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Biological Control of *Rhizoctonia solani* Causing Sugar Beet Damping - Off

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ABSTRACT

Rhizoctonia solani causing damping –off in sugar beet, which is one of the most destructive diseases in this crop worldwide. In this work, twelve bacterial isolates were isolated from rhizosphere of sugar beet crop of which six isolates showed antifungal activity against these phytopathogen, were identified as *Bacillus* spp. , *Bacillus amyloliquefaciens* and *Bacillus pseudomycodies* by standard tests and the application of biolog system .The most efffective and selected bacterial isolate (No. 8) obtained from sugar beet rhizosphere was identified using 16S rRNA .Moreover , three species of fungi as *Trichoderma* spp.were successfully used by several investigators to control sugar beet damping - off . *In vivo,* results of seed soaking with tested *B. amyloliquefaciens* showed that the most effective in controlling damping –off disease(93.33%) followed by *T. hamatum* (90.0%). While *B.pseudomycodies* recorded value of survival plants (86.67%), *Bacillus* sp.(6) , *Bacillus* sp. (1) , *T. viride* , *Bacillus* sp.(2) , *T. harzianum* and *Bacillus* sp. (3) (76.67%, 76.67%,66.67%, 63.33%, 60.00%, 56.67% ,respectively) indicated that seed coating with *T.harzianum* was the most effective in controlling disease (83.33%), followed by *T. hamatum*, *B. amyloliquefaciens*, *B.pseudomycodies* , *T. viride* , *Bacillus* sp.(2), *Bacillus* sp. (1), *Bacillus* sp. (3) and *Bacillus* sp.(6) (76.67%, 70.00%, 66.67%, 60.00%, 60.00%,36.67%, 33.33%, 26.67% , respectively) in soil infested with *Rhizoctonia solani.*

Keywords: *Rhizoctonia solani*, *B. amyloliquefaciens*, *B. pseudomycodies*, *Trichoderma* spp.

INTRODUCTION

Sugar-beet (*Beta vulgaris*) is one of the most important sugar crops all over of the world. In Egypt, due to the great consumption of sugar, the production of sugar-beet must be increased to cover the requirement of sugar which depending sugar cane(Abo-Elnaga ,2014).

Seedling diseases can be caused by several common soil borne genera, such as *Pythium, Fusarium* and *Rhizoctonia*. 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 (Vincelli, 2008). *Bacillus* spp. in particular are gaining recognition as safe biocontrol agents in a variety of crops, specifically as seed protectants and antifungal agents (Haggag, 2008). Moreover, they are spore-formers, which impart a natural formulation advantage over other microorganisms.

Trichoderma spp. are fungi that occur worldwide. Recent studies show that they are not only parasites of fungal plant pathogens but also can produce antibiotics. In addition, certain strains can induce systemic and localized resistance to several plant pathogens also , some strains may enhance plant growth and development(Anita *et al.* , 2012).In general, *Trichoderma* spp. are very effective biocontrol agents and controlling seedling disease in suger beet (Afify, *et al.,* 2017 & 2018) .

The aim of the present study was to study the possibility of controlling sugar beet damping –off disease by using some bioagents (*Bacillus* spp. and *Trichoderma* spp.) in laboratory and greenhouse conditions.

MATERIALS AND METHODS

Soil samples collection

The soil preserved in fridge and examined within week of collection. Rhizosphere sugar beet soils were collected and removed by shaking of plant roots El – pana,(2018).

Isolation and purification of bacteria

Ten grams of soil samples was suspended in 90 ml of sterile tap water and by serial dilutions plate method were made. Three replicates were prepared from each dilution. Colony forming units were obtained after two days of incubation at 30^0C. The bacteria were isolated and purified on nutrient agar (NA) medium (Harry and Paul , 1989).

