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Integrated management of corn diseases using biological and natural products

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Abstract The problems of corn production caused severe losses of foliar diseases eg. streak, leaf blight, gray leaf spot and rust. The study was done to reduce disease incidence and increase the productivity of corn grown in the new reclaimed region. The application of biocontrol agents (*Rhodotorula glutnis*, *Paenibacillus polymyxa*, *Bacillus subtilis*, *Pseudomonas putida* ,*Trichoderma harzianum* and *Marin actimycete*) and natural products **(**potassium silicate and amino acids eg. L-arginine-L-methionine, L-ornithine were clearly investigated. All biological products were actively antagonized the tested fungal pathogens which isolated from corn. Biological and natural products application are improved disease management, yield and quality**.** *P. polymyxa* and *P. putida* are significantly promoted the growth of corn. *P. putida*, *P. polymyxa* and natural compounds were proved to be effectively increased total phenols, total soluble protein, chitinase and peroxidase. The protection level of biological treatments in combination with application of natural products was as effective as the application of biological agents alone.

Keywords: Biological products, Natural products, Corn

Introduction

Maize or corn (*Zea mays*) is the second most important grain crop in Egypt (Haggag, 2013; Haggag, 2018). Recently, there is a worldwide interest in corn production because the demand continuously increases. Yield and quality of maize are at risk due to pathogens (Oerke, 2006). The corn crop can be attacked by several pathogens which cause severe loss in productivity and yield (Costa *et al.*, 2012). Brito *et al*. (2011) reported that fungal infestation in corn can reduce productivity of up to 80%. The common diseases are: leaf spot, brown eye spot, rusts, leaf anthracnose and *Diplodia*. Disease management in corn is commonly carried out by using resistant varieties or cultivars and/or the use of fungicides to control pathogens thereby increasing crop yield (Barros, 2011). In our previous studies, we found that application of biological and/or natural products could contribute efficiently in reducing diseases (Haggag *et al.,* 2017). Several species of *Trichoderma*, *Bacillus* and *P. polymyxa* are known to be good biocontrol agents. They promote growth, produce several secondary metabolites: peptide antibiotics, phytohormonas and solubilize phosphate in the soil (Hayat *et al.*, 2010). The mechanism of action of these bacterial

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species is mainly due to their capability to produce antibiotics, lipopolysaccharides (LPS) and iron regulated metabolites (Raaijmakers *et al.*, 2002). Several species of actinomycetes, such as in the genus *Streptomyces,* are known as biocontrol agents which inhibit or lyse many fungal pathogens. *Streptomyces* sp. is being used in industry because it produces secondary metabolites, special antibiotics and others bioactive compounds. *Rhodotorula glutinis* has long been known to reduce the plant fungal diseases and has been considerably important as beneficial biocontrol agent. Several researches have been conducted to study the mode of action of Plant Growth Promoting Rhizobacteria (PGPR) with regards to seed germination, improving crop quality, performance and yield, resistance to biotic and abiotic stresses as well as prevent postharvest losses. Silicon has been known to play an important role in the management of environmental stresses and increase plant resistance against fungi and bacteria (Wang *et al.*, 2017). Silicon is taken up by the plant through the roots as silicic acid ($Si(OH)_4$). It passes through the plasma membrane facilitated by two Si transporters: Lsi1 and Lsi2 (Wang *et al.*, 2017). Another study pointed out that the effect of amino acids on plant pathogens varies according to their structure and their optical isomerism (Hasabi *et al.*, 2014). The objective was to control multiple diseases of corn crop, combat pre and postharvest losses and increase the productivity using integrated management.

Materials and Methods

Isolation of pathogens

The fungi associated with corn was determined using two kinds of seeds: unsterilized and sterilized with 1% Clorox for 4-5 min. In both cases, the seed were washed in sterile water, dried on sterile filter paper and sown on media Czapek medium (SIGMA). The plates were incubated at 25 ℃ for 7 days. Then, the fungal colonies that emerged were individually transferred onto Potato Dextrose Agar medium.

Biocontrol agents

The bio-control agents used in this study were *Rhodotorula glutnis, Paenibacillus polymyxa, Bacillus subtilis, Pseudomonas putida, Trichoderma harzianum* and a marine actinomycetes.

