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Development and application of biotechnological products for sustainable corn and soybean production under stresses condition

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Abstract The productivity of strategic crops as corn and soybean grown in new reclaimed region was increased and reduced their losses causesd by biotic and abiotic stresses using biotechnological products of *Rhodotorula glutnis*, *Paenibacillus polymyxa*, *Bacillus subtilis*, *Pseudomonas putida*, *Pseudomons aeruginsa*, *Trichoderma spp and Marin actimycete*. So, we are modified bioproducts by formulated in natural components to improve the plant resistance to both of biotic stress as diseases and environment stress, climate change and increased in yield productivity. Kaolin clay, Bentonite formulation were more effective as formulations in all microorganisms that viability was high untill 12 months. The experiments showed that under stress condition, bio- products reduced disease incidence significantly of all crops, whereas *P. putida* and *P. polymyxa* showed significant potential against all diseases as well as increased yield of three crops in both regions i.e. Sahl El Tena and Bohera. *Pseudomonas putida* and *P. polymyxa* also were more effective in increasing total phenols, peroxidase, chitinase and total soluble protein. Using modified formulations of bio elicitors as kaolin and bentonite gave possibility to provide a guarantee to obtain ecological pure products.

Keywords: Corn, Soybean, Biotechnological products, Stresses

Introduction

The world's population is continuing on developing and the demand for food and renewable crude materials are needed which must be delivered on a constrained amount of agricultural lands. The fertile soils of the Nile Valley are among the most productive in the world in terms of agricultural yield and mild Egyptian winters created conditions conducive to year-round cultivation of many cereal and horticultural crops. In spite of its productivity, the limited arable land leaves the country heavily dependent on imported food products. Soil information is needed for a wide range of environmental and agricultural applications (Dobos and Montanarella, 2007). Nevertheless, a number of biotic (fungi, bacteria) and abiotic (drought, salinity) stresses are severely affecting

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the yield of these crops. The country's agricultural lands are increasingly strained due to limited water resources and significant urban encroachment. Recently, there is a worldwide interesting in in green corn production and valuable frying oil. Yield and quality of maize are at risk by animal pathogens (Oerke 2006). *Aspergillus niger, Penicillium* spp., *Aspergillus flavus and Fusarium* Ear Rot, Anthracnose leaf blight and *Cercospora zeae-maydis* are the pathogens of corn (White. 1999, Bade and Carmona, 2011, Haggag, Wafaa, 2013 and Sartori *et al.*, 2017). Soybean is a main world source of edible oil and protein ("Global Soybean Production, 2006). In Egypt, soybean is one of the export and locally consumed crops. The total soybean production for the world during 2006 was 220.4 million ton (USDA. 2006). Unfortunately, yields in major soybean producing countries have been suppressed by diseases in the past (Wrather *et al.*, 2010), and income derived from this crop has been less than optimal. This financial loss is important to rural economies and to economies of allied industries. Soybean is subjected to many diseases caused by soil borne fungi are charcoal rot (*Macrophomina phaseolina* (Tassi) Goid), collar rot (*Sclerotium rolfsii* Sacc.), Rhizoctonia root rot (*Rhizoctonia solani*) and Fusarium root rot (*Fusarium solani*) (Hartman et al., 1999, Wrather *et al.*, 2010). Most soybean diseases were managed by resistant cultivars and fungicides (Chawla *et al*., 2013).