Fungal strains as bioagents

Three fungal strains namely;*T.viride, T. harzianum* and *T. hamatum* were obtained from Plant Pathology Research Institute, Agric. Res. Center (A.R.C), Giza, Egypt.

Pathogenic fungus

The pathogen , *Rhizoctonia solani* was used in these experiments namely soil-borne fungus. The standard culture of this fungus was obtained from Agric. Res. Center (A.R.C), Plant Pathology Research Institute, Mycology Research& Plant Disease Survey Department, Giza, Egypt.

Host plant

Sugar beet (*Beta vulgaris* L.) cultivar Sultan provided by Sugar Crops Dis. Res. Dept., Plant Pathol. Res. Instit., Agric. Res. Center (A.R.C), Giza, Egypt.

***In vitro* experiment**

Antagonism between the isolated bacteria, *Trichoderma* spp. and the causal pathogenic fungus

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

Identification of bacterial isolates

The isolates of bacteria were selected that gave comparable results *in vitro*. These bacterial isolates were identified by standard tests according to Bergey's Manual of Systematic Bacteriology (2005), by the application of biolog system in the Cairo MIRCEN , Fac. Of Agric. ASU. Egypt (Biolog ,2013) and by molecular identification .

Molecular identification of the selected bacterial isolate

In order to confirm morphological identification of the most efffective and selected bacterial isolate (No. 8) this obtained from rhizosphere sugar beet.Molecular identification was done by Sigma Scientific Services Co.using 16S rRNA gene. The resulted nucleotide sequences was blasted in National Center for Biotechnology Information database (NCBI) (www . ncbi.nlm.nih .gov /blast) to identify the DNA sequence.To functionally characterize the isolated DNA fragment, similar sequence of ITS was used in many bacteria related

to our targets species and phylogentic trees were deduced.The nucleotide sequence of 16S RNA gene of *B. amyloliquefaciens* sub sp. *plantarum* FZB42T .

Greenhouse experiments

Soil infestation technique

Glass bottles of 500 ml capacity containing 100 g barley grain and 100 ml water were autoclaved for 30 minutes at 1.5 atm, then inoculated with 7- day old pathogenic fungus culture and incubated at 28 + 1°C for 15 days. Sandy-clay soil was prepared by mixing sand and clay (1: 2) and sterilized 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 ratio of 2% of potted soil and the pots were moisted with water for one week before sowing (Abo-Elnaga ,2014).

Disease assessment

Readings of seedling and plant stands were taken at 15 and 45 days of planting. Disease assessment was carried out by recording the percentage of pre, post-emergence damping-off after 15 and 45 days and survived plants after sowing, respectively, as follow:

$$\text{Pre-emergence damping-off\%} = \frac{\text{No.of non germinated seeds}}{\text{Total cultivated seeds}} \times 100$$

$$\text{Post-emergence damping-off\%} = \frac{\text{No.of dead seedling}}{\text{Total cultivated seeds}} \times 100$$

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

Seeds treatment and cultivation

Seeds of sugar beet was treated with bioagents by soaking and coating. Seeds were cultivated in infested soil (10 seeds/pot). Three replicate pots (No. 35 cm diameter) were used and uninfested soil acted as a control (Singh and Mehrotra , 1980 & Kommedahl *et al.,*1981).

Detection 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

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

4-Chitinase

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

Statistical analysis

The obtained data were subjected to analysis of variance (ANOVA) (Steel and Terrie 1960). Duncan's multiple range test (MRT) was applied for comparing means under the study (Duncan, 1955).

RESULTS AND DISCUSSION

Antagonistic effect of different bacterial isolates against *R. solani in vitro*

The results obtained are presented in Table 1 . All twelve bacterial isolates were tested *in vitro* against *Rhizoctonia solani* causing damping-off. Six bacterial isolates (No. 3,5,6 8,10 & 11) were chosen because they gave better results for inhibition pathogenic fungus (Sagahón *et al.,* 2011).

