 25%, followed by *F. solani* with 20% then*M. phasolina* with 18%and *F. equiseti* with 15%, respectively, (Fig. 1).

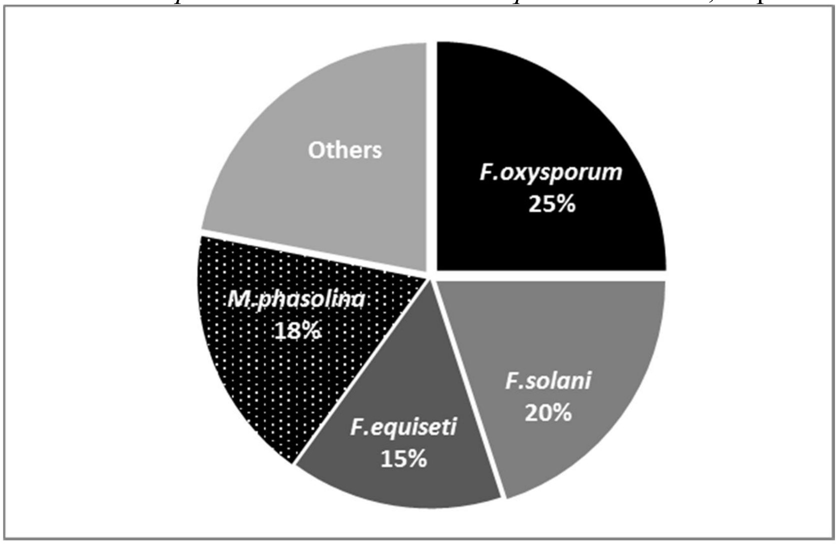

Fig. 1: Percentage of fungi isolated from roselle crown and roots grown in Siwa Oasis

1.1. Pathogenicity test:

All the fungal isolates which were tested in a pathogenicity test and induced severe lesions were further identified to pathogenic fungi; *F. oxysporum, F. solani*, *F. equiseti* and *Macrophomina phasolina* (Table 1). They recorded pre and post emergence damping off to roselle seedlings with significant differences among them. *M. phasolina* and *F. equiseti* recorded the lowest pre and post emergence damping off of seedlings, while the highest ones were recorded with *F. oxysporum.* The highest root-rot incidence of roselle plants were recorded with *F. solani* followed by *M. phasolina*, and *F.equiseti* respectively, two months after sowing. On the other hand, *M. phasolina* followed by *F. oxysporum* gave the highest crown rot incidence compared to other pathogenic fungal isolates.

Table 1: Effect of some soil born fungi on the incidence of pre and post emergence damping off, root-rot and survival of roselle plants:

Fungal isolates	**Damping- off %**		**Root rot incidence (%)**	**Crown rot Incidence (%)**	**Survival (%)**
	Pre-emergence	**Post-emergence**			
F.oxysporum	32.3	29.6	33.6	64.5	38.1
F.solani	26.7	23.5	66.6	46.6	49.8
F.equiseti	13.5	17.8	38.7	14.5	68.7
M.phasolina	11.6	18.6	58.6	67.5	69.8
LSD (0.05%)	1.2	1.5	2.8	2.3	2.7

2. Effect of seed dressing and foliar application as well as their interaction treatments on:

2.1. Damping- off, root and crown rot diseases:

Results presented in Fig.2 indicate that, both seed dressing treatments were significantly reduced pre & post emergence damping-off and root-rot diseases incidence compared to untreated seeds during two successive seasons. Furthermore, Table (2) illustrated that, Bio-Mix treatment was superior to Row Kaolin for reducing damping- off, root and crown rot disease incidence and severity by seed treatments compared with the control treatment. Meanwhile, the four foliar application treatments had significantly reduced root and crown rot incidence as well as severity during two successive seasons compared with untreated plants. Also, Bio-Mix was the most effective treatment that reduced root and crown rot incidence and severity compared with other treatments under study.

