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Biological Control of Maize Damping - off Disease by Microorganisms

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ABSTRACT

Pythium ultimum is important soil born fungus causing damping - off of maize. Twelve bacterial isolates were obtained from naturally rhizosphere maize. *In vitro* four bacterial isolates gave comparable results against the pathogen fungus. Two bacterial isolates which showing highly antagonism *in vitro* and also *in vivo*,it was identified by standard tests and by the application of biolog system belonging to *Microbacterium maritypicum* and *Bacillus subtilis*, while three fungi of *Trichoderma* spp. were successfully used by several investigators to control .Under greenhouse conditions , the highest percentage of survival plants with *Microbacterium maritypicum* , *Bacillus subtilis, Trichoderma viride, T. harzianum* and *T. hamatum were(60%,60%,43.33%,56.67%,43.33%)*respectively in the case of kernels soaking . Hence these bacteria and fungi can be used as an effective biological control agents in maize cultivation.

Keywords:*Microbacterium maritypicum*, *Bacillus subtilis* , Biological control, *Pythium ultimum*, *Trichoderma* spp.

INTRODUCTION

Maize (*Zea mays*L.) is considered the third cereals crops after wheat and rice all over the world for production and consumption. Maize is multipurpose crop, provides food for the human, beings feed for animals, poultry and fodder for livestock. Moreover, it is also used for industrial purposes such as glue, soap, paint, insecticides, toothpaste, shaving cream, rubber tries, rayon, molded plastics, fuels and other (White and Johnson, 2003). It has high nutritional value as it contains about 72% starch, 10% proteins, 4.8% oil, 8.5% fibers, 3.0% sugar and 1.7% ash (Chaudhary, 1983).

Several *Pythium* species including *P. aphanidermatum , P. irregular* and *P. ultimum* are know to cause damping- off in greenhouses on different plant species (Chen and Nelson, 2008). Seedling diseases can be caused by several common soil – born organisms, such as *Pythium, Fusarium*, *Rhizoctonia*, and plant parasitic nematodes. Seedling diseases are often difficult to diagnose because they have similar symptoms. Diagnosis of a specific disease may be of limited value because management may be similar for several seedling diseases. At least 14 species of *Pythium* have been previously identified that can cause seedling blight and root rot (Vincelli, 2008).

Biological control of plant pathogens is becoming important for plant disease management and several successful attempts have been made to control the pathogens by microorganisms including Actinomycetes , *Bacillus* spp., *Pseudomonas* spp. and *Trichoderma* spp. (Cook and Granados, 1991).

The aim of the present study was planned to study the possibility of controlling maize damping –off disease by using some bioagents in laboratory and greenhouse conditions.

MATERIALS AND METHODS

Source of soil samples:

The soil samples were collected from two different governorates namely; El-Dakahlia and El-Gharbia governorates in Egypt. These samples were taken for microbiological examination and preserved in fridge and examined within week from collection. Rhizospheric soils removed by shaking of plant roots were collected.

Isolation and purification of bacteria:

The bacteria were isolated and purified on NA medium(Skerman, 1967) .

Fungal strains:

Three fungal strains namely: *Trichoderma viride, T. harzianum* and *T. hamatum* were obtained from Agric. Res. Center, Giza, Egypt.

Fungal pathogen strain:

The pathogen *Pythium ultimum* was used in these experiment namely soil-born fungi. The standard culture of this fungs was obtained from Agric. Res. Center, Giza, Egypt .

Host plant:

Maize (*Zea mays* L.) cultivar Balady it provided by Agric. Res. Center, Giza , Egypt .

***In vitro* experiment**:

Antagonism the bacterial isolates , *Trichoderma* spp. and the pathogen fungus:

This experiment was carried out to study the relationship between the tested pathogenic fungus (*P.ultimum*) and bioagents according to Ferreira *et al.*, 1991 .