Table 1. Selecting of different bacterial isolates to antagonism against *Rhizoctonia solani* .

Bacterial isolates No.	Inhibition zone (mm) *R. solani*
1	0.0
2	0.0
3	0.16[e]
4	0.0
5	0.33[d]
6	0.33[d]
7	0.0
8	1.46[a]
9	0.0
10	0.76[b]
11	0.60[c]
12	0.0
Control	0.0

Mean within acolumn with the same letter are not significantly different (P<0.05)

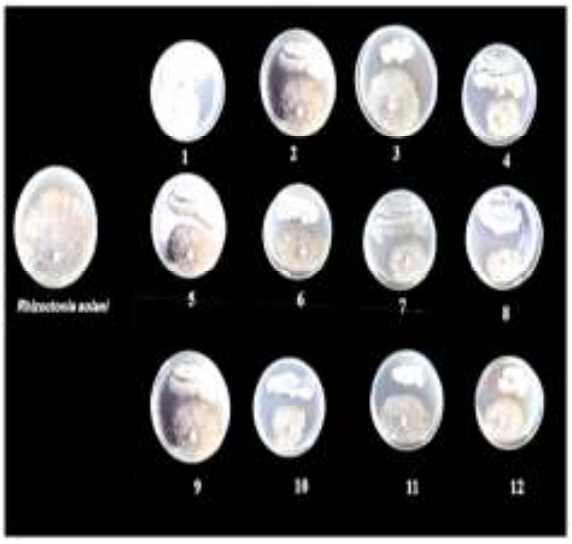

Photo1 . Inhibation of *Rhizoctonia solani* by antagonistic bacterial isolates

Effect of *Trichoderma* spp. on growth of *Rhizoctonia solan*

Data presented in Table 2 indicated that all *Trichoderma* spp. actively affected the growth of the pathogen under study and slight differences between them were observed. *Trichoderma viride, T. harzianum* and *T. hamatum* were the most potent inhibitors to the growth of *R. solani* (Moussa, 2002; Kazempour, 2004 and Abo-Elnaga, 2014).

Table 2. Effect of *Trichoderma* spp. on growth of *Rhizoctonia solani*

Trichoderma spp.	*Rhizoctonia solani*
T. viride	++
T.harzianum	++
T. hamatum	++

(++) inhibition of pathogen by over growth

Identification of bacterial isolates

Data in Table 3 showed six isolates of bacteria were identified by morphological and biochemical characteristics tests. The isolates are (No. 3, 5, 6, 8,10 &11) belonging to the genus *Bacillus*.

Table 3 . Some morphological and biochemical characteristics of the effective biocontrol bacterial isolates

Tests	Bacterial isolates No.					
Morphological characters	3	5	6	8	10	11
Gram stain	+	+	+	+	+	+
Spore forming	+	+	+	+	+	+
Motility	+	-	+	+	+	-
Capsule formation	-	-	-	-	-	-
Cell diameter (µm)	(1x5)	Sequence of morphological changes	(1.5x4-5)	(4x1.2)	(1.5x(3-4))	(4x1)
Biochemical characters						
Indole production	-	-	-	-	-	-
Voges- proskauer test	+	+	+	+	+	+
Methyl Red test	+	+	+	+	+	+
Citrate utilization	-	+	+	+	+	+
Catalase production	+	+	+	+	+	+
Starch hydrolysis	+	+	+	+	+	+
Casein hydrolysis	+	+	+	+	+	+
Gelatin liquefaction	+	+	+	+	+	+
Cellulase production	-	-	-	-	-	-
Sugars assimilation						
Glucose	+	+	+	+	+	+
Mannitol	-	+	-	-	-	+
Sucrose	+	+	+	+	+	+
Fructose	+	+	+	+	-	+
Lactose	-	-	-	-	-	-
Dextrin	-	-	-	-	-	-
Xylose	-	-	-	-	-	-
Glycerol	-	-	-	-	-	-

Identification of bacterial isolates by biolog system

After identification of the bacteria by morphological and biochemical methods according to Bergey,s Manual of Systematic Bacteriology (2005). Results in Table 4 showning the two bacterial isolates (No. 8 &10) that the most effective towards fungal pathogen were identified by biolog system and isolate (No. 8) was also , identified by 16S rRNA and phylogenetic analysis was presented in Fig.1. The final results of identification are presented in Table 4 .