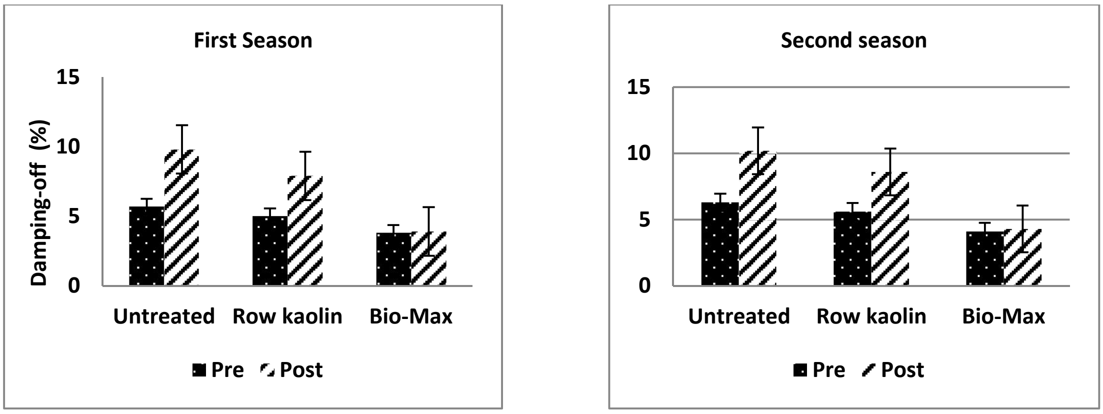

Fig 2: Effect of seed dressing on damping- off disease

Table 2: Effect of seed dressing, foliar application and their interaction treatments on root rot and crown rot diseases of roselle (*Hibiscus sabdariffa*, L.) plants during two seasons (2015 and 2016).

Seed dressing (S) / Foliar application(F)	Control	Row Kaolin	Bio-Mix	Means (S)	Control	Row Kaolin	Bio-Mix	Means (S)
	First season				Second season			
	Root rot disease incidence (%)							
Control	33.4	28.5	23.7	28.5	32.5	27.6	22.7	27.6
Row Kaolin	27.6	23.9	17.7	23.1	29.1	24.6	19.8	24.5
Trecer	27.5	22.4	18.0	22.6	28.7	23.6	18.6	23.6
Kenz	25.7	22.1	18.5	22.1	29.2	24.2	19.0	24.1
Bio-Max	23.8	18.9	13.8	18.8	23.4	18.5	13.5	18.5
Means(F)	27.6	23.2	18.3		28.6	23.7	18.7	
L. S. D. at5% for	(S)=1.2		(F)=1.4	(S*F)= 2.0	(S)=1.3	(F)=1.5	(S*F)=2.3	
	Root rot disease severity							
Control	4.0	3.4	2.5	3.3	3.5	3.0	2.5	3.0
Row Kaolin	3.4	2.1	1.4	2.3	3.0	2.7	2.2	2.6
Trecer	2.5	1.6	1.0	1.7	2.4	2.0	1.5	2.0
Kenz	2.0	1.0	1.0	1.3	1.6	1.2	1.6	1.5
Bio-Mix	1.5	0.7	0.5	0.9	1.2	1.0	0.4	0.9
Means(F)	26.8	17.6	1.3		2.3	2.0	16.4	
L. S. D. at 5%	(S)=0.05		(F)=0.07	(S*F)=0.12	(S)=0.06	(F)=0.07	(S*F)=0.11	
	Crown rot disease incidence (%)							
Control	27.4	24.8	19.8	24.0	30.8	26.2	21.6	26.2
Row Kaolin	25.4	21.3	16.4	21.3	26.2	21.9	18.5	22.2
Trecer	24.5	20.9	15.4	20.5	25.7	21.3	17.8	21.6
Kenz	24.7	20.5	15.7	20.4	25.7	21.3	17.3	21.4
Bio-Mix	21.6	16.6	13.4	17.2	23.3	18.9	12.7	18.3
Means(F)	24.7	20.8	16.1		26.3	21.9	17.6	
L. S. D. at 5%	(S)=0.9		(F)=1.1	(S*F)=1.7	(S)=1.0	(F)=1.2	(S*F)=1.8	
	Crown rot disease severity							
Control	2.5	2.2	1.6	2.1	2.5	2.1	1.6	2.1
Row Kaolin	2.3	2.0	1.1	1.8	1.9	1.5	1.3	1.6
Trecer	1.6	1.0	1.0	1.2	1.8	0.9	0.5	1.1
Kenz	1.0	1.0	0.8	0.9	1.4	0.5	0.1	0.7
Bio-Mix	0.5	0.5	0.5	0.5	0.6	0.0	0.0	0.2
Control	1.6	1.3	1.0		1.6	1.0	0.7	
Means(F)	1.6	1.3	1.0		1.6	1.0	0.7	
L. S. D. at 5%	S)=0.05	F)=0.06	(S*F)= 0.1		(S)=0.02	(F)= 0.03	(S*F)= 0.06	