Identification of bacterial isolates:

The isolates of bacteria were selected that gave comparable results were identified by morphologicals and biochemicals tests according to Bergy's Manual of Systematic Bacteriology (2005), and by the application of Biolog system in the MIRCEN, Cairo , Egypt (Biolog, 2013).

***In vivo* experiment:**

Soil infestation technique:

Sandy-clay soil was prepared by mixing sand and clay (1: 2) and sterilizing by 5% formalin solution. The pots (35 cm diameter) supplied with 5 kg of the prepared soil were used. Infestation was carried out by fungs under the study at the rate of 2% of potted soil and the pots were moisted with water for 1 week before sowing.

Disease assessment:

Disease assessment was carried out by record the percentage of pre, post-emergence after 15 , 45 days and survived plants after sowing, respectively as follow**:**

Pre-emergence damping-off%=

$$\frac{\text{No.of non germinated seeds}}{\text{Total cultivated seeds}} \times 100$$

Post-emergence damping-off%=

$$\frac{\text{No.of dead seedling}}{\text{Total cultivated seeds}} \times 100$$

Survival plants%= $\frac{\text{No.of stand seedling}}{\text{Total cultivated seeds}} \times 100$

Kernels treatment and cultivation:

Kernels of maize was treated with bioagents by soaking. Bioagents bacterial or fungal antagonists, were grown in shaking nutrient broth for three days for bacterial cultures or potato dextrose broth for five days for fungal cultures at 28 ± 1°C . After the incubation period, cultures were filtered through filter paper and centrifuged at 5000 rpm for twenty minutes. The supernatants were taken and used for soaking kernels. Soaking was done for over night and kernels were immediately sown . Bacteria or fungi free media were incubated at the same conditions, centrifuged to get the supernatant and used for soaking kernel as acontrol. While kernels coating were moistened with a volume of an aqueous solution of the bioagents sufficient to moist the kernel surface. Talc powder and few drops of solution from arabic gum assisted in coating kernels and air dried before planting. Kernels were cultivated in infested soil (10 kernels/pot). Three replicate pots (No. 35 cm diameter) were used and uninfested soil acted as a control (Kommedahl *et. al.*, 1981)and (Singh *et. al*., 1980).

Parameters of antagonistic compounds :

1-Hydrogen Cyanide (HCN) :

Production of HCN was detected according to the method of Lorck (1948)

2-Indole Acetic Acid (IAA):

Production of IAA was detected according to the method of Patten and Glick (2002).

3-Cellulase production:

Aerobic cellulose decomposition was determined using Dubos medium (Allen, 1959).

4-Chitinase production:

Colloidal chitin was prepared according modified method as described by Faramarzi *et al*., (2009).

Statistical analysis:

Duncan's multiple range test (MRT) was applied for comparing means under the study (Duncan, 1955).

RESULTS AND DISCUSSION

Effect of different bacterial isolates against fungus pathogen under laboratory conditions:

Tweleve bacterial isolates were recovered from soils samples and tested *in vitro* antagonism against *P. ultimum* caused damping-off, the results obtained are presented in Table (1) .Two bacterial isolates (No. 10&11) were selected because gave better results (Sagahón,*et al.*,2011).

Data presented in Table (2) showed that *Trichoderma* spp. actively affected the growth of the pathogen under the study and slight differences between them were observed. *T. hamatum* was the most potent inhibitor to the growth of *Pythium ultimum* than *T. viride* and *T.harzianum* (Moussa,2002 ;Kazempour, 2004 and Abo-Elnaga,2013).

Table 1. Selecting of different bacterial isolates to antagonism against *Pythium ultimum* .

Bacterial isolates No.	*Pythium ultimum* Inhibition zone (cm)
1	0.0
2	0.33d
3	0.0
4	0.0
5	0.0
6	0.0
7	0.0
8	1.63 a
9	0.0
10	0.63c
11	1.23b
12	0.0
Control	0.0

Means within a column with the same letter are not significantly different at 5% level.

