RESEARCH ARTICLE
Published 31th Desember 2023
https://doi.org/10.21070/nabatia.v11i2.1623

Biocontrol of Rhizoctonia Solani in Cucumber Plants Using Endobacteria: Effects On Disease Severity and Chlorophyll Content

Khaldoon Faris Saeed[1*], Abdullah Mahmood Othman[2]
Tikrit university- Agriculture college-plant protection department

Abstract. *This study aimed to investigate the potential of endobacteria isolated from different parts of cucumber plants (roots, stems, and leaves) to inhibit the growth of the pathogenic fungus Rhizoctonia solani. Molecular identification confirmed the presence of R. solani in cucumber plants displaying symptoms of wilting and yellowing. Laboratory experiments revealed variations in citrate consumption and gram stain results among different endobacterial isolates. In field experiments, treatments involving Bacillus subtilis with Tricozone and Pseudomonas fluorescens with Tricozone demonstrated significant reductions in disease severity and increased chlorophyll content in cucumber plants compared to the pathogen control. Additionally, Acinetobacter baumannii with Hymazole at the first concentration showed superior effects on leaf area. These findings highlight the potential of endobacteria as biocontrol agents against R. solani, with implications for improving cucumber plant health and productivity.*

Keywords: *Endobacteria, Rhizoctonia solani, Biocontrol, Cucumber plants, Disease severity*

I. INTRODUCTION

The cucumber plant belongs to the cucurbit family, Cucurabitaceae, and it is one of diffusively vegetable crops in the world [1]. Many fungal pathogens affect cucumber plants, the most important of which are Pythium aphanidermatum, Rhizoctonia solani, Fusarium oxysporum, and Macrophomina phaseolina [2, 3, 4]. Rhizoctonia solani is a soil fungus that is diffusively in regions of the world, causing great losses, as it can attack plant seeds and seedlings as well as crop roots of different plant hosts[5].

The fungus R. solani has several plant families, including the Cucurabitaceae family, to which the cucumber plant belongs, causing many problems in the different stages of plant growth and losses may reach 100% in Green houses [6]. In view of the recent trends of researchers in the use of safe alternatives to chemical pesticides, efforts have been directed to soil microorganisms to use them to combat pathogens residing in the soil [7].

Among the biological control factors for many pathogenic plant pathogens is the bacterium Bacillus subtilis through the production of antibiotics, including Subtilin and Bacillomycin, and enzymes that break down fungal cell walls, including the enzyme Chitinase [8, 9]. Also, the bacteria Pseudomonas spp is of great importance in biological control, as it showed good effectiveness in combating fungi that cause seedling fall and root rot [10], through its indirect effect by inducing resistance in the host plant, or its direct effect by secreting enzymes and toxins, or through parasitism [11]. The study aimed to apply some combinations of biological and chemical control processes in the management of Rhizoctoni crown and root rot disease.

II. MATERIALS AND METHODS

Bacteria isolation

Cucumber plant samples were washed well with distilled water (sterilized), then cut into small pieces of 1 cm, each part separately (root _ stem _leaves), and the pieces were transferred to petri dishes containing distilled water to complete the washing process, then they were superficially sterilized with sterile sodium hypochlorite solution (Clorox 6%) at a concentration of (3%) after being transferred to the aforementioned solution for one minute. After that, the remnants of the solution (sodium hypochlorite) were removed by transferring the cut plant parts to dishes containing distilled water, and then dried using filter paper. The plant parts were distributed on different mediums PDA, NA, MAa(Potato Dextrose Agar, Agar Nutrient, MacConkey Agar) , and 5 pieces were placed in each Petri dish for three replicates for each part of the plant (root-stem-leaves). Then the dishes were placed in the incubator for 48-72 hours. Petri dishes containing NA medium were prepared, these dishes were inoculated by taking a smear from the growing bacterial colonies with a sterile needle, and the dishes were placed in the incubator for 48 hours. After the expiration of the incubation period, the bacterial isolates were divided according to some phenotypical characteristics such as size, color, and edges of the bacterial colony, and microscopically by staining with Gram stain.

Motion test

The method of hanging drop (Drop Hanking) was used to test the ability of bacteria to move by growing the bacterial isolates in the liquid activation medium (NB), and at the age of one day for the bacteria, the test was carried out by drawing a drop with a medical syringe and placing it on an examination slide under a light microscope and observing its movement [12].