Table 4 .Scientific name of bacterial isolates

Bacterial isolates No.	Scientific name	Identifcation
3	*Bacillus* sp. (1)	standerd tests
5	*Bacillus* sp. (2)	standerd tests
6	*Bacillus* sp. (3)	standerd tests
8	*B. amyloliquefaciens*	biology system + 16S rRNA
10	*B. pseudomycodies*	biology system
11	*Bacillus* sp. (6)	standerd tests

Fig .1. Phylogenotic analysis of the nucleic acid sequences of 16S rRNA of the Gram positive bacteria with antifungal activity

Greenhouse experiments

In greenhouse conditions, statistical analysis of data indicated significant differences in pre-and post-emergence damping –off and also, survival plants for two methods of seed treatments. All of the tested bioagents for all methods applications are effective in reducing pre- and post-emergence damping-off and increased survival plants caused by *R. solani* (Table 5) of sugar beet. Results of seed soaking with tested *B. amyloliquefaciens* showed that the most effective in controlling damping –off disease (93.33 % survival plants) followed by *T . hamatum* and *B. pseudomycodies* (90.00% and 86.67% survival plants, respectively). While, *Bacillus* sp. (3) recorded the lowest value of survival plants (56.67%) and *T . harzianum* (60.00 %) the tested bioagent fell in between compared with the control (33.33%). Also, data presented in (Table 5) indicated that seed coating with *T. harzianum* was the most effective in controlling disease, hence it gave the highest survival plants(83.33 %) , followed by *T. hamatum* and *B. amyloliquefaciens* (76.67% and 70.00 % survival plants, respectively).On the other hand *B.pseudomycodies*, *Bacillus* sp.(6) and *Bacillus* sp.(3) were the lowest in controlling damping – off and reducing survival plants (66.67%, 26.67% and 33.33% respectively) *R. solani* compared with the control(23.33 %). Generally, data in (Table 5) showed that seed soaking with bioagents was the most effective in controlling damping –off disease compared with seed coating on controlling sugar beet damping – off disease caused by *R.solani* in greenhouse conditions.

Table 5. Effect of bioagents with two methods of seeds application on controlling sugar beet damping – off disease caused by *R. solani* in greenhouse conditions

Bioagents used	Seed soaking			Seed coating		
	Damping- off (%)		Survival (%)	Damping- off (%)		Survival (%)
	Pre- emergence	Post- emergence		Pre- emergence	Post- emergence	
Bacillus sp. (1)	10.00^{bcd}	13.33^{bcd}	76.67^{ab}	46.67^{a}	16.67^{abc}	36.67^{c}
Bacillus sp.(2)	13.33^{bcd}	23.33^{ab}	63.33^{bc}	26.67^{bc}	13.33^{bc}	60.00^{b}
Bacillus sp. (3)	23.33^{b}	23.33^{ab}	56.67^{c}	43.33^{ab}	23.33^{abc}	33.33^{c}
B.amyloliquefaciens	0.00^{d}	6.67^{d}	93.33^{a}	16.67^{cd}	13.33^{bc}	70.00^{ab}
B.pseudomycodies	6.67^{cd}	6.67^{d}	86.67^{a}	20.00^{cd}	13.33^{bc}	66.67^{ab}
Bacillus sp.(6)	3.33^{d}	20.00^{abc}	76.67^{ab}	43.33^{ab}	26.67^{ab}	26.67^{c}
T. viride	13.33^{bcd}	20.00^{abc}	66.67^{bc}	16.67^{cd}	23.33^{bc}	60.00^{b}
T. harzianum	20.00^{bc}	20.00^{abc}	60.00^{bc}	6.67^{d}	10.00^{c}	83.33^{a}
T. hamatum	0.00^{d}	10.00^{cd}	90.00^{a}	10.00^{cd}	13.33^{bc}	76.67^{ab}
Control	40.00^{a}	26.67^{a}	33.33^{d}	46.67^{a}	30.00^{a}	23.33^{c}

