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DIAZOTROPH – BIOAGENT – PATHOGEN PANORAMA TOWARDS THE PRODUCTION OF ENVIRONMENTALLY SAFED CUCUMBER

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ABSTRACT

The interactions among some bacterial bioagents, diazotrophs and phytopathogenic fungi were in vitro examined adopting the dual plate culture technique. *Pseudomonas aurogonosa* inhibited all the examined diazotrophs with an average inhibition zone measured 8 mm in diameter. In contrast, all diazotrophs could persist the antagonistic effect of *Pseudomonas putida*. *Azotobacter chroococcum* was the most sensitive to mixed cultivation with the bioagents wherease *Bacillus polymyxa* was the most tolerant. The bioagents antagonized all the examined fungal pathogens. *Pseudomonas fluorescense* exhibited the highest antagonistic capabilities against all pathogens with an average inhibition zone diameter of 21 mm followed by *Bacillus subtilis* (A) and (B). Interestingly, the examined diazotrophs suppressed growth of the pathogens; the measured inhibition zones averaged 12, 9 and 9 in diameter with *Azotobacter chroococcum*, *Azospirillum brasilense* and *Bacillus polymyxa*, respectively. The bioagents and diazotrophs were in vitro screened for IAA, gibberellins, sidrophores and hydrogen cyanide production. All the examined bacteria were active producers of IAA, gibberellins and siderophores, while hydrogen cyanide was exclusively detected with the bacteria of the genus *Pseudomonas*. In the greenhouse, the bioagents and diazotrophs improved plant survival, shoot biomass, nitrogen uptake and yield of cucumber plants. The improvements in the measured parameters were more pronounced when the bioagent mixture, composite of diazotrophs and their combinations were used for combating the root – rot disease in cucumber. With these inocula, the disease incidence was diminished to a minimum of 10 % whether the soil was infested with the pathogens or not. Shoot – dry matter production by cucumber plants was greatly affected by all inocula particularly, when both bacterial groups were combined in an inoculum. Such inoculum increased shoot biomass by 145 and 94 % over control plants in plain and fungi–infested soil, respectively. Moreover, the yield was maximized to reach < 2 and 4-folds higher than control plants grown with or without fungi, respectively. The obtained results refer to a possible integration of the examined diazotrophs as biofertilizers and bioagents in a combined inoculum for biological control of root–rot disease and improved growth and yield of cucumber plants under greenhouse conditions.

Keywords:*Diazotrophs, bacterial biagents, phytopathogenic fungi, interactions, cucmber growth, yield and root-rot incidence*.

INTRODUCTION

Biological control offers a powerful and environmentally safe alternative to the use of pesticides for combating plant diseases (Elizabeth *et al.,* 2000).

Biofertilizers, particularly those of diazotrophs, are well documented to furnish a portion of plant N demand (Döbereiner *et al.,* 1993; Hegazi *et al.,* 1998). Development of biocontrol agents for soil-borne plant pathogens has been attempted by many investigators (Halverson *et al.,* 1993; Zhang *et al.,* 1996) to improve stand establishment and seedling vigour. However, the effect of biocontrol agents on soil microorganisms other than the target pathogens is poorly understood.

Soil microorganisms that colonize roots and promote plant growth represent a subset of rhizosphere bacteria, which can produce direct or indirect effects on host plants. Indirect effects are those related to the production of metabolites, such as antibiotics, siderohores or HCN. These metabolites improve plant growth by decreasing the activities of pathogens or deleterious microorganisms (Kloepper *et al.,* 1991; Duberikovsky *et al.,* 1993). The direct effects include production of plant growth regulators as auxins, IAA, GA and cytokinins that promote plant growth and enhance seedling emergence rates (Amara *et al.,* 1996; Sedik, 1998; Garcia de Salamone *et al.,* 2001). Several bacteria of the genera *Azotobacter, Azospirillum, Bacillus, Enterobacter, Klebsiella, Sarcina* and *Pseudomonas* isolated from the rhizosphere of various plants have been reported to produce growth promoting substances (Forlani *et al*., 1995; Amara *et al.,* 1996**).**