Isolation of pathogenic fungus

Isolation of the pathogenic fungus Rhizoctonia solani from cucumber plants that showed infection symptoms such

as wilting and yellowing in the crown area.

Molecular diagnosis of fungus

DNA extraction from fungus

The nucleic acid was extracted after taking a swab weighing ((100-50 mg wet weight) from a pure, newly developed fungal colony (5 days old) to which 750 µl of extraction solution was added to detect the result of the PCR reaction while the standard nucleic acid was present, according to the instructions of the processing company ZR / Fungal American / Bacterial / Yeast DNA Mini Prep TM, using solutions and tools prepared to extract genomic DNA Separate the product of the PCR reaction by electrophoresis on a 1.5% agarose gel, then the bands were shown by an ultraviolet light source with 336 nm after placing the gel in a water bath It contains 3 µL of DNA staining solution and 500 mL of distilled water.

Table 1. Prefixes used in the reaction

Primer	Sequence	Tm (°C)	GC (%)	Product size
Forward	5′- TCCGTAGGTGAACCTGCGG -3′	60.3	50 %	500-650 base pair
Reverse	5′ TCCTCCGCTTATTGATATGC-3′	57.8	41 %	

Detection of ITS genes using PCR technology:

Detection of the ITS4_ITS1 gene was performed using primers to amplify a portion of the ITS in DNA by the instructions of the attached leaflet and the additions shown in the table to the reaction mix**.**

Table 2. Components of the Maxime PCR PreMix Kit (i- Taq)

Material	Volume
i-Taq DNA Polymerase	5U/µl
DNTPs	2.5mM
Reaction buffer (10X)	1X
Gel loading buffer	1X

Table 3. Reaction mixture specific for gene diagnosis

Components	Concentration
Taq PCR PreMix	5µl
Forward primer	10 picomols/µl (1 µl)
Reverse primer	10 picomols/µl (1 µl)

DNA	1.5µl
Distill water	16.5 µl
Final volume	25µl

Determine the sequences of nitrogenous bases

The sequences of the nitrogenous bases of the polymerase chain reaction product of the gene (5.8 S rRNA) for the fungi to be diagnosed were identified for the product of the PCR reaction,

The sequences obtained from the Korean company Bioneer were analyzed using the National Center for Biotechnology Information (NCBI).

Infection criteria

Estimate the severity of the injury

Readings were taken from the plants at the beginning of the nodes and for each treatment, and the plants that showed symptoms of infection were recorded, and the severity of infection of the treatments was estimated according to the Mckinney equation (1923)[13], according to the following pathological evidence prepared by Gao et al. (1995)[14]:

0 _ A healthy plant (no symptoms of infection appear).

1_ Yellowing of a limited number of leaves and slight discoloration of the roots.

2 _ Yellowing of the entire leaves of the plant with discoloration of the roots.

3 _ The extension of the discoloration of the roots of the plant to the bases of the stems.

4 _ plant wilting and death.

The severity of the injury= (The number of infected plants in grade 0 × 0 + ... the number of plants in grade 4 × 4 / The total number of plants examined × the highest category in the pathological index) × 100

Estimate the percentage of infection

The percentage of infection with the pathogen *R. solani* was estimated according to the equation :

The incidence percentage = (The number of plants infected with the pathogen / The total number of plants examined) × 100

vegetative standards

Chlorophyll content measurement : Using a Chlorophyll_meter (Spad), the chlorophyll content of three leaves of three randomly selected plants was measured at flowering and for each experimental unit.

Paper area measurement : Using a device (Leaf area meter), the leaf area was measured by taking three leaves from each three plants that were chosen randomly at flowering and for each experimental unit.

statistical analysis: Laboratory trials were designed as a complete randomized design (CRD). As for the field experiment, it was applied according to the randomized complete block design (RCBD), and the data were analyzed according to the GenStat program for statistical analysis, and the significant difference between the means was tested using (LSD) Least Significant Deference, the least significant difference at the probability level (0.05).

III. RESULT AND DISCUSSIONS

Molecular diagnosis of pathogenic fungus

The results of the amplification of the ITS region through the polymerase chain reaction of DNA showed the diagnosis of the fungus *Rhizoctonia solani* to the species level.