Fungicide applications made throughout the corn canopy prior to the spread of a disease can help to increase yield potential when environmental conditions may cause high disease pressure. Fields containing foliar diseases should also be scouted for stalk health as the reduction in photosynthesis can predispose corn plants to stalk lodging. Identification of foliar diseases can help to determine the need for changes in management practices such as tillage, crop rotation, and the selection of more resistant corn products to help to reduce incidence of disease next season. Numerous options have been recommended for the control and management of maize diseases. Microbial elicitors derived from some fungal endophytes promote biomass and induce terpenoid biosynthesis and production in plant suspension cells. Many groups of fungi as *Trichoderma spp.*, *Coniothyrium minitans*, *Acremonium alternatum*, marine Actinomycetes, algae, *Bacillus*, *Paenibacillus polymyxa*, *Pseudomonas aeruginosa* , *Burkholderia* sp. well known as biocontrol agents (Haggag, Wafaa *et al*. 2014 a,b and Bahrouna *et al*., 2018) and some of them are importance of biofilms formation in biocontrol initiation (Haggag Wafaa and Timmusk, 2007). Activators of the plant defense response include signaling molecules such as SA, ethylene and jasmonic acid (Haggag, Wafaa and Abd-El-Kareem, 2009**)**. Bio and organic fertilizers are safe for both the environment and human health and it is used to improve soil properties and making the soil easier to cultivate by encouraging root development, providing plant nutrients and enabling their increased uptake by plants as organic fertilizers and green algae (Saber *et al*., 2016). Also, biofertilizers thus include the following, asymbiotic free nitrogen fixers, algae biofertilizers, phosphate solubilising bacteria, mycorrhizae, organic fertilizers and green algae (Saber *et al*., 2016). Many studies have been conducted to determine the using of algae as biofertlizer, modes of action for a list of benefits including early seed germination and establishment, improved crop performance and yield, elevated resistance to biotic and abiotic stress, and enhanced postharvest shelf-life of perishable products (El-Sayed *et al*., 2012). Cooperation with other scientist's wills the potential of integrated management for reducing losses in food that is the focus of this grant and selection of a proper crop pattern and irrigation management. These technologies include GIS, bioremediation of saline soils using chemical, biological products and crop cycle is followed by the application of clean biological farming system, executive weed control and proper crop pattern. The aim was to innovate technologies for a clean farming system be able to produce economically feasible and sustainable harvest from selected strategic crops as soybean and corn free from agrochemical residues in a better environment, combating and diseases losses using biological products.

Materials and methods

Production and formulation of Bio-control agents and elicitors

The most effective of bio-control agents as using biotechnological products of *Rhodotorula glutnis*, *Paenibacillus polymyxa*, *Bacillus subtilis*, *Pseudomonas putida*, *Pseudomons aeruginsa*, *Trichoderma spp and Marin actimycete* were optimized for biomass production using some locally, easier and cheap materials available for yielded production, suitability and shelf life enhancement of bio-fungicides products.

Dry mass measurement

The content of the above mentioned bottles were boiled for 10 min to dissolve the agar content. The warm suspensions were filtered through Wattman paper No.40. The fungal mass were washed once and dried at 50°C for 24 hours.

Formulation studies

Viability study of microorganisms was done every month interval throughout the 6 months of storage period at 302℃ on formulation of Kaolin clay, zeolite, bentonite earth, alginate, glycerol additive materials powdered talc (TAL) and compared to the viability of non-formulated cells (control). Bio-formulation was sampled (1g) and re-suspended in 10 mL of sterile water and serial dilution was then done to 10^5. A 0.05 of diluted sample was pipetted and spread onto agar plates contain LB media and incubated for 4 days at room temperature (25±2 ℃). After incubation, colonies formed were assessment.

Integrated Management (Clean farming system)

Field Application

The field experiments were applied at new reclaimed regions during 2017 and 2018, Egypt. A randomized complete block design and split plot design in three replications were applied for each group of experiments. Maize grains cv. Baladi and soybean cv. Giza 35 were managed with recommended agronomic practices (sowing, fertilizer, irrigation, weed control and different agricultural practices) using drip irrigation. Different elicitors formulation was performed using biotechnological products of *R. glutnis*, *P. polymyxa*, *B. subtilis*, *P. putida*, *P. aeruginsa*, *Trichoderma spp and Marin actimycete*. They were tested either as combined treatments of seed soaking and foliar spray or as a single foliar spray treatment. The foliar spray application was done 45 and 60 days after sowing and studied its effects on yield, yield attributes and oil quality characters and after harvesting. Seeds without treatment were used. Drip irrigation was used .The efficiency of replacing pesticides with biological control technique were evaluated.