Strategies for integrating both biofertilization and biological control of plant diseases in agricultural practicing have recently received increasing attention among agriculturalists. The present study was planned to study the possible interactions amongst biofertilizers, biological control agents and some soil-borne fungal pathogens. The dual interactions between the pathogenic fungi, diazotrophes and biological control bacteria were in-vitro studied. Production of plant growth promoting substances by rhizobacteria was also in-vitro assayed. In addition, a pot experiment was carried out to study the effect of integrated application of biofertilizers and bioagents on growth and development of cucumber plants infected with some soil-borne pathogenic fungi.

MATERIALS AND METHODS

Microorganisms

Three diazotrophs namely, *Azotobacter chroococcum, Azospirillum brasilense, Bacillus polymyxa* were kindly provided by Soils, Water and Environment Research Institute, Agricultural Research Center (ARC), Giza, Egypt. For subculturing, propagation and inocula preparation of *Azotobacter chroococcum, Azospirillum Brasilense* and*, Bacillus polymyxa*, the modified Ashby's medium (Higazi and Niemela, 1976), Döbereiner medium (Döbereiner and Day, 1976) and Hino and Wilson medium (Hino and Wilson, 1958) were utilized, respectively.

The biocontrol agents *Bacillus subtilis* (A), *Bacillus subtilis* (B), *Pseudomonas fluorescense, Pseudomonas putida, Pseudomonas aerugenosa* were obtained from Plant Pathology Research Institute, ARC,

Giza, Egypt. *Pseudomonas* and *Bacillus* were cultivated on Nutrient agar medium (CAIM, 1987) and King's medium (King *et al.,* 1954), respectively.

Fusarium oxysporum, Macrophomina phasiolina and *Rhizoctonia solani* responsible for root-rot disease in vegetables were obtained from Plant Pathology Research Institute, ARC, Giza, Egypt. Potato dextrose agar (PDA) was used for cultivating these fungi.

Plant material

Cucumber seeds (*Cucumis sativus* L.cv. Delta Star F1) were obtained from El-Bosaily Protected Cultivation Experimental Farm,ARC, Behaira Governorate.

Soil

A sandy soil from Giza was used in the greenhouse experiment. The soil is characterized by the following: sand, 94.5%; Silt, 3.5%; Clay, 2.0% .The EC and pH of the soil paste (1:10) were 2 dSm^{-1} and 7.3, respectively. (Jackson, 1973).

Fungicide

Vitafax 75% was added in the rate 1 gm/litre wp (active substrate: thiram + carboxeen).

Experimentation

***In–vitro* antagonistic activities of the bacterial strains against pathogenic fungi**

The various bacterial candidates were tested for their antagonistic activity against *Fusarium oxysporum, Macrophomina phasiolina* and *Rhizoctonia solani* using the dual plate assay method (Silo - Suh *et al*., 1994).

Production of plant growth promoting substances by rhizobacteria

Bacterial strains were tested for their capabilities to produce indole acetic acid (Bric *et al.*, 1991), siderophores (Alexander and Zuberer, 1991) and hydrogen cyanide (Bakker and Schippers, 1987) on Tryptone Soya Agar Medium (TSA) (Difco, 1984).

Quantitative determination of indole acetic acid was preformed according to Glickmann and Dessoux (1995). Total gibberellins were measured adopting the procedure of Udagwa and Kinoshita (1961).

Microbial inocula preparation

Fungal inocula strains were separately incubated in autoclaved sorghum-sand medium at 25 ◌̊C for 15 days according to Arafa (1994).

For bacterial inocula, conical flasks (250 ml) containing 100 ml of the specific liquid media were inoculated with a loop-full of the examined rhizobacteria or bioagent and incubated at 28-30◌̊ C.

Greenhouse experiment

The experiment has been carried out at El- Dokki location (CLAC) in El – Giza Governorate.