Table 2. Effect of fungal isolates on the linear growth *against Pythium ultimum*

Fungal strains	*Pythium ultimum*
T. viride	+
T.harzianum	+
T. hamatum	++

(++)inhibition of pathogen by over growth
(+)inhibition of pathogen

Identification of bacterial isolates:

Data in Table (3) showed two isolates of bacteria were identificated by morphological and biochemical characteristics tests according to Bergy's Manual of Systematic Bacteriology (2005) and (Biolog, 2013). The isolate (No. 10) belonging to *Microbacterium maritypicum* and isolate (No. 11) was *Bacillus subtilis.*

Table 3. Morphological and biochemical characteristics of the effective bacterial isolates

Tests	Bacterial isolates No.	
Morphological characters	10	11
Gram stain	+	+
Spore forming	+	+
Motility	+	+
Capsule formation	-	+
Measurement (µm)	(1x6)	(1x4)
Biochemical characters		
Indole production	-	-
Voges proskauer test	+	+
Methyl Red test	+	+
Citrate utilization	+	+
Catalase production	+	+
Starch hydrolysis	+	+
Casein hydrolysis	+	+
Gelatin liquefaction	+	+
Cellulase production	-	-
Glucose assimilation	-	+
Manitol assimilation	-	+
Sucrose assimilation	+	-
Fructose assimilation	+	+
Lactose assimilation	-	-
Dextrin assimilation	-	-
Xylose assimilation	-	-
Glycerol assimilation	-	-

Greenhouse experiments:

Data in Table (4) showed significant differences between the bioagents tested in controlling damping-off for two methods of seed treatments. All of the tested bioagents for both methods applications are effective in reducing pre- and post-emergence damping-off; and increased survival plants caused by *Pythium ultimum* . Results of kernels soaking with tested by *Microbacterium maritypicum* and *B.subtilis* showed that the most effective in controlling damping –off disease with the same percent of survival plants (60.00%) followed by *T . harz*ianum (56.67%) , other bioagents fell in between compared with the control (13.33%). Also, data presented in (Table 4) indicated that kernels coating with *T. harzianum* was the most effective in controlling disease, hence it gave the highest survival plants(53.33 %) , followed by *T. viride* (43.33 %) . On the other hand *Microbacterium maritypicum* was the lowest in controlling damping – off and reducing survival plants (36.67%) compared with the control(20.00 %).

Table 4. Effect of bioagents with two kernels methods of application on controlling maize damping – off disease caused by *P.ultimum.*

Treatments	Kernels soaking			Kernels coating		
	Damping- off %		Survival %	Damping- off %		Survival %
	Pre-emergence	Post-emergence		Pre-emergence	Post-emergence	
Microbacterium maritypicum	16.67^{c}	23.33^{bc}	60.00^{a}	33.33^{ns}	30.00^{a}	36.67^{ab}
B.subtilis	26.67^{bc}	13.33^{c}	60.00^{a}	40.00^{ns}	16.67^{ab}	43.33^{ab}
T. viride	20.00^{c}	36.67^{ab}	43.33^{a}	46.67^{ns}	10.00^{b}	43.33^{ab}
T. harzianum	20.00^{c}	23.33^{bc}	56.67^{a}	30.00^{ns}	16.67^{ab}	53.33^{a}
T. hamatum	33.33^{abc}	23.33^{bc}	43.33^{ab}	33.33^{ns}	30.00^{a}	36.67^{ab}
Control	46.67^{a}	40.00^{ab}	13.33^{c}	50.00^{ns}	30.00^{a}	20.00^{b}

In the same column, means followed by the same letter are not significantly different at 5% level

The results in the greenhouse are in agreement with Abada, 1994, El-Kazzaz *et al.,*2000; Moussa and Rizk, 2002; Islam *et al.,* 2007; and Eid 2014. Beet root rot was found also, throughout the present investigation to be affected by bioagent treatments. It was reported that treatment seed with biocontrol agents is the most effective and economical method of introducing the bioagents against seed and soil - borne pathogens. They prevent seed decay, seedling blight or pre-emergence damping off diseases. *Trichoderma* spp.and *Bacillus subtilis* were successfully used by several investigators to control some major diseases that affect field crops such as a sugar beet by seed treatments Moussa (2002).