Field application

Field experiments were conducted at 2 new reclaimed regions (Behira and Sahl El Tena, Egyptt in 2018 and 2019. The experiments were laid out in randomized complete block and split plot designs. Corn crops were managed applying common agronomic practices (sowing, fertilizer, irrigation, weed control and so on). Drip irrigation was used to water the plants. Biological agents (*Rhodotorula glutnis*, *Paenibacillus polymyxa*, *Bacillus subtilis*, *Pseudomonas putida*, *Trichoderma harzianum* and Marine *actimycete)* and natural products (20g/L of potassium silicate (KSi) and 25mM of solutions of amino acids (L-arginine, L-methionine, L-ornithine) were applied either singly or in combination for seed soaking and foliar spray. Foliar spray applications were done at 45 and 60 days after sowing. The effect on yield, yield attributes, oil quality characters was determined. Seeds without treatment were used as control. Three replicates were used for each treatment. The diseases incidence was determined using the scale proposed by Chester (1950). The corn productivity was determined in mature stage using randomly marked plants. All the corncobs were collected. The seeds were separated from the cobs and the moisture content was adjusted to 13% for the weight determination of 100 grains.

Chemical assays

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

Phenol content: Conjugated and free phenols were measured in plant sprayed with chemical elicitors using the Folin–Danis reagent (AOAC 1975). Phenols were measured through High Performance Liquid Chromatography using a reverse phase C8 column and compared with a catecol standard (Sigma chemicals).

Protein content: Extraction of soluble protein was done and measured according to the methods of Bollag and Eldelstein (1992).

Grain quality tests

In harvest stage, data on corn grain yield was recorded as flour yield. It was measured on a laboratory mill (e.g., Buhler or Miag Multomat) under a standard operating procedure.

Postharvest (storage experiments)

One of the main goals of the project is reduce the losses during all the stages of production, harvesting, drying and storage. Study on post-harvest losses covered losses due to pathogens and other factors.

The effect of bioproduct treatments was evaluated under different storage conditions: temperature, humidity, chemical and biological aspects. Also, the use of different types of containers to estimate different qualitative parameters of seed germination was studied. The following were used to calculate the different parameter as follows:- germination percentage after 12 days, seedling dry weight after 12 days, vigor index = germination % X average seedling length (shoot-root), and dry weight of pods and seeds at the end stage.

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

Results

Fungi associated with corn seeds

It was found fungi associated with corn seeds were *Aspergillus niger, Penicillium* spp., *Aspergillus flavus and Fusarium* (Ear Rot) as seen in Figure 1.