Bacterial strains were evaluated for their efficiencies to promote cucumber growth and control root-rot disease caused by *Fusarium*

oxysporum, *Macrophomina phasiolina* and *Rhizoctonia* solani in the greenhouse during the season extended from May to Aug. 2004.

Cucumber seeds (*Cucumis sativus* L.cv. Delta Star F1) were sown in multi-pot transplant trays filled with a peat moss-vermiculite mixture (1:1w/w). Three weeks after sowing, cucumber seedlings were transplanted to the greenhouse (6x40 m^2).

Plastic bags (30 cm diam. x 30cm height) containing 10 kg sandy soil were infested or not with either *Fusarium oxysporum*, *Macrophmina phasiolina* , *Rhizoctonia saloni* or their mixture. The rates of inocula application were 2% (w/w) for *Fusarium* or *Macrophomina* and 3% for *Rhizoctonia*. In case of mixed inoculum, equal volumes of individual fungal material were mixed to obtain a homogeneous composite. In order to enhance fungal growth, the infested soil was moistened with water 7 days prior to seedling transplantation.

Bacterial inoculation was performed by soaking the transplanted seedling roots in the bacterial inoculum. After transplanting, 10 ml of the examined bacterial inoculum were added to each bag. Mixed bacterial inocula were made from equal aliquots of pure liquid cultures of the designated bacterial strains.

The amounts of fertilizers recommended per greenhouse by Ministry of Agriculture, Central Administration for Agricultural Extension Services (1999) were added (Table, 1) through the drip-irrigation system by fertigation. Commercial grades of ammonium nitrate (33.5 % N), super phosphate (15.5% P_2O_5), potassium sulphate (50% K_2O) and magnesium sulfate were used for fertilization. Foliar fertilizers containing micro-elements were added to all plants when being necessary throughout a three-month season. Pre- and post-emergence damping-off and plant survival were recorded. Dry weight of shoots and total plant yield were determined at the end of the season. Total shoot nitrogen content was determined by micro Kjeldahl method (Jackson, 1973).

Table (1): Chemical fertilizers added per greenhouse

Fertilizers	Amounts (kg)
Ammonium nitrate (33.5 % N) Potassium sulfate (50% K_2O)	25 50
Ca Super Phosphate (15.5% P_2O_5)	75
Magnesium sulfate	10

Disease assessment

Survival rate of seedlings after 30 days and disease assessment for incidence of pre- and post–emergence damping off seedlings were calculated according to Phillips and Hayman (1970).

$$\text{Pre emergence damping off (\%)} = \frac{\text{No. of non emerged}}{\text{No. of sown seeds}}$$

$$\text{Post emergence damping off (\%)} = \frac{\text{No. of infected plants}}{\text{Total number of plants}}$$

$$\text{Survival rate (\%)} = \frac{\text{No. of healthy survival plants}}{\text{Total number of plants}}$$

Experimental design and Statistical analysis

Three hundred bags of transplanted seedlings were arranged in the greenhouse in a completely randomized design with 3 replicates for each treatment. Data were statistically analyzed by least significant difference by the method of Snedecor and Cochran (1980).

RESULTS

Antagonistic activities of the bioagents against diazotrophs

The antibiosis of the bioagents against diazotrophes was in vitro examined in dual agar plate cultures (Table, 2).

Table (2): Effect of biocontrol agents on growth of diazotrophs (inhibition zone diameters, mm)

Biocontrol agents	*Azospirillum brasilense*	*Azotobacter chroococcum*	*Bacillus polymyxa*
Bacillus subtilis (A)	11	1	0
Bacillus subtilis (B)	1	19	0
Pseudomonas fluorescence	0	12	0
Pseudomonas putida	0	0	0
Pseudomonas aerugenosa	7	1	18