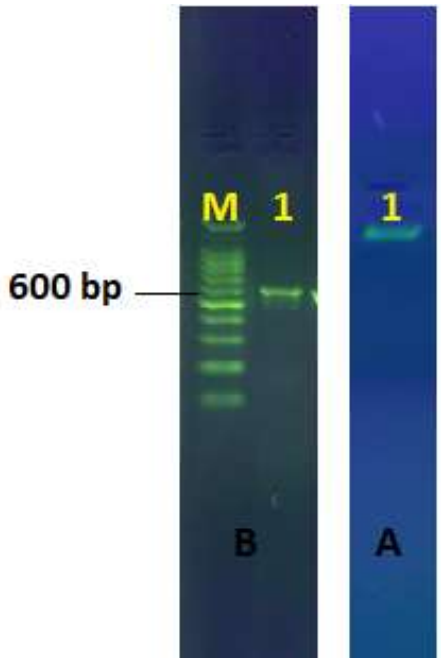

Figure 1. Electrophoresis of genomic DNA bundles of a fungus isolate

And through the results of the molecular diagnosis of the fungus isolate based on the percentage of matching of the 5.8S r RNA gene sequences with the strains of fungi in the global genetic bank at the NCBI site, Table (4) shows the similarity percentage of the mushroom isolate from the cucumber plant and its conformity with fungal species registered with international numbers and the country that isolated from him .

Table 4. shows the results of the molecular diagnosis of the fungus isolate

similarity%	Country	world number	The most closely related type of fungus
97.92	Turkey	MT478424.1	*Rhizoctonia solani isolate 59_394_AG-4-HGI_RHIZ*

Test of consumption of citrate, gram dye and motility of bacteria isolated from the inside of the plant

Several tests were conducted for bacteria isolated from the cucumber plant, where table (5) shows the consumption of citrate for the two isolates HA1 and B2 as a source of carbon and ammonia as a source of nitrogen. The medium is transported into the cells and breaks down the citrate molecules to benefit from them as an energy source. The two isolates were positive for Gram stain with the appearance of a blue color and were mobile. It was found to belong to the genus *Bacillus* [15],

As for the HA2 bacteria, they were citrate-consuming, mobile, and negative for the gram stain, with the appearance of pink color. According to the results, they belong to *Enterobacter* [16], As for the isolate HA6, it was not consuming citrate, non-motile, and negative for gram-staining, indicating that it belongs to the genus *Acinetobacter*. As for the bacteria IS6, it was consuming citrate, as it gave a positive result by the appearance of blue color, motile and negative for gram-staining, and it turned out that it belongs to the genus Pseudomonas. of citrate, but it is mobile and gram-positive, and it was found to belong to the genus *Bacillus* [15].

Table 5. Shows the consumption of citrate, gram stain and motility of bacterial isolates from the plant

bacteria	Gram stain test	Motion test	citrate test	Bacterial isolation symbol
B. subtilis	+	+	+	HA1
E. cloacae	–	+	+	HA2
A. baumannii	–	–	–	HA6
P. fluorescens	–	+	+	IS6
B. licheniformis	+	+	–	IS7
B. pumilus	+	+	+	B2

positive result (+) , negative result (-)

Results of the infection criteria of the pathogen

The impact of treatments on the mean rate and severity of infection with the pathogen *R. solani* on cucumber plants

Table (6) shows the effect of treatments with selected bacterial isolates, Tricozone biofungal preparation, Topsin and Hymazol at the recommended concentration and the half recommended, and the compatibility between the different treatments under conditions of infection with the pathogen in the percentage and severity of infection, where all treatments gave a clear effect in reduction the ratio and severity of infection with the pathogen fungus. Compared with the control treatment with pathogen fungus, the treatment of seeds with *B. subtilis* with a biological fungus civilizing