Chemicals Assays

Plant analysis

Two months after sowing, ten leaves per plant were separately collected, frozen for 36 h, dried and powdered. Generally, 100 mg dried sample were used for analysis.

Determination of phenol content

Free and conjugated phenols were determined in treated leaves, 15 days after plant spraying with chemical elicitors according to (AOAC 1975) using the Folin–Danis reagent. Phenols were identified by HPLC using a reverse phase C8 column and compared with a catecol standard (Sigma chemicals).

Protein content

Soluble protein extraction was carried out according to Bollag and Eldelstein (1992).

Yield

All the plants of different treatments were harvested and data on yield and its components was recorded.

Statistical analysis

Data were analyzed using an ANOVA of square- transformed data. Significant differences were assessed by comparison the differences between means using LSD value at 0.05.

Results

Formulation studies

The viability of bio-formulated of using biotechnological products of *Rhodotorula glutnis*, *Paenibacillus polymyxa*, *Bacillus subtilis*, *Pseudomonas putida*, *Pseudomons aeruginsa*, *Trichoderma spp and Marin actimycete* using Kaolin clay, zeolite, bentonite earth, alginate, powdered talc and glycerol additive materials was determined until 12 months . Data in Tables (1-5) indicated that all formulation are suitable for microorganisms until 12 months. However, viability of microorganisms in the some bioformulation during storage at 30 ℃ for 12 months were more effective. Kaolin clay formulation was more effective as formulation in all microorganisms. Also, Bentonite was more effective one as bioformulation untill 12 months. Alginate was the best formulation for *R. glutnis.*

Integrated Management (Clean farming system)

Field Application

Effective of different biotechnological products of *R. glutnis*, *P. polymyxa*, *B. subtilis*, *P. putida*, *P. aeruginsa*, *Trichoderma spp* and *Marin actimycete* on controlling of diseases of strategic crops i.e. corn and soybean were evaluated in two different environmental conditions i.e. new reclaimed areas in Sahl El Tena and compared in normal location, Behira. Data showed that the all diseases incidence were higher in untreated of corn and soybean plants in both regions. Leaf spots and blight are the most important and theater diseases of corn that cause severe infection. (Table 6).

Our experiments showed that under stress condition, all bio- products reduced diseases incidence significantly, whereas *P. putida* and *P. polymyxa* showed significant potential against all diseases of crop in both regions (Table 6). *R.glutinis* was more effective against spots. Analysis of data indicated that all treatments significantly reduced disease incidence under normal and saline conditions in corn plants. Significant differences were obtained among treatments and untreated control. The control plant developed a strong disease incidence. This may be to increase plant defense compounds against pathogens. In salt soil, most of the tested isolates showed good effects.

Data also showed that the all diseases incidence were higher in untreated of Soybean plants in both regions. Seedling decay (*Rhizoctonia solani*, *Fusarium oxysporum*), Leaf Spot eye spot (*Cercospora sojina*) , Rust and *Colletotrichum* pod blight are the most important diseases of soybean that cause severe infection, (Table 7). Leaf Spot eye spot (*Cercospora sojina*) , Rust and *Colletotrichum* pod blight are the most important that cause severe losses in Behira Governorate and low in Sahl El Tena . Also, experiments showed that under stress condition, bio- products reduced diseases incidence significantly, whereas *P. putida* and *P. polymyxa* showed significant potential against all diseases in both regions.

Biochemical changes associated with induced resistance

Plant treatment with bioproducts induced resistance of corn and soybean plants against diseases with substantial reduction in disease incidence (Tables 8 and 9). The induction of resistance was associated with many biochemical changes viz. increase in total phenols, peroxidase, chitinase and total soluble protein in two regions. *Pseudomonas putida* and *P. polymyxa* were more effective in increasing total phenols, peroxidase, chitinase and total soluble protein. At the same time, results clearly show that ascorbic acid markedly increase the total phenols, peroxidase, chitinase and total soluble protein in both varieties.