Growth of *Bacillus polymyxa* was not affected by the examined bioagents and only an inhibition zone of 18 mm was observed when this bacterium was plated with *Pseudomonas aerugenosa*. *Azotobacter chroococcum* appeared the most affected by the bioagents, in particular *B. subtilis* (B) and *Ps. fluorescence* which indicated by the development of inhibition zones measured 19 and 12 mm in diameter, respectively. In *Azospirillum brasilense* plate cultures, *Bacillus subtilis (A) and Pseudomonas aerugonesa* showed higher antagonistic capabilities, meanwhile the other bioagents had no effect on growth of such diazotroph. It is worth mentioned that no growth inhibition zones were recorded with dual cultures of *Ps. putida* and any of the examined rhizobacteria indicating the lack of antagonism between these bacterial candidates.

The antifungal activities of bioagents and diazotrophs

The sensitivity of the examined phytopathogens to the bacterial bioagents and diazotrophs was studied in dual agar plates (Table, 3). In general, bioagents belonging to the genera *Bacillus* and *Pseudomonas*

exhibited a wider range of antifungal activities compared with the other bacterial candidates. An inhibition zone of 30 mm was developed in the culture of *Ps. flourescence* and *Macrophomina Phasiolina*. The other *Pseudomonas* bioagents had negligible effect on growth of *Macrophomina phasiolina*. The examined diazotrophs, surprisingly, showed different antagonistic activities against the fungi. This was evidenced by growth inhibition zones of 24, 12 and 13 mm recorded in cultures of *Fusarium* with either *Azotobacter, Azospirillum* or *Bacillus,* respectively.

Table (3): Antibioses of bacterial strains against pathogenic fungi (inhibition zone diameter, mm)

Bacterial strains	Pathogenic fungi		
	Fusarium oxysporum	***Macrophomina phasiolina***	***Rhizoctonia solani***
Bacillus subtilis (A)	20	19	20
Bacillus subtilis (B)	15	12	14
Pseudomonas fluorescense	15	30	17
Pseudomonas putida	14	-	-
Pseudomonas aerugenosa	-	+	15
Azotobacter chroococcum	24	12	+
Azospirillum brasilense	12	+	15
Bacillus polymyxa	13	+	15

Production of plant growth promoting substances, siderophores and hydrogen cyanide by the different bacteria

In vitro production of indol acetic acid, gibberellins, siderophores and hydrogen cyanide by the different bacterial strains are present in Table (4).

Table (4): *In vitro* production of indole acetic acid (IAA), gibberellins (GA), siderophores and hydrogen cyanide (HCN) by bacterial strains

Strains	*G. P. S. (mg/l)		Siderophores	HCN
	IAA	GA		
Bacillus subtilis (A)	149.9	566.0	+	-
Bacillus subtilis (B)	192.0	851.0	+	-
Pseudomonas fluorescense	163.0	509.0	+	+
Pseudomonas putida	107.0	638.0	+	+
Pseudomonas aerugenosa	112.0	427.0	+	+
Azotobacter chroococcum	145.0	629.7	+	-
Azospirillum brasilense	102.0	619.0	+	-
Bacillus polymyxa	167.0	584.0	-	-

*** G.P.S. = Growth Promoting Substances.**

All the examined strains were active producers of indole acetic acid and GA, particularly the biocontrol agent *Bacillus subtilis* which produced as high as 192 and 851.0 mg l^{-1} of IAA and GA, respectively. Bacterial bioagents of the genus *Pseudomonas* produced quantities of IAA and GA varying from 107 and 163 to 427 and 638 mgl^{-1} respectively. On the other hand, associative diazotrophs formed up to 167 and 629.7 mgl^{-1} of IAA and GA, respectively.

The majority of strains simultaneously produced sidrophores except *Bacillus polymyxa.* However, hydrogen cyanide producing capability appears to be a specific trait of the bacteria of the genus *Pseudomonas* and no hydrogen cyanide could be detected in cultures of bacteria from the other genera.