It is the highest effect among the treatments with an infection rate of 14.31% and an infection severity of 0.12, followed by the treatment of seeds with *Pseudomonas fluorescens* bacteria with Tricozone bio-fungus preparation without any differences between them, with an infection rate of 15.41 and an infection severity of 0.11, and this indicates that there is a synergy between the action of the enhanced bacteria and the biological resistance fungus. , while the lowest effect was in reducing the rate and severity of infection when treated with *A. baumannii* with the pesticide Topsin at half the recommended concentration, where the infection rate was 54.31% and the severity of infection was 0.47. The treatment of seeds and soil with the bacteria *Pseudomonas fluorescens* recorded a higher effect in reducing the percentage and severity of infection than the rest of the bacterial isolates, without a significant difference between them and the treatment with *Bacillus subtilis*. This proves the existence of a high antagonistic ability of the two bacterial isolates through its ability to produce antibiotics, including Subtilin and Bacillomycin, and enzymes that break down fungal cell walls, including the enzyme Chitinase [7, 8]. These results converge with the study of Kamil et al. (2007) [17], where it was found that Bacillus sp has high efficiency in controlling the fungus *R. solani* and reducing the percentage of infection with the fungus to 25%. As for treating the soil with Trichozone, it achieved good efficiency in controlling the pathogenic fungus and reducing the infection rate and severity. As for the coefficients of interaction with pesticides, the treatment with the pesticide Topsin at the recommended concentration with the bacteria *Bacillus subtilis* was the most efficient in the control, with an infection rate of 17.69, and an infection severity of 0.28, then followed by treatment with *Acinetobacter baumannii* with the pesticide Hemazole, with a percentage and severity of infection of 25.11, 0.21 without a significant difference between them. The effect is due to the efficiency of the active substance of the pesticide Topsin (methyl thiophanate) and the pesticide Hemazole (Hymxazol) and its inhibition of the disease while achieving compatibility with the treatment of bacteria by watering the soil and weakening the efficiency of the pathogen in condensing its pollen and preventing the division of its cells. The pathogen control treatment gave the highest rate and infection intensity on cucumber plants amounted to 71.05, 0.66%, and this is due to the ability of the fungus *R. solani* produces many enzymes that degrade the outer cell walls of the plant host, such as pectinase, cellulose, pectinlyase, pectin methyl esterase, and phosphatase [18].

Table 6. Average effect of the treatments on the ratio and severity of infection with the fungi *R. solani* on

cucumber plants

injury severity%	injury rate%	Treatments	injury severity%	injury rate%	Treatments
0.38	47.10	B2 W F2 h	0.23	28.21	B1
0.19	21.40	B2 W Tri	0.25	31.06	B2
0.39	43.50	B3 W F1 W	0.36	44.67	B3
0.47	54.31	B3 W F1 h	0.18	22.65	B4
0.21	25.11	B3 W F 2 W	0.20	20.62	F1 W
0.31	37.64	B3 W F2 h	0.37	51.30	F1 h
0.12	16.01	B3 W Tri	0.25	32.33	F2 W
0.26	35.20	B4 W F1 W	0.41	50.13	F2 h
0.38	44.10	B4 W F1 h	0.24	26.05	Tri
0.49	49.03	B4 W F 2 W	0.28	17.69	B1 W F1 W
0.40	46.17	B4 W F2 h	0.34	38.15	B1 W F1 h
0.11	15.41	B4 W Tri	0.29	32.02	B1 W F 2 W
0.66	71.05	Control w dis	0.22	30.45	B1 W F2 h
0.00	0.00	Control w out d	0.12	14.31	B1 W Tri
			0.45	49.35	B2 W F1 W
			0.42	45.33	B2 W F1 h
			0.40	44.30	B2 W F 2 W
نسبة الاصابة = 9.379		شدة الاصابة = 0.10294			LSD 0.05

B1 =*Bacillus subtilis* , B2 =*Enterobacter cloacae* , B3 =*Acinetobacter baumannii*
B4 =*Pseudomonas fluorescens* , F1w = Topsin at the recommended concentration
F1h = The pesticide Topsin is half recommended ,Tri =The preparation trichozone
F2w =Hemazole at the recommended concentration ,F2h = The pesticide Hemazole is half recommended
Control w dis = Pathogen control treatment ,Control w out d =Proper control treatment

Results of estimation of vegetative parameters

The effect of treatments on the rate of chlorophyll/seed content and leaf area/cm2 of cucumber plants under conditions of infection with the pathogen *R. solani*.