S= Seed dressing treatments F= Foliar application treatments

As indicated in Table (2) all interactions between seed dressing and foliar application treatments decreased root and crown rot incidence as well as disease severity during two successive seasons. The highest reduction of root and crown rot incidence in addition to disease severity were obtained from the interaction between (Bio-Mix as seeds dressing × Bio-Mix as foliar application) during two successive seasons.

2.2 Yield and its Components:

It was clear from data presented in Tables (3 and 4) that the treatments of seed dressing (Row-Kaolin or Bio-Mix products) tended to a significant increase in all yield parameters (fruits number/plant, dry weight of sepals g/plant, dry yield of sepals kg/fed, seed weight g/plant and seed yield kg/ fed.) in both seasons compared with untreated plants. However, seed dressing with Bio-Max gave the highest values of all these parameters than Row-Kaolin and control treatments in both seasons. Meanwhile, all treatments of foliar application (Row Kaolin, Trecer, Kenz or Bio-Mix)led to increase in this regard compared with untreated plants during both seasons. The highest values of these parameters were obtained from the treatment of foliar application by Bio-Mix product in both seasons in comparison with the other treatments under study.

Table 3: Effect of seed dressing, foliar spraying and their interaction treatments on number of fruits / plant, dry weight of sepals / plant and / feddan of roselle (*Hibiscus sabdariffa*, L.) plants during two seasons (2015and 2016).

Seed dressing (S) / Foliar Application (F)	Control	Row Kaolin	Bio-Mix	Means (S)	Control	Row Kaolin	Bio-Mix	Means (S)
	First season				Second season			
	Number of fruits / plant							
Control	33.00	38.00	41.67	37.65	63.03	67.00	71.33	67.12
Row Kaolin	43.33	45.67	48.67	45.89	74.67	81.89	84.67	80.41
Trecer	51.67	55.33	59.00	55.33	87.42	91.06	103.67	94.05
Kenz	62.67	66.33	75.00	68.00	107.33	117.67	126.00	117.00
Bio-Max	78.00	101.33	123.33	100.89	127.67	130.50	182.00	146.72
Means(F)	35.73	61.33	69.53		92.02	97.62	113.53	
L. S. D. at 5%	(S)=2.30		(F)=3.75	(S*F)=5.63	(S)=0.53	(F)=0.67	(S*F)=1.17	
	Dry weigh of sepals / plant (g)							
Control	24.49	25.65	26.29	25.48	20.41	23.17	25.68	23.09
Row Kaolin	27.43	28.17	28.68	28.09	26.60	29.10	31.27	28.99
Trecer	29.62	30.37	31.17	30.39	35.98	39.05	42.06	39.03
Kenz	32.91	33.58	35.27	33.92	43.62	46.22	51.59	47.14
Bio-Mix	37.16	43.76	53.54	44.82	53.46	56.66	69.23	59.78
Means(F)	30.32	32.31	34.991		36.02	38.84	43.97	
L. S. D. at 5%	(S)=0.51		(F)=1.79	(S*F)=2.02	(S)=0.32	(F)=0.39	(S*F)=0.70	
	Dry weight of sepals / fed (kg)							
Control	269.43	282.15	289.15	280.24	224.51	254.83	282.52	253.95
Row Kaolin	301.69	309.87	315.48	309.01	292.60	320.10	344.01	318.90
Trecer	325.78	334.03	342.91	334.24	395.82	429.59	462.66	429.35
Kenz	362.05	369.38	387.97	373.13	479.82	508.42	567.45	518.56
Bio-Mix	408.80	481.36	588.98	493.04	588.06	623.26	761.53	657.62
Means(F)	333.55	355.36	384.90		396.16	427.24	483.63	
L. S. D. at5%	(S)=5.66	(F)=19.66	(S*F)=22.18		(S)=3.54	(F)=4.29	(S*F)=7.74	