Greenhouse experiment

Incidence of root-rot disease in cucumber as affected by bioagents and diazotrophs

Data in Table (5) indicate the high frequency of disease incidence among 70 % of control plants. The disease severity reached a peak in control plants seeded in fungi-infested soil, which led to the loss of up to 80% damping off seedlings. Fungicide application induced disease resistance in control plants reflected by improved plant survival in soils with or without the pathogens. Application of N-fertilizer to control plants enhanced as well, their survival, particularly when the full-dose of N-fertilizer was incorporated into soil.

The introduced bioagents significantly contributed to the well-being of cucumber plants and suppressed the pathogencity of the fungi. In some cases, pre- or post- emergence damping off disease was completely eliminated by an individual bioagent or their mixture. Accordingly, 90% of plants were disease-free compared to 60 or 70% achieved with fungicide treatments in soils with or without fungal infestation, respectively. As a bioagent, *Bacillus subtilis (A)* exhibited a superior antagonistic capabilities towards the native or introduced fungi while the activities of some other candidates, e.g., *B. subtilis (B)* were not consistent. The latter bioagents showed lower antagonistic activities in the presence of the pathogens.
Azotobacter chroococcum improved plant survival up to 90 and 70 % in the soil with and without infestation, respectively. *Azospirillum brasilense* and *Bacillus polymyxa* had a similar influence on restricting the disease incidence in both soils. Both diazotrophs could increase the plant survival to 80 and 90 % in fungi–infested and uninfested soils, respectively. The composite diazotroph inoculum had a consistent disease suppressive ability with improved plant survival of 90 % whether the soil was infested or not.

Control plants had as low as 12.3 g. plant^{-1} biomass of shoots (Table, 6). N-fertilized plants showed enhanced dry matter production being approximately 2 folds higher than those received the full-dose of nitrogen-fertilizer. The individual bioagents stimulated dry matter production to an overall maximum of 32.3 g. plant^{-1} when *Pseudomonas aerugenosa* was applied to soil without fungal infestation. The stimulative effect of the bioagents on dry biomass was more noticeable when they were added in a composite inoculum, regardless of fungi infestation.

In both soils, the examined diazotrophs exerted a remarkable impact on the virulence of pathogens and ameliorated growth of cucumber plants, which eventually led to improve plant vigor *Azotobacter chroococcum* and *Azospirillum brasilense* were superior in this regard promoting the recovery of plant vigor with significantly increased shoot dry weight of 30.3, 26.3, 28.00 and 29.3 g.plant^{-1} in infested and un-infested soils, respectively. The positive effect of diazotroph individuals on plant growth was maximized when they were applied in a mixed inoculum. This could be observed by comparing shoot biomass of plants received single candidates and those inoculated with composite diazotroph inocula. The latter inocula magnified biomass accumulation in cucumber shoots by 122 % compared with controls. Moreover, a synergistic effect was pronounced when both bioagents and

diazotrophs were combined in an inoculum. Such inoculum resulted in increased dry shoot biomass of 31.3 and 28.1 g.plant^{-1} in un-infested and infested soils, respectively.

Despite the little improvement in shoot biomass of control plants received half–dose of N fertilization, heavier shoots of 27.7 g .plant^{-1} were produced by the full N – dressed ones. Nevertheless, N– fertilization appeared to have a suppressive influence on the growth promoting capacity of the diazotrophs in their mixture or combined inoculum with the bioagents. The diazotrophs mixture promoted plant growth vigor with 31.1 g dry shoot.plant^{-1} when applied in the absence of nitrogen fertilizer and 27.4 g. plant $^{-1}$ in the presence of fertilizer. This negative effect was less pronounced with the combined inoculum which suggests the synergistic functioning of the bioagents and diazotrophs.

The expected injury in plant growth account for the applied pathogens was constricted by the examined fungicide, which eventually resulted in a significant increase in shoot dry weight by 75 %. Such targeted outcome of disease control was effectively scored by any of the individual biological agents with additional growth promoting capabilities in the composite, mixture or combined inocula.