Yield and Quality analysis

Yield of corn and soybean are highly inter-related and both are significantly influenced by salt stress in Sahl El Tena than in normal soil (Tables 10 and 11). According to presented data in Tables 10 and 11, it could be noticed that the yield had a very significant influence. There was a highly significance difference between control and treated plants with different bio products. Overall, the results suggest that the corn and soybean yield were negatively influenced under saline soil that influence yield performance, meanwhile it’s improved under treated conditions. Corn and soyabean plants treated with *P. putida, P. polymyxa* resulted in a significantly higher increase in the grain yield. The highest grain yields were obtained with the application of *P. polymyxa or R. glutinisthe* gave the highest grain yields either in new reclaimed soils in Sahl El Tena or arable land in Behira Governorate. These results are exact with both corn and soybean cultivars as well as in both seasons. It is worthy to mention that all treatments increased significantly the yield components in comparison to untreated control.

Table 1 . Viability of *Rhodotorula glutnis* in the bioformulation during storage at 30 ℃

Formulation	Viability (cfu/g) of *Rhodotorula glutnis*					
	On Time	**2 Months**	**4 Months**	**6 Months**	**8 Months**	**12 Months**
Kaolin + glycerol	5.0×10^5	5.0×10^5	5.0×10^5	4.0×10^5	4.0×10^5	4.0×10^5
zeolite+ glycerol	5.0×10^5	5.0×10^5	5.0×10^4	3.0×10^4	3.0×10^4	2.0×10^4
Bentonite + glycerol	5.0×10^5	5.0×10^5	5.0×10^4	3.0×10^5	3.0×10^5	3.0×10^5
Talc+ glycerol	5.0×10^5	5.0×10^4	5.0×10^3	2.0×10^3	1.0×10^3	1.0×10^3
Alginate+ glycerol	5.0×10^5	5.0×10^5	5.0×10^5	5.0×10^5	5.0×10^5	3.0×10^5
Liquid formulation	5.0×10^7	3.0×10^5	2.0×10^4	2.0×10^2	2.0×10^2	0.5×10^2

Table 2. Viability of *Paenibacillus polymyxa* in the bioformulation during storage 30 ℃

Formulation	Viability (cfu/g) of *Paenibacillus polymyxa*					
	On Time	**2 Months**	**4 Months**	**6 Months**	**8 Months**	**12 Months**
Kaolin + glycerol	5.0×10^7	5.0×10^7	5.0×10^7	4.0×10^7	4.0×10^7	4.0×10^7
zeolite+ glycerol	5.0×10^7	5.0×10^7	5.0×10^7	3.0×10^7	4.0×10^7	3.0×10^7
Bentonite + glycerol	5.0×10^7	5.0×10^7	5.0×10^7	3.0×10^7	3.0×10^7	3.0×10^7
Talc+ glycerol	5.0×10^7	5.0×10^7	5.0×10^7	3.0×10^7	2.0×10^4	1.0×10^4
Alginate+ glycerol	5.0×10^7	5.0×10^7	5.0×10^7	5.0×10^5	5.0×10^5	5.0×10^5
Liquid formulation	5.0×10^7	3.0×10^5	2.0×10^4	2.0×10^2	1.0×10^2	0.5×10^2

Table 3. Viability of *Pseudomons putida* in the bioformulation during storage 30 ℃

Formulation	Viability (cfu/g) of *Pseudomons putida*					
	On Time	**2 Months**	**4 Months**	**6 Months**	**8 Months**	**12 Months**
Kaolin + glycerol	5.0×10^7	5.0×10^7	5.0×10^7	5.0×10^7	4.0×10^7	4.0×10^7
zeolite+ glycerol	5.0×10^7	5.0×10^7	5.0×10^7	5.0×10^7	4.0×10^7	3.0×10^7
Bentonite + glycerol	5.0×10^7	5.0×10^7	5.0×10^7	5.0×10^7	3.0×10^7	3.0×10^7
Talc+ glycerol	5.0×10^7	5.0×10^7	5.0×10^5	4.0×10^5	2.0×10^4	1.0×10^4
Alginate+ glycerol	5.0×10^7	5.0×10^7	5.0×10^7	3.0×10^5	5.0×10^5	5.0×10^5
Liquid formulation	5.0×10^7	3.0×10^5	2.0×10^4	2.0×10^2	1.0×10^2	0.5×10^2