S= Seed dressing treatments F= Foliar application treatments

Table 4: Effect of seed dressing, foliar application and their interaction treatments on seed yield / plant and / feddan of roselle (*Hibiscus sabdariffa*, L.) plants during two seasons (2015and 2016)

Seed dressing (S) / Foliar Application (F)	Control	Row Kaolin	Bio-Mix	Means (S)	Control	Row Kaolin	Bio-Mix	Means (S)
	First season				Second season			
	Seed yield / plant (g)							
Control	17.13	23.65	26.85	22.55	20.77	25.04	26.21	24.01
Row Kaolin	28.02	28.32	29.83	28.72	27.01	28.43	31.36	28.94
Trecer	31.18	32.98	33.84	32.67	41.13	46.80	47.79	45.24
Kenz	35.98	39.13	41.83	38.98	52.14	54.38	55.56	54.03
Bio-Mix	46.94	59.60	72.01	59.52	56.65	57.41	60.50	58.19
Means(F)	31.85	36.74	40.87		39.54	42.41	44.28	
L. S. D. at 5%	(S)=1.43	(F)=3.40	(S*F)=4.28		(S)=0.78	(F)=1.00	(S*F)=1.73	
	Seed yield /fed (kg)							
Control	188.47	260.19	295.35	248.00	228.47	275.44	288.27	264.06
Row Kaolin	308.26	311.48	328.09	315.94	297.15	312.77	344.96	318.29
Trecer	342.89	362.78	372.28	359.35	452.43	514.80	525.65	497.63
Kenz	395.74	430.39	460.09	428.74	573.54	598.18	611.20	594.31
Bio-Mix	516.30	655.60	792.15	654.68	623.15	631.55	665.46	640.05
Means (F)	350.35	404.09	449.59		434.95	466.55	487.11	
L. S. D. at 5%	(S)=15.72	(F)=37.44	(S*F)=47.12		(S)=8.54	(F)=11.01	(S*F)=19.06	

S= Seed dressing treatments F= Foliar application treatments

Data presented in the same Tables clearly show that the fruits number/plant, dry weight of sepals g/plant, dry yield of sepals kg/fed, seed weight g/plant and seed yield kg/ fed. Of roselle plants significantly increased by all interaction treatments between seed dressing (Row-Kaolin or Bio-Mix products) and foliar application (Row-Kaolin, Trecer, Kenz or Bio-Mix) compared to the control treatment during the two seasons. The highest values in this respect were resulted from the interaction treatment between seed dressing by Bio-Mix product and spraying with Bio-Mix in the two seasons compared to the other ones.

2.3. Chemical Constituents:

The data in Tables (5and 6) showed that, all treatments of seed dressing led to increase total chlorophyll, anthocyanin content (mg/100g), vitamin A, vitamin C content (mg/g) in sepals and enzyme activities (perioxdase and chinase) of roselle compared to the control during both seasons. All studied parameters of roselle increased gradually by using Row-Kaolin followed by Bio-Mix products treatments. Bio-Mix product was the superior treatment in this concern compared to the other treatments under study. Furthermore, spraying plants by Row Kaolin, Trecer, Kenz or Bio-Mix tended to a significant increase in all chemical parameters under study during the second season compared with untreated plants. The highest values in this respect were obtained by treatment of spraying plants by Bio-Mix product. Also, the maximum increment in this respect was obtained from the treatment of Bio-Mix followed by Kenz, Trecer then Row-Kaolin products compared to control treatment. Moreover, the interaction between seed dressing and foliar application had significant increased in all the chemical constituents parameters during the second season compared to the other ones under study. The highest increment in this respect was recorded by the combined treatment between Bio-Mix as seed dressing and Bio-Mix as a foliar application in both seasons.

Table 5: Effect of seed dressing, foliar application and their interaction treatments on total chlorophyll and Anthocyanin content (mg/100g) of roselle (*Hibiscus sabdariffa*, L.) plants during two seasons (2015and 2016).