Interestingly, the examined bioagents stimulated nitrogen biosynthesis by cucumber plants. *Pseudomonas aerugenosa* and the bioagent mixtures were the most potent in stimulating increases of as high as 1195.1 and 810 mg N. shoot $^{-1}$ in the absence of the pathogens and 591.8 and 930 mg N. shoot $^{-1}$ in the presence of the fungi, respectively.

However, a stunning gain in shoot nitrogen was attributed to diazotrophs, in particular, the composite inoculum which resulted in a maximum value (1212.9 mg N shoot $^{-1}$) of shoot nitrogen. High amount of shoot nitrogen (1220.7 mg) was also recorded with plants inoculated with combined inoculum of diazotrophs and bioagents. A total shoot nitrogen content of 843 mg. plant $^{-1}$ was attributed to the combined inoculum when introduced to cucumber infested plants. A trend could be tentatively drawn with the majority of the examined bacteria refers to a reduced growth promoting activities and biological control capacity in the presence of the fungi infestation. A half–dose of N fertilization accompanying composite inoculation of cucumber plants did help the recovery of shoot nitrogen despite the presence of pathogens.

As a result of pathogen infestations, cucumber yield was drastically reduced from 1.6 to 0.7 Kg. plant^{-1}. Moreover, fruit production of the inoculated plants was higher when grown without the pathogen with a maximum productivity of 3.8 Kg.plant^{-1} scored by plants inoculated with the composite diazotrophs. Among plants received bioagents, the most productive ones were those inoculated with the mixed bioagent inoculum. Their yield was in excess of 2– and 4–folds higher than their respective controls without or with pathogens, respectively. The presence of diazotrophs in the root environment of cucumber appeared necessary for maximized yield. In most cases, the yield was higher with diazotrophs–inoculated plants than the others.

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In response to combined inoculation, cucumber productivity was as high as 3.6 Kg.plant^{-1} without the fungi and 2.8 Kg.plants^{-1} in the presence of infestation. Nitrogen fertilizer application did not improve the yield of inoculated plants. However, the yield of control plants was increased from 1.6 to 1.9 and 2.7 Kg. plant $^{-1}$ due to the half– and full– doses of N fertilizer. High productivity could also be noticed with fungicide–treated plants although production was higher with biologically–treated candidates.

DISSCUSSION

Successful recruitment of bacteria for disease control and plant growth promotion is a possible approach in agro ecosystem requires sustained manifestation of the interactions among the introduced bacteria, pathogens and the plant. Other biotic and abiotic factors contribute to or impair the disease suppression and growth promotion by these bacteria. For many bacterial bioagents, the disease suppression was attributed to antibiosis which was considered as the most important mechanism in biological control of root pathogens (Weller and Thomashow, 1993). The antagonistic merit of a bacterium could be *in vitro* authenticated adopting the dual plate culture technique which provided a criterion for selecting pioneer bioagents in cucumber (Mourhofer *et al*., 1992) mandarin (Ziedan and Farag, 2002) fababean (Fayez *et al*., 2004) and beans (Refae *et al*., 2006).

The present study revealed that the bacterial bioagents had varied antagonistic activities against diazotrophs. *Pseudomonas aerugenosa* inhibited all the examined diazotrophs with an average inhibition zone of 8.5 mm**.** The diazotrophs showed no sensitivity to mixed cultivation with *Pseudomonas putida. Azotobacter chroococcum* was the most sensitive while *Bacillus polymyxa* was the most persistent against the antagonistic effect of the bioagents. On non–targeted soil microflora including diazotrophs, the inhibition of such particular group of soil biota was attributed to the antibacterial substances produced by the biocontrol agents (Fayez *et al*., 2004)

All the examined pathogens particularly, *Fusarium oxysporum* showed high sensitivity to the different bioagents. *Pseudomonas flourscense* was the most potent antagonist inhibiting growth of the fungi in an average inhibition zone of 21 mm in diameter followed by *Bacillus subtilis* (A) (20 mm) and *Bacillus subtilis* (B) (14 mm)**.** Surprisingly, the associative diazotrophs successfully antagonized the tested fungi. The measured zones of inhibitions averaged 12, 9 and 9 mm in diameter with *Azotobacter chroococcum, Azospirillum brasilense* and *Bacillus polymyxa***,** respectively.