Table 4. Viability of *Trichoderma spp* in the bioformulation during storage 30 ℃

Formulation	Viability (cfu/g) of *Trichoderma spp*					
	On Time	**2 Months**	**4 Months**	**6 Months**	**8 Months**	**12 Months**
Kaolin + glycerol	5.0×10^4	5.0×10^4	5.0×10^4	4.0×10^4	4.0×10^7	4.0×10^7
zeolite+ glycerol	5.0×10^4	5.0×10^4	5.0×10^4	4.0×10^4	3.0×10^7	2.0×10^7
Bentonite + glycerol	5.0×10^4	5.0×10^4	5.0×10^4	3.0×10^4	3.0×10^7	3.0×10^7
Talc+ glycerol	5.0×10^4	5.0×10^4	5.0×10^3	3.0×10^2	1.0×10^4	1.0×10^4
Alginate+ glycerol	5.0×10^4	5.0×10^4	5.0×10^3	5.0×10^2	3.0×10^5	2.0×10^5
Liquid formulation	5.0×10^4	3.0×10^5	2.0×10^4	2.0×10^2	1.0×10^2	0.5×10^2

Table 5. Viability of *Marin actimycetes* in the bioformulation during storage at 30 ℃

Formulation	Viability (cfu/g) of *Trichoderma spp*					
	On Time	**2 Months**	**4 Months**	**6 Months**	**8 Months**	**12 Months**
Kaolin + glycerol	5.0×10^6	5.0×10^6	5.0×10^6	4.0×10^6	4.0×10^7	4.0×10^7
zeolite+ glycerol	5.0×10^6	5.0×10^6	5.0×10^6	4.0×10^5	3.0×10^7	2.0×10^7
Bentonite + glycerol	5.0×10^6	5.0×10^6	5.0×10^6	3.0×10^5	3.0×10^7	3.0×10^7
Talc+ glycerol	5.0×10^6	5.0×10^6	5.0×10^6	3.0×10^6	2.0×10^4	1.0×10^4
Alginate+ glycerol	5.0×10^6	5.0×10^6	5.0×10^6	5.0×10^6	3.0×10^5	2.0×10^5
Liquid formulation	5.0×10^6	3.0×10^5	3.0×10^4	2.0×10^3	2.0×10^2	1.0×10^2

Table 6. Diseases incidence of treaded Corn plants with bioformulation of bioagents, and grown in Sahl El Tena , compared with normal condition in Behira during of 2017 and 2018 seasons

Treatments	Seedling decay		Leaf Spot		Leaf Blight		Ear rots	
	Behira	Sahl El Tena	Behira	Sahl El Tena	Behira	Sahl El Tena	Behira	Sahl El Tena
Rhodotorula glutnis	0.4	0.5	8.8	6.8	5.8	4.6	1.1	0.0
Paenibacillus polymyxa	0.0	0.0	3.3	0.0	0.0	0.0	0.0	0.0
Bacillus subtilis	1.7	0.6	8.2	7.7	5.6	4.6	1.5	0.9
Pseudomonas putida	0.0	0.0	3.7	1.2	0.8	0.6	0.4	0.0
Pseudomons aeruginsa	2.3	0.3	7.6	9.3	5.6	4.8	1.9	1.0
Trichoderma spp	0.8	0.76	10.3	3.7	7.6	6.6	2.4	1.4
Marin actimycete	0.0	0.0	3.6	2.5	2.8	1.8	1.4	0.8
Untreated control	2.3	5.7	17.8	28.8	36.8	32.0	4.0	7.7
LSD	0.7	0.6	0.83	1.3	1.1	1.2	0.9	0.8