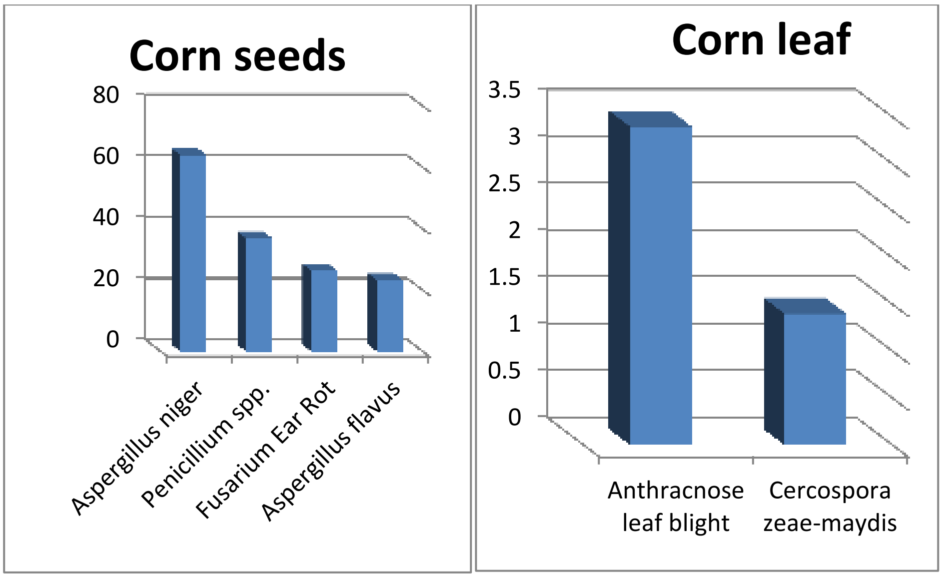

Figure 1. Fungal percentage isolated from corn plants grown in Beheria

In vitro bioassay

The result on the effect of antagonists against fungal isolates *in vitro* using PDA medium is presented in Table 1. All tested microorganisms showed capability to antagonize the fungal pathogens. However, some of them showed specific ability to antagonize pathogenic fungi. For instance, *Rhodotorula glutnis* showed the ability to reduce seed-borne pathogens such as *Aspergillus niger, Penicillium and Fusarium*. The marine *actinomycetes, Paenibacillus polymyxa and Pseudomonas* were effectively reduced the growth of all pathogenic fungi.

Field application

Results indicated that disease incidence of seedling decay, leaf spot, leaf blight and ear rots was higher in untreated corns in both regions (Table 2). Leaf spot and leaf blight were the most important cause of severe losses in Behira Governorate. Our experiments showed that under stress condition, biological products reduced diseases incidence significantly. *P. putida*, *P. polymyxa* and *Streptomycetes* showed significant against all diseases in both regions. The protection level of biological treatment in combination with natural products was as effective as application of biological products only.

Biochemical changes associated with induced resistance

Plants treated with bio-products increased defense mechanism and induced resistance of corn plants against seedling decay, leaf spot, leaf blight and ear rots (Table 3). The induction of resistance was associated with many biochemical changes viz. increase in total phenols, total soluble proteins, chitinase and peroxidase activities in both regions. *P. putida*, *P. polymyxa*, *Sterptomyctes* and *Trichoderma* were more effective in increasing total phenols, total soluble protein, chitinase and peroxidase activities. The induction of resistance of biological treatment in combination with natural products was as effective as the application of biological products only.

Yield and storage quality analysis

Treatment of corn plants with *P. putida* and *P. polymyxa* resulted to a significantly higher increase in the grain yield in new reclaimed soils in Sahl El Tena and arable land in Behira Governorate. These antagonists also protected the seeds during storage for three months in terms of germination, dry weight and vigor (Figure 2). Result indicated that the incidence of pathogenic fungi i.e. *Aspergillus niger, Penicillium* and *Fusarium* in seeds which harvested from plants treated with the different biological and natural products in Beheria were greatly decreased when compared with the untreated plants after three months of storage (Figure 3). Generally, the corn yield and seed quality from plant treated with biological and natural products showed as effective as application of biological products only (Figure 4).