These observations are in harmony with the report indicating the broad–spectrum antifungal activities of the genus Bacillus and Pseudomonas. As a group of biocontrol agents, Bacillus offer several advantages over the genus Pseudomonas. Of these, the ability to form endospores of extraordinary persistence against harsh conditions (Dal – Soon *et al.*, 1997). Some effective Bacillus bioagents are known to produce various antibiotics (Stabb *et al*., 1994). On the other hand, it was reported that *Fusarium oxysporium* was the most susceptible to biocontrol preparation

while*, Macrophomina phasiolina* successfully restricted the harmful effect of antagonists (Fayez *et al*., 2004).

The bioagent–diazotroph–pathogen interactions are so complicated to be explained by a single factor. Mechanisms, other than antibiosis, have been proposed to account for the disease suppressive effect of bioagents. Of these are competition for iron and infection sites, production of secondary metabolites as antibiotics and hydrogen cyanide (Buchenaure, 1998) and (Cook *et al*., 1995). Therefore, the examined bacteria were screened for production of growth promoting substances, siderophores and hydrogen cyanide. The overwhelming majority of the tested bacteria are active producers of IAA, gibberellins and siderophores while HCN production was exclusively detected in plate culture of *Pseudomonas spp*.

Hydrogen cyanide is a general biocide forming stable compounds with divalent ions and inhibiting cytochrome oxidase of many organisms (Voisard *et al*., 1994). Production of HCN by *Pseudomonas spp*. was reported by Egamberdiyeva (2005). Indole acetic acid, gibberellins and siderophores production has been suggested for explaining the antagonism against plant pathogens exhibited by *Azotobacter*, *Pseudomonas* (Ahmed *et al*., 2004), *Azospirillum* (Young *et. al*., 1991) and *Bacillus* (Sarhan *et. al*., 2001).

In the greenhouse, the examined soil harbored subsistent populations of virulent pathogens causing root diseases in cucumber. Owing to their virulence, the disease severity harmed 70 % of control plants while the exogenous fungi increased this percentage by 10 %. In general, the obtained results refer to reduced plant survival, growth and yield of all plants grown in infested soil. This, of course, could be attributed to the complement pathogenicities of the native and high virulent–exogenous pathogens.

In addition to their well–known N_2–fixing and plant growth promoting capabilities, diazotrophs seemed necessary for combating damping off in cucumber. Their importance as effective disease–suppressive agents was more pronounced when their composite inocula were applied. The overall maxima of cucumber yield (3.8 and 3.0 Kg plant^{-1}) were scored by plants received the composite diazotroph inocula. The combined application of bioagents mixture with the composite diazotrophs inoculum supported plant disease resistance and maximized plant growth vigor as well as shoot N-content which indicates the synergistic functioning of these two types of bacteria.

Significant negative correlation could be observed (Fig., 1) between root rot disease incidence and cucumber yield (r =0.5165) and positive ones between shoot dry weight and shoot N-content (r = 0.7943), cucumber yield and shoot nitrogen (0.5969) as well as yield and shoot dry weight (r = 0.5535).

The fungicide–treated plants could withstand the disease severity account for native or introduced fungi with improved plant growth, shoot N–content and yield. However, this outcome when compared with the biologically achieved ones was not a promising target. Any of the examined bacterial inoculum could excessively fulfill the biocidal requirements for cucumber to withstand the pathogenicity of the native and/or introduced fungi with additional advantage of being a plant growth promoting treatment.

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The obtained results as well, refer to a possible biological control of cucumber damping off using the combined utilities of bioagents and diazotrophs in an inoculum containing individuals from both bacterial types. Thus, the disease severity could be diminished; the plant growth and productivity could be additionally improved. This overall strategy of integrated biofertilizer application with biological control of plant diseases fits in well with the recent concerns of sustainable agriculture relying on renewable resources for crop production.