Table 7. Diseases incidence (%) of treaded Soybean plants with bioformulation of bioagents, and grown in Sahl El Tena, compared with normal condition in Behira during of 2017 and 2018 seasons

Treatments	Seedling decay		Leaf Spot		Leaf Blight		Ear rots	
	Behira	Sahl El Tena	Behira	Sahl El Tena	Behira	Sahl El Tena	Behira	Sahl El Tena
Rhodotorula glutnis	0.0	0.0	1.8	3.3	5.5	6.8	1.3	2.7
Paenibacillus polymyxa	0	0.4	0	0.0	0.0	3.3	0.0	0
Bacillus subtilis	2.3	3.3	0	1.3	5.7	7.2	2.1	4.7
Pseudomonas putida	0.0	1.3	0	0.0	1.0	2.7	0.0	0
Pseudomons aeruginsa	9.7	11.3	1.2	0.8	4.3	6.4	7.8	11.6
Trichoderma spp	7.3	9.2	2.9	3.4	10.8	12.3	3.5	7.8
Marin actimycete	1.9	2.2	2.3	3.3	3.3	6.6	0.0	0
Untreated control	15.3	19.4	9.7	15.7	23.8	54.0	13.5	18.7
LSD	0.7	0.9	0.5	0.98	1.7	2.3	0.7	1.2

Table 8. Chemical compositions of treaded corn plants, with bioformulation of bioagents, and grown in Sahl El Tena , compared with normal condition in Behira during of 2017 and 2018 seasons

Treatments	Phenol content (mg catechol/g F.W.)		Enzymes activities				Soluble protein (mg g^{-1} F .W.)	
			Peroxidase		Chitinase			
	Behira	Sahl El Tena	Behira	Sahl El Tena	Behira	Sahl El Tena	Behira	Sahl El Tena
Rhodotorula glutnis	30.5	32.5	20.4	20.1	4.8	5.9	22.0	24.0
Paenibacillus polymyxa	39.5	40.5	22.1	22.7	6.9	9.6	38.3	39.1
Bacillus subtilis	30.4	32.1	29.0	30.1	9.6	9.9	37.2	37.2
Pseudomonas putida	36.4	36.5	27.1	27.1	9.7	10.7	39.8	39.5
Pseudomons aeruginsa	34.0	35.3	23.1	24.2	8.0	8.6	31.9	33.7
Trichoderma spp	30.3	32.3	23.1	19.7	6.2	7.7	35.9	36.4
Marin actimycete	37.8	39.1	24.5	23.5	7.5	7.9	34.7	38.3
Untreated control	23.1	25.1	12.2	14.0	5.7	5.9	14.0	16.0

Table 9. Chemical compositions in leaves of Soybean plants, cv Giza 35 treaded with bioformulation of bioagents, and grown in Sahl El Tena, *compared with normal condition in* Behira during of 2017 and 2018 seasons

Treatments	Phenol content (mg catechol/g F.W.)		Enzymes activities				Soluble protein (mg g^{-1} F .W.)	
			Peroxidase		Chitinase			
	Behira	Sahl El Tena	Behira	Sahl El Tena	Behira	Sahl El Tena	Behira	Sahl El Tena
Rhodotorula glutnis	25.8	28.2	21.3	22.4	4.6	4.9	22.2	24.0
Paenibacillus polymyxa	26.2	39.7	22.3	27.7	9.0	9.6	34.1	39.2
Bacillus subtilis	35.1	35.1	26.7	23.9	7.2	7.8	28.1	37.2
Pseudomonas putida	35.3	37.7	29.7	27.4	9.0	9.9	37.1	38.5
Pseudomons aeruginsa	30.0	31.0	22.1	21.9	6.6	7.8	23.0	26.7
Trichoderma spp	17.1	28.3	20.3	21.7	5.5	5.9	35.3	37.9
Marin actimycete	26.2	27.5	26.8	21.5	9.1	9.8	30.2	32.1
Untreated control	11.1	13.5	11.7	13.0	5.1	5.8	15.2	17.0