Table (7) shows the effect of different treatments on the chlorophyll content and the leaf area of the cucumber plant, where the treatment with the bacteria *Pseudomonas fluorescens* with the preparation Trichozone excelled under the conditions of infection with the pathogen, as the chlorophyll content reached 18.96 spad, followed by the treatment with the bacteria *Bacillus subtilis* with the bio-fungus preparation Trichozone, where the content reached Chlorophyll 18.80 SP, *Enterobacter cloacae* were also recorded with Trichozone preparation, average chlorophyll for the treatment was 18.26 sap, without significant differences between the aforementioned treatments, with its superiority over the pathogen control treatment with highly significant differences. While the treatment with the pesticide Topsin at half the concentration recorded the lowest chlorophyll content, reaching 10.40 spad, without a significant difference between it and the control treatment with the pathogenic fungus, which amounted to 9.86 spad. As for the treatment

with bacterial isolates, the treatment with *Pseudomonas fluorescens* gave the highest chlorophyll content of 17.60 SPAD without any significant differences between it and the treatment with *Bacillus subtilis* and *Acinetobacter baumannii*, where the chlorophyll content of the two isolates was respectively 16.39 SPAD and 15.68 SPAD, and the high chlorophyll content when treated with bacterial isolates was attributed The ability of internal bacteria to stimulate plant growth and enhance the level of chlorophyll [19]. As for the treatment with fungicides, the pesticide Hemazole, at the recommended use rate, recorded the highest chlorophyll content of 16.89 sap, with significant differences from the pathogen control treatment. As for the impact of the treatments on the mean leaf area of the plant, the treatment with *A. baumannii* with the pesticide Hymazole at the recommended use rate under the influence of the pathogen fungi treatment was the highest in effect at a rate of 160 cm 2 and with highly significant differences between it and the control treatment of the pathogen, which amounted to 92.8 cm 2, and the reason for the superiority of the treatment is due to Bacteria produce some compounds that stimulate plant growth, such as auxins, and supply the plant with some basic elements present in the soil and increase its absorption capacity, such as phosphorus, potassium, and nitrogen [20], and the synergistic action of the pesticide has a role in reducing the virulence of the pathogenic fungus on the plant host. Followed by treatment with *Acinetobacter baumannii* with Trichozone preparation, with an average leaf area of 144.5 cm2, which recorded highly significant differences between it and the pathogen control treatment. While the treatment with *Enterobacter cloacae* with the pesticide Hemazole gave the least effect ratio at a rate of 91.5 cm 2 without any significant differences between it and the treatment of the control of the pathogen compared to the test of the least significant difference. As for the effect of the bacterial isolates, the treatment with *Pseudomonas fluorescens*, under the conditions of infection with the pathogen, gave the highest average leaf area, reaching 141.1 cm 2, without a significant difference between it and the treatment with *Acinetobacter baumannii*, with the effect of all treatments being superior.Bacterial isolates on the control treatment of pathogenic fungi. As for the treatment with fungicides under the influence of infection, Topsin pesticide recorded the highest average leaf area of 140 cm2, while the lowest effect of Hemazole was half of the recommended and an average of 97.2 cm2, with no superiority over the pathogen treatment. Trichozone was also superior to the soil treatment under the influence of infection over the pathogen treatment at a rate of 134.2 cm 2 .

Table 7. The effect of treatments on the rate of chlorophyll content/spad and leaf area/cm2 of cucumber plants under conditions of infection with the pathogenic *R. solani*

Paper area / cm2	Plant chlorophyll/ spad	Treatments	Paper area / cm2	Plant chlorophyll/ spad	Treatments
115.1	14.69	B2 W F2 h	112.8	16.39	B1
141.8	18.26	B2 W Tri	116.7	14.44	B2
129.5	13.61	B3 W F1 W	126.6	15.68	B3
117.9	11.28	B3 W F1 h	141.1	17.60	B4
160	15.05	B3 W F 2 W	140	13.30	F1 W
144	13.30	B3 W F2 h	122.8	10.40	F1 h
144.5	16.63	B3 W Tri	97.2	16.89	F2 W
108.6	14.11	B4 W F1 W	125.5	13.66	F2 h
114	12.34	B4 W F1 h	134.2	18.74	Tri
119.2	17.64	B4 W F 2 W	111	15.98	B1 W F1 W
113.2	15.31	B4 W F2 h	136.2	12.45	B1 W F1 h

110.9	18.96	B4 W Tri	110.5	18.11	B1 W F 2 W
92.8	9.86	Control w dis	123.7	14.66	B1 W F2 h
155.9	18.66	Control w out d	136.5	18.80	B1 W Tri
			117.7	14.60	B2 W F1 W
			101.5	11.32	B2 W F1 h
			91.5	16.57	B2 W F 2 W
Plant chlorophyll = 2.228		Paper area / cm2 =18.49			LSD 0.05