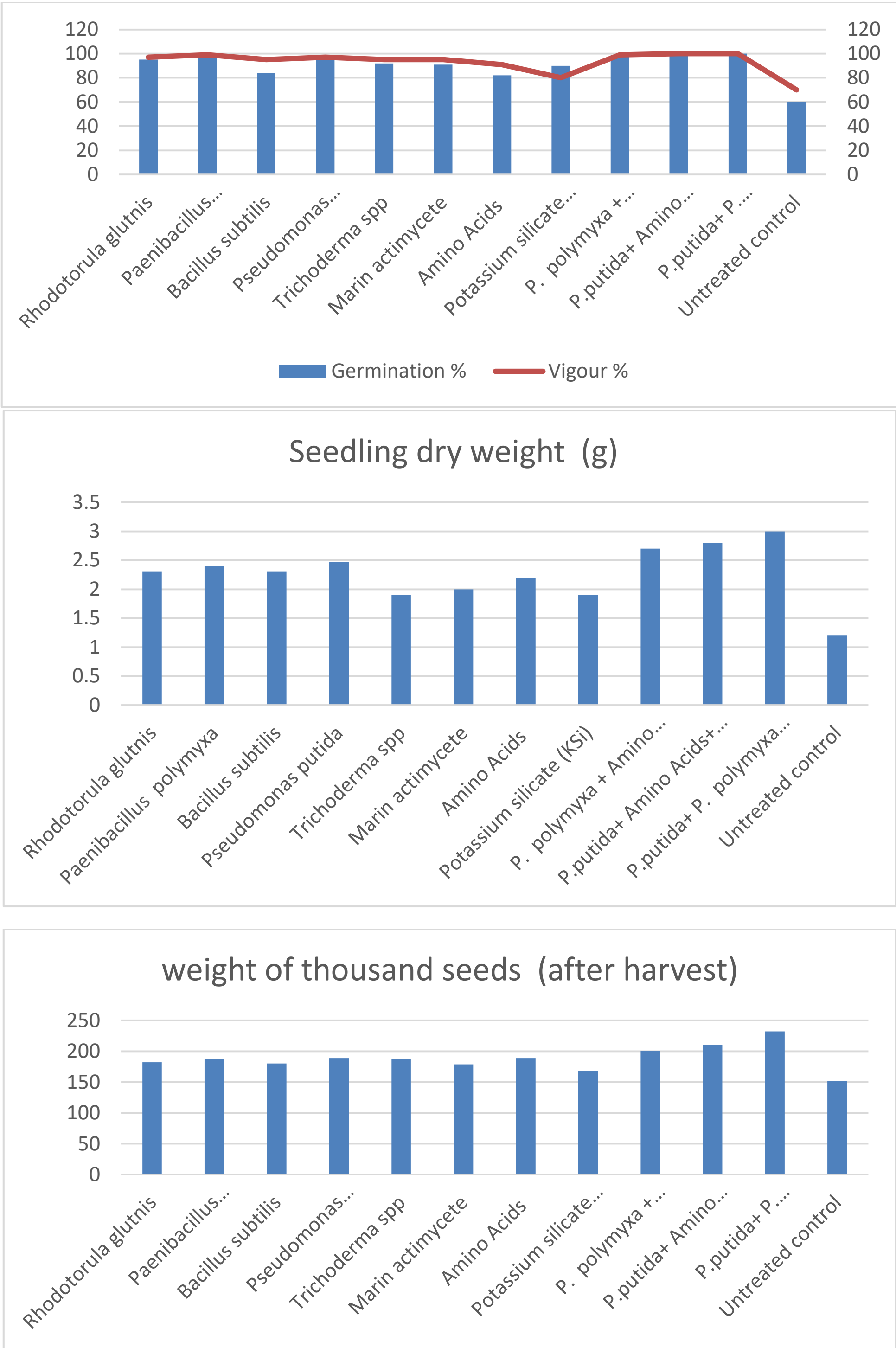

Figure 2. Analysis for corn germination dry weight, and vigor for evaluation after three months from harvested seeds subjected to different applications of biological and natural products in Beheria

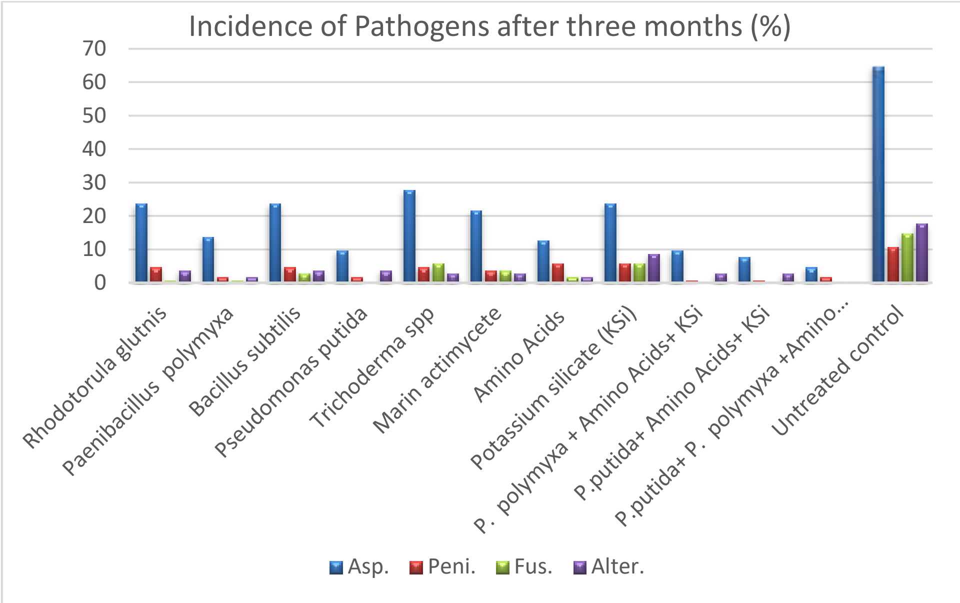

Figure 3. Pathogenic fungi of corn after three months from harvested seeds subjected to different applications of biological and natural products in Beheria