Table 10. Yield (ardab/fed.) of treaded corn plants, with bioformulation of bioagents, and grown in Sahl El Tena , compared with normal condition in Behir during of 2017 and 2018 seasons

Treatments	Grain yield (kg/fad)		Grains Wight /Plant (g)	
	Behira	Sahl El Tena	Behira	Sahl El Tena
Rhodotorula glutnis	4671.3	4291.9	220.8	213.`
Paenibacillus polymyxa	4834.5	4523.9	244.8	234.4
Bacillus subtilis	4692.3	4564.1	230.7	224.7
Pseudomonas putida	4933.4	4412.3	233.8	223.3
Pseudomons aeruginsa	4724.2	4321.6	228.8	220.3
Trichoderma spp	4841.5	4564.6	239.8	225.8
Marin actimycete	4801.9	4498.6	242.9	232.4
Untreated control	4631.7	4297.6	200.7	180.7
LSD	11.2	9.5	8.7	7.6

Table 11. Yield (ardab/fed.) of Soybean plants, cv Giza 35 treaded with bioformulation of bioagents, and grown in Sahl El Tena , compared with normal condition in Behira during of 2017 and 2018 seasons

Treatments	Weight of pod (Mg fed-1)		Weight of 100 seeds (g)	
	Behira	Sahl El Tena	Behira	Sahl El Tena
Rhodotorula glutnis	1.349	1.302	19.8	19.3
Paenibacillus polymyxa	1.296	1.223	20.8	20.7
Bacillus subtilis	1.287	1.143	18.8	17.8
Pseudomonas putida	1.377	1.323	20.43	20.0
Pseudomons aeruginsa	1.056	1.087	18.87	17.7
Trichoderma spp	1.237	1.234	20.09	19.7
Marin actimycete	1.231	1.310	20.7	19.6
Untreated control	1.049 1	0.778	17.8	13.7
LSD	0.54	0.47	1.1	0.9

Discussion

The growing demand for food consumption in developing countries alone is predicted to increase by around 1.3% per annum until 2020. Egypt, the most populous country in the Arab World, is also by far the largest importer of soyabean and corn globally. The increase in imports in the coming marketing year is expected in response to the increased local crushing capacity with the objectives of producing affordable, high quality blended oil and high-protein soy meal for the feed industry.

Applications of fungicides are the main way diseases are managed in both conventionally and organically-produced crops. Resistant varieties, unfortunately, are not available for foliar diseases that have been affecting production. The agriculture industry is in need of novel biofungicides and development of large-scale production of biofungicides, either microbial cells themselves or cell-free microbial components. An increasing number of biocontrol agents (BCAs) are being developed for control of plant pathogens. Once a biocontrol agent has shown potential for disease control, production of an effective biomass becomes a major concern. Commercial biomass production of antagonists requires large-scale (Haggag Wafaa and Singer, 2012). Our results indicated that formulation and delivery systems has resulted in prototype formulations that exhibit good viability over periods up to one 12 months and that have good efficacy at 302℃. Kaolin formulation is more effective one for all microorganisms. Development and implementation of bioformulations in agriculture will improve the quality of production of ecological pure products and will enhance income stability on high temperature as 302℃. Thus, our priority is to introduce plant disease bioformulations, which will reduce a damage of crop capacity more than 30-35% on the plots. The effective species were *P. putida*, *B. polymyxa*, resulted in a significantly greater decrease in the diseases incidence moreover, increased of total soluble protein, total phenols, chitinase and peroxidase in plants grown in dry and normal regions. Several species of *Bacillus* including *B. subtilis* and *B. polymyxa* are widely known for their biocontrol and growth promotion abilities as they produce several antibiotics and phytohormones, solubilization of phosphate, releasing ammonia from nitrogenous organic matter (Hayat et al. 2010). Mechanisms of these bacterial species is mainly due to their potentially to produce antibiotics, lipopolysaccharides (LPS) and iron regulated metabolites (Raaijmakers *et al.* 2002, Yunus et al., 2016 and Bahrouna *et al*., 2018). The biological protection of crops, using bioformulations, gives possibility to exclude chemical preparations and provides guarantee to obtain ecological pure products (Haggag Wafaa and Singer, 2012; Haggag Wafaa *et* *al*., 2017 and and Bahrouna *et al*., 2018). The plants defense strategy against biotic stress as diseases showed different types of stress proteins with different protective functions (Gianinazzi *et al.,* 1970). The applied approach could promote and encourage additive or synergistic inhibitory effects resulting from use of bioformulation of bio elicitors (Choudary *et al.,* 2007). The antagonistic efficiency has often been corresponding to production of different secondary metabolites, hormonal stress, biological and molecular characterization of microorganisms. These are useful as biocontrol agents or as producers of bioactive compounds, which are of noteworthy relationship for the echo-environment compatible agriculture. The potentially of a bio-elicitors to encourage resistance to disease has also been noted as a mechanism of controlling and management of plant diseases.