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 those obtained by Jorjani *et al.,(* 2012); Sedki and El-Mohamady , (2012) & Eid , (2014). Beet root rot was also, found 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 soilborne 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 sugar beet by seed treatments (Abo-Elnaga , 2014).

Microbiological parameters for antagonistic

Production of HCN, IAA and some enzymes by bioagents

Data presented in Table 6 indicated that bacterial isolates were all negative for HCN production, and cellulase. Similar results were obtained by Singh *et al*., (2008).

Table 6 . Production of antagonistic properties from bacteria and fungi

Microorganisms	HCN	IAA	Chitinase	Cellulase
B. amyloliquefaciens	-	+	+	-
B. pseudomycodies	-	+	+	-
T.viride	+	+	-	-
T. harzianum	+	+	-	-
T. hamatum	+	+	-	-

All bacterial isolates were positive for IAA and chitinase . In case of *Trichoderma* spp. , who was reported that all isolates were positive for HCN and IAA while negative for both cellulase and chitinase were found . The results are in agreement with those obtained by Arora *et al., (*1991); Ashour and Afify , 2017and Bayoumy *et al*., (2017)

Finally, this shows that genera *Bacillus* and *Trichoderma* are quite important for effective as biocontrol agents.

REFERENCES

Abo-Elnaga H.I.G.(2014). Photosynthetic efficiency promotion of sugar beet by formulation of *Trichoderma* and control of some sugar beet disease seedling. Agrotechnol. ,93: 127.

Arora, D.K.; K.G. Mukerji and E.H. Marth (1991).Handbook of Applied Mycology, 3: Foods and Feeds. Marcel Dekker, New York.

Allen, O.N. (1959).Experiments in Soil Bacteriology . Bargess Pubi.Co.Minneapoles 15, Minnesota.

Anita, P.; A. Laddha; A. Lunge; H. Paikrao and S. Mahure (2012). *In vitro* antagonistic properties of *Trichoderma* species against Tomato root rot causing *Pythium* species. Int. J. Sci, Environ. Technol. 1(4): 302-305.

Afify ,Aida, H. ; A. B. B. El-Sayed and Seham, E. M. El-pana (2017). Biological control of Maize damping - off disease by microorganisms J. Agric. Chem. and Biotech., Mansoura Univ. 8 (11): 277 – 280.

Ashour, A.Z.A. and Aida, H. Afify (2017). Antagonistic effect of plant growth promoting rhizobacteria (PGPR) as biocontrol of plants damping-off. J. Agric. Chem. and Biotech., Mansoura Univ. 8 (4) :112-119.

Afify, Aida, H. ; A. B. B. El-Sayed ; Samia , M. M. B auomy and Samer , S. A. Elshal (2018) . Biological control of sugar beet pathogens damping – off by *Trichoderma* spp. The second Internat. Sci. Conf. on the Environ. and sustainable Develop. (ISCESD).Our Resources….Our Childern Life , AL.Azhar Univ. Cairo , Egypt.

Bayoumy, Samia, M.M.; Aida, H. Afify; A.B.B. El-Sayed and Samar, S.A.Elshall (2017). Antagonistic effect of *Bacillus* spp. against sugar beet pathogens *Fusarium* wilt. J. Agric. Chem. and Biotech. 8(6): 177-181.