Integrated **Control**

Figure 4. Treated Corn with P.putida+ P. polymyxa +Amino Acids+ KSi in the 2019 crop year at Beheri

Table 1. In vitro evaluation of the antagonistic capability against the pathogenic Fungi

Biocontrol (Bio-elicitors)	***Aspergillus niger***	***Aspergillus flavus***	***Fusarium graminearum***	***Penicillium* spp.**	***Colletotrichum graminicola***	***Cercospora zeae-maydis***	***Rhizoctonia solani***
Rhodotorula glutnis	29.6	17.6	28.3	28.9	26.7	11.5	15.4
Paenibacillus polymyxa	19.3	18.5	20.7	19.9	25.7	19.5	18.4
Bacillus subtilis	11.3	11.5	13.7	10.9	14.7	10.5	13.2
Pseudomons putida,	17.3	16.4	17.3	16.4	22.1	26.4	22.4
Trichoderma harzianum	11.3	11.4	17.5	9.9	20.3	16.8	12.7
Marin actimycete	12.9	13.3	16.6	14.5	19.9	15.5	13.9
LSD	2.3	1.5	2.2	2.4	2.4	2.1	2.3

Table 2. Diseases incidence of treaded corn plants, treated with bioproducts and grown under dry condition of Sahl El Tena and compared with normal condition in Behira during 2018 and 2019 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	0.9	0.53	8.3	6.3	5.4	4.9	1.5	1.3
Paenibacillus polymyxa	0.7	0.7	4.6	1.0	1.3	0.6	0.9	0.3
Bacillus subtilis	1.6	0.9	7.6	7.6	6.9	4.9	1.6	0.9
Pseudomonas putida	0.3	0.6	3.6	1.6	1.3	0.9	0.8	0.3
Trichoderma spp	1.3	1.3	9.6	3.6	7.9	6.9	2.3	1.8
Marin actimycete	0.9	0.6	3.3	2.6	2.6	2.6	1.6	0.6
Amino Acids	3.4	3.0	8.9	7.4	6.4	6.0	1.3	1.0
Potassium silicate (KSi)	3.7	3.3	8.7	8.3	6.0	5.7	1.0	0.8
P. polymyxa + Amino Acids+ KSi	0.3	0.2	7.6	8.3	1.0	0.9	0.8	0.6
P.putida+ Amino Acids+ KSi	0.3	0.2	6.3	6.0	1.0	0.8	0.7	0.5
P.putida+ P. polymyxa + Amino Acids+ KSi	0.0	0.0	0.0	0.3	0.6	0.5	0.0	0.0
Untreated control	7.1	9.3	23.0	26.2	23.3	18.3	5.3	2.6
LSD	0.9	0.9	1.9	1.8	1.9	1.4	0.9	0.9

Table 3. Chemical compositions of treaded corn plants treated with bioproducts and grown under dry condition of Sahl El Tena and compared with normal condition in Behira during 2018 and 2019 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	35.5	38.5	22.1	22.7	6.9	9.6	38.3	39.1
Bacillus subtilis	30.4	32.1	29.0	30.1	8.6	9.9	37.2	37.2
Pseudomonas putida	36.4	36.5	27.1	27.1	8.7	10.7	39.8	39.5
Trichoderma spp	34.0	35.3	23.1	24.2	8.0	8.6	31.9	33.7
Marin actimycete	30.3	32.3	23.1	19.7	6.2	7.7	35.9	36.4
Amino Acids	37.8	39.1	24.5	23.5	7.5	7.9	39.7	38.3
Potassium silicate (KSi)	36.8	39.6	26.6	31.0	9.8	9.8	39.9	40.0
P. polymyxa + *Amino Acids*+ KSi	38.8	40.7	29.5	33.2	9.3	9.6	40.6	43.5
P.putida+ *Amino Acids*+ KSi	38.6	40.7	29.5	35.9	9.3	9.8	40.3	44.9
P.putida+ *P. polymyxa* +*Amino Acids*+ KSi	39.5	33.2	30.7	36.7	9.8	10.4	45.7	46.7
Untreated control	23.1	25.1	12.2	14.0	5.7	5.9	14.0	16.0
LSD	2.4	2.6	1.6	1.7	0.7	0.9	1.6	1.8