IV. CONCLUSION

Isolasi jamur patogen dari tanaman mentimun yang menunjukkan gejala layu dan menguning, dan setelah dilakukan diagnosa molekuler diketahui merupakan jamur patogen Rhizoctonia solani. Beberapa isolat bakteri dipilih untuk mempelajari pengaruhnya dalam menghambat pertumbuhan jamur patogen R. solani dengan mengisolasinya dari berbagai bagian tanaman mentimun (akar, batang, daun).Sampel dikumpulkan dari berbagai lahan di Distrik Al-Shirqat - Kegubernuran Saladin Hasil percobaan laboratorium menunjukkan konsumsi sitrat sebagai sumber karbon pada beberapa isolat endobakteri HA1, HA2, IS6, dan B2, juga menunjukkan variasi pada uji pewarnaan gram, dimana isolat HA1, IS7, dan B2 positif pewarnaan gram, sedangkan uji motilitas menunjukkan semua isolat positif kecuali isolat HA6. Percobaan lapangan yang dilakukan meliputi pengujian pengaruh endobakteri dalam memberantas penyakit busuk Rhizoctonia pada tanaman ketimun melalui perlakuan benih dan tanah dengan suspensi isolat bakteri, perlakuan tanah dengan jamur biologis Trichoderma harzianum dan pestisida kimia Hemazol SL 30%. pada konsentrasi pertama (2 ml/liter) dan konsentrasi kedua (1 ml/L) dan Topsin WP 70% pada konsentrasi pertama (2g/L) dan konsentrasi kedua (1g/L) serta interaksi antar berbagai perlakuan . Hasil percobaan lapangan menunjukkan pengaruh pengobatan terhadap standar infeksi.Pengobatan dengan bakteri Bacillus subtilis dan obat Tricozone lebih unggul dalam mengurangi tingkat dan keparahan infeksi jamur patogen, tercatat 14,31 dan 0,12 di bawah pengaruh infeksi dibandingkan dengan perlakuan kontrol dengan patogen yang memberikan angka tertinggi yaitu 71,05 dan 0,66. Hasil perlakuan dengan bakteri internal Pseudomonas fluorescens dan sediaan Tricozone menunjukkan adanya peningkatan kandungan klorofil tanaman mentimun yang cukup signifikan, dimana rata-rata kandungan klorofil mencapai 18,96 SPAD, dibandingkan dengan kandungan klorofil terendah pada perlakuan kontrol dengan pemberian Tricozone. patogen yaitu sebesar 9,86 SPAD. Sedangkan untuk pengaruh perlakuan terhadap rata-rata luas daun, perlakuan dengan bakteri Acinetobacter baumannii dan pestisida Hemazole pada konsentrasi pertama lebih unggul dibandingkan perlakuan dengan jamur patogen, dengan luas rata-rata 160 cm2, dengan signifikansi yang signifikan. selisih pengaruh terendah pada perlakuan kontrol dengan jamur patogen yaitu sebesar 92,8 cm2.

REFERENCE

[1] A. A. M. Hassan, "Produksi sayuran musim hangat dan panas di lahan gurun," Rumah Arab untuk Penerbitan dan Distribusi, 1994, hal. 288.

[2] N. S. El-Mougy, N. G. El-Gamal, dan M. M. Abdel-Kader, "Control of wilt and root rot incidence in Phaseolus vulgaris L. by some plant volatile compounds," Journal of Plant Protection Research, vol. 47, hal. 255-265, 2007.

[3] S. N. Hussein, "Integrated management of the Fusarium vascular wilt disease of Cucurbita pepo in Iraq," Journal of Agricultural and Marine Sciences, vol. 23, hal. 40-47, 2019. DOI: 10.24200/jams.vol23iss1.pp40-47.

[4] P. H. Binagwa, C. K. Bonsi, S. N. Msolla, dan I. Ritte, "Morphological and molecular identification of Pythium spp. isolated from common beans (Phaseolus vulgaris) infected with root rot disease," African Journal of Plant Science, vol. 10, hal. 1-9, 2016.