Bergey's Manual of Systematic Bacteriology (2005).Don, J.; Noel, R.K. and James, T.S.2nd ed. Vol.2. George, M. U.S.A., 325-340.

Biolog, D.B. (2013). Biolog GP Data Base. Release 15G Hayward, CA: Biolog.

Duncan, D.B. (1955). Multible Range and Multible F-tes.Biometrics. 11:1-42.

Eid, K. E. (2014). Biological control of bean damping – off caused by *Sclerotium rolfsii*. Egypt. J. Phytopathol., 42(1):179-191.

El-pana , Seham , E. M. I. (2018). Microbes as bioagents for seedlings diseases in some plants . M . Sc . Thesis , Fac . Agric . Mansoura Univ . , 104 pp .

Faramarzi, M.A.; M. Fazeli; M.T. Yazd ; S. Adrangi; K. J. Al-Ahmadi ; N. Tasharrofi and F. A. Mohseni, (2009). Optimization of cultural condition for production chitinase by soil isolate of Massiliatimonae. Biotechnol. 8, 93-99.

Ferreira, J.H.S.; F.N. Mathee and A.C. Thomas (1991). Biological control of Eutyptalota on grapevine by an antagonistic strain of *Bacillus subtilis*. Phytopathol., 81: 283-287.

Haggag, W.M.(2008). Isolation of bioactive antibiotic peptides from *Bacillus brevis* and *Bacillus polymyxa* against *Botrytis* grey mould in strawberry. Archives of Phytopathology and Plant Protection 41, 477–491.

Harry , W. Seeley , Jr. and Paul , J . Van Demark (1989) . Microbes in action a laboratory manual of microbiology 3rd ed . First Published in the United States by W. H. Freeman and Company New York and Oxford .

Jorjani, M.; A. Heydari; H. R. Zamanizadeh; S. Rezaee; L. Naraghi and P. Zamzami (2012). Controlling sugar beet mortality disease by application of new bioformulations.J. Plant Protec. Res., 52(3):303-307.

Kazempour, M.N. (2004). Biological control of *Rhizoctonia solani*, the causal agent of rice sheath blight by antagonistic bacteria in green house field conditions. Plant Pathol.J., 3 (2):88-96.

Kommedahl, T.;C.E.Windels;G. Sarbini; and H.B. Wiley (1981). Variability in performance of biological and fungicidal seed treatments in corn, peas, and soybeans. Protection Ecology 3, 55-61.

Lorck, H. (1948). Production of hydrocyanic acid by bacteria. Physiol. Plant. 1: 142–146.

Moussa, A.A. (2002). Studies on biological control of suger beet pathogen *Rhizoctonia solani* Kuhn.J. of Biol. Sci. 2(12):800-804

Patten, C. and B. Glick (2002).Role of *Pseudomonas putida* indole acetic acid in development of the host plant root system. Appl. and Environ. Microbiol. 68: 3795–3801.

Sagahón I.P.; M.A.A. Reyes; H.V. Silva;R.A. Cuenca; A.T. Jurado; I.O.C. Álvarez and Y.M. Flores (2011). Isolation of bacteria with antifungal activity against the phytopathogenic fungi *Stenocarpella maydis* and *Stenocarpella macrospora* . Int. J. Mol. Sci., 5522-5537.

Sedki, R. and R. El-Mohamady (2012). Biological control of *Pythium* root rot of broccoli plants under greenhouse conditions. J. of Agric.Technol., 8(3): 1017-1028.

Singh, P.J. and R.S. Mehrotra(1980).Biological control of *Rhizoctonia bataticola* on germ by coating seed with *Bacillus* and *Streptomyces* spp. and their influence on plant growth . Plant and Soil, 56: 475-483.

Singh, N.; P. Pandey; R. C.Dubey and D. K. Maheshwar (2008). Biological control of root rot fungus *Macrophomina phaseolina* and growth enhancement of *Pinus roxburghii* (Sarg.) by rhizosphere competent *Bacillus subtilis* BN1. World, J. Microbiol .Biotechnol., 24: 1669-1679.