Table 4. Yield (ardab/fed.) of treaded corn plants treated with bioproducts and grown under dry condition of Sahl El Tena and compared with normal condition in Behira during 2018 and 2019 seasons

Treatments	**Grain yield (kg/fad)**		**Grains Wight /Plant (g)**	
	Behira	**Sahl El Tena**	**Behira**	**Sahl El Tena**
Rhodotorula glutnis	4734.4	4351.1	234.1	227.0
Paenibacillus polymyxa	4865.6	4603.3	256.3	239.1
Bacillus subtilis	4754.4	4541.5	238.1	230.3
Pseudomonas putida	4998.3	4632.7	267.1	233.3
Trichoderma spp	4734.7	4451.1	221.3	221.9
Marin actimycete	4828.1	4523.2	230.1	234.8
Amino Acids	4812.4	4423.1	247.4	236.1
Potassium silicate (KSi)	4643.1	4412.2	228.4	229.5
P. polymyxa + Amino Acids+ KSi	5153.4	4701.4	274.5	263.5
P.putida+ Amino Acids+ KSi	5214.7	4812.5	271.6	261.3
P.putida+ P. polymyxa +Amino Acids+ KSi	5643.1	4945.1	292.5	276.6
Untreated control	4134.0	3851.4	204.1	177.0
LSD	12.5	9.7	8.9	7.9

Discussion

Egypt, the most populous country in the Arab World, is also by far the largest importer of corn globally (USDA, 2006 and World Food Situation, 2016). Since the 1930's, chemical farming based on chemical pesticides and mineral fertilizers had started in many parts of the world to combat food deficiency. The application of agrochemicals increased gradually until it reaches hazardous levels. Application of fungicides is the most common methods to control disease. Chemical farming, however, may lead to serious environmental deterioration and unsafe harvests. Still, resistant varieties are not available for management of foliar diseases of corn plant. Bioorganic farming excludes the use of agrochemicals in agriculture and replace them with safe biological inputs. Bioorganic farming depends mainly on biopesticides, biofungicides and or biotechnology products. The agricultural industry is in need of novel bioproducts as biofungicides or biopesticides. Development of large-scale production of biofungicides by using either microbial cells themselves or cell-free microbial components is needed to increase the number of available biocontrol agents (BCAs). Large-scale production of commercial biomass from antagonistic microorganisms is required . Our results on the application of biological products such as *P. putida* and *P. polymyxa* resulted in a significantly decrease of diseases incidence moreover, increased total soluble proteins, total phenols, peroxidase and chitinase activities in corn plants grown in dry and normal regions. Several species of bioagents including *P. putida* and *P. polymyxa* are known for their biocontrol effect and growth promoting factors because they produce several antibiotic peptide and phytohormones (Hayat *et al.*, 2010). The bioactive echanisms of these microorganisms are mainly due to their ability to produce lipopolysaccharides (LPS) and iron regulated metabolites (Raaijmakers *et al*. 2002). The present results of our research is in corroboration with our past research, wherein we found bean rust disease suppression due to over-accumulation of conjugates and free polyamine. The biological agents mixed with natural products: amino acids and or potassium silicate, give possible guarantee to obtain ecologically safe and pure products. Plant defense response to infection include signaling molecules such as SA, ethylene and jasmonic acid. An applied approach to promote and encourage plant response is through the use of additive or synergistic formulations as bielicitors (Choudary *et al.,* 2007). The antagonistic efficiency has often been corresponding to the production of different secondary metabolites, hormonal stress, biological and others characteristics of microorganisms. These are useful biocontrol agents which produce several bioactive compounds. They are environmentally compatible with agriculture. The potential of a bio-elicitors to encourage resistance to disease has also been noted as a method of management for plant diseases control. The application of biotechnological products and/or biocontrol agents reduce plant pathogens, induce plant resistance to diseases, reduce environment stress, adapt to climate change, increase yield productivity, prevent pre-harvest losses thereby increase the quality and quantity of the products. The results show that it could be possible to replace traditional chemical pesticides with bio-elicitors; it is safe, environmentally friendly and thus provided economic and ecological values.

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