Seed dressing (S) / Foliar Application (F)	Control	Row Kaolin	Bio-Mix	Means (S)	Control	Row Kaolin	Bio-Mix	Means (S)
	First season				**Second season**			
	Total chlorophyll (mg/g.f.w.)							
Control	40.67	43.13	44.95	42.92	41.82	42.82	43.53	42.72
Row Kaolin	47.05	48.43	48.82	48.10	44.80	45.04	45.43	45.09
Trecer	50.09	50.87	51.92	50.96	45.83	46.42	46.62	46.29
Kenz	53.14	54.15	54.82	54.03	47.49	48.33	48.85	48.22
Bio-Mix	55.67	56.80	60.58	57.68	49.47	51.42	57.25	52.71
Means(F)	49.32	50.68	52.22		45.88	46.81	48.34	
L. S. D. at 5%	(S)=0.32	(F)=0.46	(S*F)=0.75		(S)=0.52	(F)=1.15	(S*F)=2.30	
	Anthocyanin content (mg/100g)							
Control	350.61	371.49	412.02	378.04	350.41	366.19	411.91	376.34
Row Kaolin	411.30	434.42	438.19	427.97	412.42	437.88	440.27	430.02
Trecer	474.44	479.53	506.72	486.90	472.61	477.39	506.62	485.62
Kenz	509.06	535.95	534.62	526.54	506.87	534.16	536.76	525.85
Bio-Mix	597.35	599.59	719.55	638.83	598.27	600.87	720.77	639.97
Means (F)	468.55	484.20	522.22		467.69	483.30	523.42	
L. S. D. at 5%	(S)=5.08	(F)=11.62	(S*F)=14.84		(S)=3.25	(F)=8.16	(S*F)=10.09	

S= Seed dressing treatments F= Foliar application treatments

Table 6: Effect of seed dressing, foliar application and their interaction treatments on vitamins contents and defense enzymes activity of roselle (*Hibiscus sabdariffa*, L.) plants during second season (2015 / 2016)

Seed dressing (S) / Foliar Application (F)	Control	Row Kaolin	Bio-Mix	Means (S)	Control	Row Kaolin	Bio-Mix	Means (S)
	Vitamins content							
	Vitamin A content (mg/g)				**Vitamin C content (mg/g)**			
Control	0.44	0.86	0.91	0.72	13.04	13.27	14.13	13.48
Row Kaolin	0.94	1.09	1.20	1.08	15.01	16.15	16.43	15.86
Trecer	1.33	1.46	1.52	1.44	16.44	17.38	17.53	17.12
Kenz	1.61	1.81	1.96	1.80	17.74	18.20	19.40	18.45
Bio-Mix	2.34	2.62	2.84	2.60	20.02	22.73	24.63	22.46
Means(F)	1.66	1.95	2.11		20.56	21.93	23.03	
	Defense enzymes activity							
	Peroxidase (mg1-1)				**Chitinase(mg1-1)**			
Control	0.16	0.29	0.22	0.22	0.09	0.49	0.68	0.42
Row Kaolin	0.22	0.25	0.33	0.27	0.24	0.61	0.71	0.52
Trecer	0.21	0.27	0.35	0.28	0.22	0.60	0.72	0.51
Kenz	0.17	0.24	0.31	0.24	0.13	0.53	0.68	0.45
Bio- Mix	0.25	0.31	0.37	0.31	0.33	0.64	0.85	0.61
Means(F)	0.20	0.26	0.33		0.20	0.57	0.73	

S= Seed dressing treatments F= Foliar application treatments

Discussion

Soil borne diseases as damping-off, root and crown rot caused by *Fusarium oxysporum*, *F. solani* , *F.equiseti*and *M. phaseolina*, are among the most important diseases attack roselle plants (*Hibiscus sabdariffa* L.) during growing season in Siwa Oasis., endanger roselle production wherever this crop is cultivated extensively (Hassan *et al*., 2014 and Lupien and Dugan, 2017). Although synthetic fungicides are often the first line of defense against fungal diseases, the global current trend has converted to safer and environmentally friendly alternative methods to control these pathogens. Natural eco-friendly materials could be used as alternative methods to fungicides and pesticides in controlling fungal diseases and insects as reported by Xu and Kim (2014).