The microorganisms in bio-fertilizers restore the soil's natural nutrient cycle and build soil organic matter. Through the use of bio-fertilizers, healthy plants can be grown, while enhancing the sustainability and the health of the soil. Since they play several roles, a preferred scientific term for such beneficial bacteria is "plant-growth promoting rhizobacteria" (PGPR). Therefore, they are extremely advantageous in enriching soil fertility and fulfilling plant nutrient requirements by supplying the organic nutrients through microorganism and their byproducts. Hence, bio-fertilizers do not contain any chemicals which are harmful to the living soil (Vessey, 2003). Bio-fertilizers provide "eco-friendly" organic agro-input. Bio-fertilizers such as Rhizobium, blue green algae (BGA) have been in use a long time. Many studies have been conducted to determine the using of algae as biofertlizer modes of action for a list of benefits, including; early seed germination and establishment, improved crop performance and yield, elevated resistance to biotic and abiotic stress, and enhanced postharvest shelf-life of perishable products (El-Sayed *et al*., 2011; 2012).

In this study, we developed a formulation of biocontrol agents for improve their efficiency and protection. Kaolin clay and Bentonite formulation was more effective as formulation in all microorganisms that viability were high till 12 months. The use of this formulation of bioagents isolates as bio-elicitors in either dry climate stress in new reclaimed soils and arable land is possible to improve the effects against diseases. Application of biotechnological products, include biocontrol agents reduce plant pathogens, plant resistance to diseases, environment stress, climate change and increase yield productivity, prevent pre harvest loss could increase the quality and quantity of the products. Application of bio-agents fungi and bacteria has been mentioned to support plant growth and elicit induced systemic resistance to plants against a range of pathogens when applied as seed treatments, soil drenches or foliar sprays. Field experiments confirmed the potentially of different bio elicitors in reducing the infection of diseases incidence and severity. The effective species were *P. putida*, *B. polymyxa*, resulted in a significantly greater decrease in the diseases incidence moreover, increased of total soluble protein, total phenols, chitinase and peroxidase in plants grown in dry and normal regions. Bio elicitors increased plant growth and yield of both cultivars. The role of bio elicitors in plant growth promotion is widely known as equally important for overall plant growth. In this study applying of bio-elicitors become more integrated into management control strategies in protection of plants from biotic stress and permits a reduction in fungicide inputs. The results show that it could be possible to replace traditional chemical fungicides with bio-elicitors; it is safe for human health, environment and thus provided potential, economic and ecological value. The results show that the used plant beneficial microorganisms as bio elicitors have improved the plant tolerance to biotic stress under environmental changes in the new reclaimed lands under arid climate condition. The biological protection of crops, using bioformulations, gives possibility to provides guarantee to obtain ecological pure products.

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