Several modes of action of *Trichoderma* spp. and *Gliocladium* spp. were proposed to explain it fungal activity. The antifungal activity of *Trichoderma* spp. and *Gliocladium* spp. was attributed to mycoparasitism, competition and antibiosis (Kazempour ,2004).

Parameters for antagonistic from bacteria and fungi:

Production of HCN, IAA and enzymes:

Data presented in Table (5) indicated that all bacterial isolates were negative forHCN and cellulase .

Table 5. Detection of antagonistic properties of bacteria and fungi :

Microorganisms	HCN	IAA	Cellulase	Chitinase
Microbacterium maritypicum	-	+	-	+
B.subtilis	-	+	-	+
T. viride	+	+	-	-
T. harzianum	+	+	-	-
T. hamatum	+	+	-	-

Similar results were obtained by Singh *et al*., (2008) while two bacterial isolates were only poitive for IAA and chitinase.On the other hand all fungal isolates were only done with HCN and IAA production .These results are agreement with Ashour and Afify (2017) and Bayoumy *et al*., (2017).

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المقاومه الحيويه لمرض موت البادرات لنبات الذره بواسطة الكائنات الحية الدقيقة

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يعتبر فطربيثيم التيمم من الفطريات الممرضه الهامه الموجوده في التربه وقد أمكن الحصول علي ١٢سلاله بكتيرية من منطقة الجذور لأصناف الذرة في مصر بالإضافه الي ثلاثه انواع من التريكودرما ،تم اختبارالتضا د الحيوى فى المعمل للفطر المسبب لموت البادرات لنبات الذره مع العزلات البكتيرية وانواع فطر التريكودرما. اشارت النتائج في المعمل أن جميع انواع التريكودرما أعطت نتائج مماثلة وأن عزلتان فقط من البكتريا أظهرت التضاد لفطر البيثيم التيمم . تم تعريف أكفا عزلات البكتريا بالاختبارات القياسية وقد وجد أن هذه البكتريا هي باسلس ساتلس و مايكرو باكتريم مارتيبكيم . وعند تطبيق أختبار المقاومه الحيويه فى الصوبه الزراعيه لكل من السلالات البكتيريه والفطريه وجد ان هذه السلالات اظهرت نتائج مختلفه فى الصوبة فكانت نسبة النباتات الباقيه والمعاملة بجنس الباسلس ساتلس و بكتريا المايكرو باكتريم مارتيبكيم(٦٠%). والترايكودرما هرزيانم (٥٦.٦٧ %) بينما اعطت ترايكودرما فيردى وهماتم نتائج مماثله وكانت النسبه (٤٣.٣٣%) فى حالة النقع لحبوب الذرة . وعندالكشف عن مواد التضاد للسلالات البكتيريه والفطريه أظهرت النتائج أن البكتيريا لها القدرة علي إنتاج أندول حمض الخليك وكذلك إنزيم الكيتينيز بينما فشلت في إنتاج سيانيد الهيدروجين والسليلوليز أما بالنسبه لأنواع التريكودرما فإن لها القدره علي إنتاج أندول حمض الخليك وسيانيد الهيدروجين بينما لاتستطيع انتاج إنزيمى الكيتينيز والسليلوليز .لذلك تعتبر ميكرو فلورا التربه أداه فعاله فى مقاومة فطر بيثيم التيمم ويمكن إستخدامها فى المقاومه الحيويه للفطريات المسببه لموت البادرات فى المحاصيل الاقتصاديه.