The use of rizobacteria (plant growth promoting rhizobacteria (PGPR)) as a biological fertilizer and bio control agents is the contribution of biotechnology in crop productivity improvement efforts. This was achieved by nutrient mobilization, growth hormone production, nitrogen fixation or activation of the mechanism of disease resistance (Wei *et al*., 1996 and Thakuria *et al.,* 2004). Application of beneficial microorganisms to seed is an efficient mechanism for replacement of microbial inoculate into soil where they will be well positioned to colonize seedling roots and protect against soil-borne diseases and pests. It is a zone of intense microbial activity, with growth of plants and microorganisms dependent on reciprocal provision of nutrients and a wide range of other compounds including plant growth regulators and antibiotics (Maureen, 2016). In the present study, results indicated that seed dressing with Bio-Mix was superior to row kaolin for reducing damping-off, and root and crown rot diseases under field condition in both seasons in addition to increase roselle yield (fruit number/plant, dry weight of sepals g/plant, dry yield of sepals kg/fed, seed weight g/plant,seed yield kg/ fed., total chlorophyll, an anthocyanin content of roselle sepals. These results are in agreement with others (Juma*et al.,*2017;Weaam*et al.,* 2014 ; Gendy*et al.,* 2012 ;Abo-Baker and Mostafa, 2011; Kahil*et al.* 2017) on roselle plants and Ketut, *et al.,* (2017) on swamp cabbage plants. Present results indicated that, foliar application treatments except Bio-Mix treatment had non significant effects to decrease root and crown rot incidence, in spite of there is significant effect in disease severity. Meanwhile, results indicated that, the best effective treatment on all the yield characters of roselle and its chemical constituents was that the Bio-Mix product as foliar application. Inoculation of Bio-Mix cultures to plant ecosystem can improve plant health, growth, yield, and quality of crops, this result is in agreement with Abdel-Ati, (2014) on wheat and Abdel-Ati, (2017) on faba bean. Moreover, Bio-Mix application led to suppress soil borne pathogens (Abdel-Ati and Elhadidy, 2013) and induce plant resistance (Elhadidy and Abdel-Ati, 2014). These results may be due to the content of Bio-Mix product which is considered a plant growth promoting rhizobacteria.

Also, these results showed that the integration between seed dressing and foliar application led to reduce root and crown rot diseases in addition to increase roselle yield and its component more than which was applied as single treatments. Beneficial microorganisms with growth-promoting activities in plants, including rhizobacteria can improve plant health in a variety of different ways.

Rhizobacteria viz. *Azotobacter, Azospirillum, Rhizobium, Pseudomonas* and *Bacillus* have been commonly employed for increasing plant growth and crop yields and improving soil health in cropping systems through colonization of roots, production of phytohormone(such as indole acetic acid and cytokinins), siderophore, biological nitrogen fixation, organic matter decomposition, solubilization of the mineral, transformation and mobilization of nutrients and improve fertility of soil, besides as bioprotectant for controlling plant diseases and pests(Srinivasarao and Manjunath, 2017andMeena*et a.l* 2017).

Results of the study showed that total chlorophyll, anthocyanin content of roselle sepals, vitamin A, vitamin C content were significantly increased by using Bio-Mix treatment as a seed dressing or/and foliar application as these results are in agreement with Gendy *et al.,* (2012) ; Weaam*et al.,* (2014) and Kahil *et al.,* (2017). Also, data showed that, Bio-Mix seed dressing led to enhancement of defense enzymes activity like peroxidase and chitinase when applied as single or integrated with foliar application treatments. This is in agreement with Konappa *et al*., (2016) who presented that, the lactic acid bacteria treated seed showed increase in germination percentage and seedling vigour index compared with control and induce the production of defense-related enzymes like peroxidase, polyphenol oxidase, phenylalanine ammonialyasein treated tomato seedlings against *Ralstonia solanacearum* causing bacterial wilt. The highest amount of peroxidase and chitinase were obtained from integration of seed treatment with Bio-Mix and foliar application with Bio-Mix, Tracer and Row Kaolin, respectively. Beneficial microbes were applied as bioformulation to may confer broad-spectrum resistance to soil borne pathogens (Patel *et al.,* 2017). Beneficial microbes modulate plant defenses against plant pathogens and insect herbivores by regulating hormone signaling including the jasmonic acid, ethylene and salicylic acid pathways, thereby leading to gene expression, biosynthesis of secondary metabolites, plant defensive proteins and different enzymes and volatile compounds, that may induce defenses against soilborne pathogens (Patel *et al.,* 2017).

Conclusion

Under Siwa Oasis condition the Bio-Mix ecofriendly product could be used as seed dressing and or foliar application with roselle plants to increase plant resistance against soil borne diseases which reflected on the increase of roselle productivity.

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