Steel, P.G.D. and J.H. Torrie (1960). Principle and Procedures of Statistics.MCG raw, Hill Co.Inc.New York, 481pp.

Vincelli, P. (2008). Seed and Seedling Diseases of Corn: Plant Pathology Fact Sheet University of Kentucky-College of Agriculture PPFS-AG-C-02.

المقاومة الحيوية لفطر ريزوكتونيا سولاني المسبب لموت بادرات بنجر السكر

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يعتبر فطر الرايزوكتونيا سولانى من الفطريات الممرضه الهامه التى تسبب موت البادرات فى نبات بنجر السكر.وقد تم في هذه الدراسه عزل ١٢ عزله بكتيريه من المجال الجذري لنبات بنجر السكر فى مصر بالإضافة الى ثلاث أنواع من فطر التريكودرما ، تم اجراء التضاد الحيوي فى المعمل للفطر المسبب لمرض موت البادرات لبنجر السكر مع العزلات البكتيريه وانواع فطر التريكودرما. أشارت النتائج في المعمل أن جميع أنواع التريكودرما أعطت نتائج مماثله وأن ستة عزلات فقط من البكتريا أظهرت التضاد لفطر الرايزوكتونيا سولانى.تم تعريف أكفأ عزلات البكتيريا وقد وجد أن جميع أنواعها تتبع جنس الباسيلس وأن أكفأ عزلتان تتبع النوعين باسيلس اميلوليكوفيكشن و باسلس سيدوميكودس طبقا للتعريف بنظام البيولوج و على المستوى الجرثيى بتقدير ال 16SrRNA و التى أكد نسبه تماثل تساوى ٩٩% مع بكتيريا الباسيلس أميلوليكوفيكشن. وعند تطبيق إختبار المقاومه الحيويه فى الصوبه للسلالات البكتيريه والفطريه بطريقتى النقع والتغليف لبذور البنجر أظهرت السلالات نتائج متباينه فكانت كالتالى : أعطت بكتريا باسيلس اميلوليكوفيكشن أعلي نسبه بطريقة النقع يليها تريكودرما هماتم ثم باسلس سيدوميكودس و باسلس (٦) و باسلس (١) و تريكودرما فيردي وباسلس (٢) و تريكودرما هرزيانم ثم باسلس (٣) وكانت النسبه كالأتى (٩٣.٣٣% - ٩٠.٠٠%- ٨٦.٦٧%-٧٦.٦٧%-٧٦.٦٧%- ٦٦.٦٧%- ٦٣.٣٣%-٦٠,٠٠%- ٥٦.٦٧%) علي التوالي. بينما بطريقة التغليف أعطت تريكودرما هرزيانم أعلي نسبه يليها تريكودرما هماتم وباسيلس اميلوليكوفيكشن وباسلس سيدوميكودس وتريكودرما فيردى وباسلس (٢)-(١)-(٣) - (٦)و وكانت النسبه (٨٣.٣٣% -٧٦.٦٧% - ٧٠.٠٠% -٦٦.٦٧% - ٦٠.٠٠% - ٦٠.٠٠%- ٣٦.٠٠ -٣٣.٣٣%- ٢٦.٦٧%) على التوالى.

وعند الكشف عن مواد التضاد البكتيريه والفطريه أظهرت النتائج أن البكتريا لها القدره علي إنتاج إندول حمض الخليك وإنزيم الكيتينيز بينما لاتستطيع إنتاج سيانيد الهيدروجين وإنزيم السيلولين. اما بالنسبه لسلالات التريكودرما فإن لها القدره علي إنتاج سيانيد الهيدروجين وإندول حمض الخليك بينما لاتستطيع إنتاج إنزيمى الكيتينيز والسليلوليز.