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بانوراما التأثير الحيوى المضاد بمثبتات النيتروجين للممرضات من أجل انتاج خيار آمن بيئياً

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فى هذه البحث تم دراسة علاقة التضاد بين بعض بكتريا المقاومة الحيوية والمثبتة للنيتروجين الجوى وبعض الفطريات الممرضة للنبات فى مزارع مزدوجة. وكان لسلالة البكتريا *Pseudomonas aurogonosa* تأثيرا مثبطا لنمو كل سلالات البكتريا المثبتة للنيتروجين وكان متوسط قطر منطقة التثبيط 8 مم . وفى المقابل استطاعت كل سلالات البكتريا المثبتة للنيتروجين الجوى مقاومة التاثير المثبط لسلالة البكتريا *Ps. putida*. وكانت اكثر السلالات حساسية للفعل المضاد لبكتريا المقاومة الحيوية هى سلالة الـ *Azotobacter chrococcm* بينما كانت أكثر السلالات مقاومة فى هذا الصدد هى سلالة الـ *Bacillus polymyxa*. كما أظهرت سلالات بكتريا المقاومة الحيوية قدرة عالية على التضاد لنمو الفطريات الممرضة والمسببة للذبول فى محاصيل الخضر. وكانت أكثر السلالات البكتيرية فعالية فى هذا الشأن هى *Ps. fluorescence* حيث كان متوسط قطر منطقة تثبيط النمو 21 مم يليها فى ذلك سلالة *B. subtilis (A)* وسلالة *B. subtilis (B)*. كما لوحظ أن لسلالات البكتريا المثبتة للنيتروجين قدرة على تثبيط نمو الفطريات الممرضة حيث ظهرت مناطق تثبيط لنمو الفطريات كان متوسط أقطارها 12 ,9.9 مم فى مزارع مزدوجة لها مع السلالات البكتيرية. *Azot chrococcum, Azosp. brasilense* و *B. polymyxa* على التوالى. وقد أختبرت قدرة السلالات فى المعمل على إنتاج إندول حامض الخليك والجبريللينات وsiderophores وسيانيد الهيدروجين حيث ظهر أن لكل البكتريا المختبرة قدرة ملحوظة على إنتاج هذه المواد ماعدا سيانيد الهيدروجين والذى اقتصر إنتاجه على سلالات البكتريا التابعة لجنس *Pseudomona*. وفى تجربة الصوبة أدى تلقيح بادرات نبات الخيار بكل السلالات البكتيرية الى تحسين مقاومة البادرات لمرض الذبول خصوصا عند إستخدام لقاحات مختلطة من سلالات بكتريا المقاومة الحيوية أو البكتريا المثبتة للنيتروجين. وعند إستخدام لقاح بكتيري يضم جميع السلالات تحت الدراسة أمكن تقليص نسبة النباتات المصابة بالذبول إلى 10% فى التربة الموبوءة بالفطريات أو الغير موبوءة. وقد كان للتلقيح تأثيرا إيجابياً على الوزن الجاف للمجوع الخضرى لنباتات الخيار بغض النظر عن السلالة البكتيرية المستخدمة فى التلقيح ولاسيما عند إستخدام لقاحات من خليط من البكتريا المثبتة للنيتروجين أو تلك المستخدمة فى المقاومة الحيوية. وكان اللقاح اللذى يضم السلالات البكتيرية المدروسة كلها تأثيرا كبيرا على زيادة الوزن الجاف للمجموع الخضرى بنسبة وصلت إلى 145 و94 % فى التربة غير الوبوءة بالفطر و الموبوءة به على التوالى. وتشير النتائج المتحصل عليها إلى إمكانية التكامل بين المقاومة الحيوية والتسميد الحيوى لمحصول الخيار المنزرع تحت ظروف الصوب بإستخدام سلالات البكتريا تحت الدراسة.