[5] B. Nasraoui, "Les champignons et pseudo-champignons pathogens des plants cultivees: Biologie, Nouvelle systematique, Interaction pathologique [Pathogenic fungi and pseudo-fungi of cultivated plants: Biology, New systematic, Pathological interaction]," 2016.

[6] M. R. Ardakani, D. Mazaheri, A. H. S. Rad, dan S. Mafakheri, "Uptake of Micronutrients by Wheat (Triticum aestivum L.) in a Sustainable Agroecosystem," Middle East J. Sci. Res., vol. 7, no. 4, hal. 444-451, 2011.

[7] S. H. Shoaei, G. H. Noor-mohammadi, R. Choukan, A. Kashani, S. H. H. Heydari, dan F. Rafiei, "Study of Nutrient Accumulation In The Aerial And Forage Yield Affected By Using Of Nitroxin, Supernitro Plus And Biophosphor In Order To Reduce Consumption Of Chemical Fertilizers And Drought-Resistant In Corn (KSC-704)," Advances in Environmental Biology, vol. 6, no. 1, hal. 125-131, 2012.

[8] S. El-mougy, M. M. Abdelkader, dan S. M. Lashin, "Fungicide," 2014.

[9] W. Nesmith, "Fungicide application guidelines for commercial vegetables in Kentucky," Plant Pathology, Fact Sheet, Cooperative Extension Service, Univ. of Kentucky-College of Agriculture, 2001.

[10] C. Pornsuriya, H. K. Wang, F. C. Lin, dan K. Soytong, "First report of pineapple root rot caused by Pythium graminicola in Thailand," Journal of Agricultural Technology, 2008.

[11] J. Hallmann, K. G. Davies, dan R. Sikora, "Biological control using microbial pathogens, endophytes and antagonists," in Root-knot nematodes, CABI, 2009, hal. 380-411.

[12] C. A. Black, "Methods of soil analysis. Part 2. Chemical and microbiological properties," Amer. Soc. Agron. Inc. Publisher, Madison, Wisconsin, U.S.A., 1965.

[13] H. H. McKinney, "Influence of soil temperature and moisture on infection of wheat seedlings by Helminthosporium sativum," Journal of Agriculture Research, vol. 26, hal. 195-218, 1923.

[14] H. Gao, G. H. Beckman, dan W. C. Mueller, "The rate of vascular colonization as a measure of the genetic interaction between various cultivars of tomato and various formae or races of Fusarium oxysporum," Physiol. Mol. Plant Pathol., vol. 46, hal. 29-43, 1995.

[15] M. A. S. Mahon, I. S. Blair, J. E. Moore, dan D. A. McDowell, "The rate of horizontal transmission of antibiotic resistance plasmids is increased in food preservation-stressed bacteria," Journal of Applied Microbiology, vol. 103, no. 5, hal. 1883-1888, 2007.

[16] M. Vos, P. J. Birkett, E. Birch, R. I. Griffiths, dan A. Buckling, "Local adaptation of bacteriophages to their bacterial hosts in soil," Science, vol. 325, no. 5942, hal. 833, 2009.

[17] Z. Kamil, M. Rizk, M. Saleh, dan S. Moustafa, "Isolation and identification of rhizosphere soil chitinolytic bacteria and their potential in antifungal biocontrol," Global Journal of Molecular Sciences, vol. 2, no. 2, hal. 57-66, 2007.

[18] B. L. Bertagnolli, F. K. Dal Soglio, dan J. B. Sinclair, "Extracellular enzyme profiles of the fungal pathogen Rhizoctonia solani isolate 2B-12 and of two antagonists, Bacillus megaterium strain B153-2-2 and Trichoderma harzianum isolate Th008. I. Possible correlations with inhibition of growth and biocontrol," Physiol Mol Plant Pathol, vol. 48, hal. 145-160, 1996.

[19] Y. Ma, M. Rajkumar, A. Moreno, C. Zhang, dan H. Freitas, "Serpentine endophytic bacterium Pseudomonas azotoformans ASS1 accelerates phytoremediation of soil metals under drought stress," Chemosphere, vol. 185, hal. 75-85, 2017.

[20] M. B. Khan, M. Farooq, M. Hussain, Shahnawaz, G. Shabir, Y. J. Kim, dan M. Wilson III, "Overview of base excision repair biochemistry," Current molecular pharmacology, vol. 5, no. 1, hal. 3-13, 2012.

***Conflict of Interest Statement**:The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.*