International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

The Activity of *Metarhizium sp*. To Control *Pythium aphanidermatum* Causal Agent of Cucumber Damping off Under Greenhouse Conditions

Neran Salem Aljarah

Plant protection dep. Agriculture College, Baghdad University, Iraq

Abstract: ***The study was conducted to evaluate the activity of the fungus Metarhizium sp., as a biocontrol agent, against Pythiumaphanidermatum causative agent of cucumber seedlings damping off in Agriculture College\ Baghdad University. Results showed a high significant increase in lateral root 13.7/ seedling and root length 9.3 cm in seedling grown on water agar (WA) inoculated with Metarhizium sp. After4 days of cultivation compared with 7.8 lateral root / seedling and 4.3cm length in control(without Metarhiziumsp.). More intense of hairs on the main root in seedling on water agar with Metarhizium sp. compared with control were observed. The increasing of root formation was found associated with high inhibition of P. aphanidermatum as proved by the reduction of mycelium growth on PDA compared with control. The presence of Metarhizium sp. with P.aphanidermatum in the soil cultivated with cucumber plants induced a significant increase in healthy seedling, 86.7 and 66.7% after 7 and 21 days of cultivation respectively compared with 50 and 40% in control respectively. The increase in healthy seedling was found associated with an increase in plant dry weight. The dry weights of root and foliage were attained to 0.56 and 0.89 g/ plant in pots inoculated with Metarhiziumsp. and P. aphanidermatum respectively compared with 0.31 and 0.45g/plant respectively in control after 30 days of cultivation.***

Keywords: *Metarhizium sp*., *Pythium aphanidermatum*, biocontrol agent, cucumber plants.

1. Introduction

The fungus *Pythium aphanidermatum* is one of the most important among soil born plant pathogen causing various disease symptoms, including seed rot, seedling damping off, root rot and wilt to a wide range of hosts (Agrios, 2005). It is characterized by rapid growth and high competitive ability associate with the production of enzymes and toxins causing heavy losses to wide range of crops including cucumber (lefshitz et al, 1984).The cucumber (*Cucumissativus* L.)is one of the most important vegetables in greenhouses, but its cultivation is limited because of seedling infection by *Pythium spp* (Abbasi and lazarovits, 2006).

The disease was effectively controlled by many fungicides, but the continuous and misuse of these fungicides caused enormous problems to the ecosystem and human health as well as resistant strains of the pathogen were developed making the use of fungicide ineffective. Therefor the research was oriented toward using beneficial bio-agents as alternative to fungicide for managing the disease.

It has been reported that rhizosphere microorganisms are found on plant roots, mainly bacteria and some fungi, excise beneficial effects on plant growth through suppressing soil borne pathogens. Their effects on pathogen can be the result of competition for nutrients or antibiosis (Bekker et al, 2013). Some of these microorganisms reduced diseases through inducing plant defense mechanisms referred to as induced systemic resistance (ISR) locally and systemically (Vanloon et al, 1998; Walters et al, 2005).ISR is characterized by restriction of pathogen growth and suppression of disease symptoms development (Hammerschmidt, 1999).

Among the beneficial fungi in the soil is *Metarhizium sp*. belong to Ascomycetes, order: Hypocreales, Family: Clavicipitaceae, characterize by its ability to grow mainly in the soil as saprophyte in the rhizosphere and on insects as parasite under different ecological conditions(Hu and ST. Léger, 2002;Bidochka and Small, 2003; ST. Légeret al, 2011).Kang et al. (1996) found that *Metarhizium sp.* showed antagonistic effects against several pathogenic fungi including, *Fusarium oxysporum*, *Botrytis cinerea*, and *Alternariasolni*.

The study was conducted to evaluate the possibility of using *Metarhizium sp.* to control cucumber seedling damping off caused by *Pythium aphanidermatum* under greenhouse condition as well as studying some aspects of the interaction between *Metarhizium sp.* with cucumber seedling and *P. aphanidermatum.*

2. Material and Methods

The fungi

P. aphanidermatum was isolated from cucumber seedling showing damping off symptoms in the Agriculture College / University of Baghdad fields. The infected seedlings stems were sectioned into small pieces (5 mm long). The pieces were surface sterilized in 2% sodium hypochlorite for 3 min., rinsed in sterile water and let too dry in isolation room. The pieces were then cultivated on potato-dextrose-agar (PDA) in 9 cm diameter Petri plates and incubated at 25±2 ^{o}c for 3 days. The growing fungus was purified by taking apart from the margin of the fungal colony on new PDA. This isolate was conserved in sterile soil (autoclaved twice at 121^{o}c and 1.5kg/cm^{2} for one hour in two successive days). *Metarhizium sp*. isolate was obtained from the organic culture center/ Ministry of Agriculture/ Iraq. The isolate was

reactivated on PDA at 25±2° c for 7 days and grown on sterilized sorghum seeds (100 gm/ flask of 200 ml, soaked in water for 30 min. and autoclaved at 121° c and 1.5kg/cm² for one hour) at 25±2° c.

Effect of *Metarhizium* sp. on cucumber seeds germinations

Cucumber seeds (AL-Moktar\ Iraqi type) were surface sterilized in 2%sodium hypochlorite for 3 min., rinsed with sterile water and cultivated on WA (12g agar/ L water) in 9cm petri-plates, 25 seeds/ plate at 1cm from the margin. A disc of 5mm from *Metarhizium* sp.mycelium on PDA, 7days old, was placed in the plate center and incubated at 25±2 °c for 7 days. The treatment was replicated 4 times with 25 seeds in each replication. The plates were distributed as complete randomized design (CRD), and the percent of germination was determined. Five seedlings from each replication were randomize selected for root length and a number of lateral roots/ seedling calculation, as well as samples of the roots were microscopically observed. Pieces of seedling roots, stems, and cotyledons were surface sterilized, as previously described, and cultivated on PDA. The fungal growth was observed after 4 and 7 days of cultivation.

Inhibition activity of *Metarhizium sp.* against *P. aphanidermatum* growth on PDA:-

Dual culture technique on PDA in 9 cm diameter petri plates was adopted. The inoculated plate between the two fungi was determined.

The activity of *Metarhizium sp.* against *P. aphanidermatum* in pots under greenhouse conditions:

Inoculum preparation:-

1) *P. aphanidermatum*: four plates of the pathogen growth on PDA, 4 days old, were mixed with one liter of distilled water in the electrical mixer for one minute. The mixture was maintained at 4°c for 45- 60 min. and used as a fungal inoculum.
2) *Metarhizium sp.*: sterilised sorghum seeds in 250 ml flasks (100gm/ flask), were inoculated with 5 discs of 1cm diameter, 7 days old, of the fungus growth on PDA and incubated at 25 ±2 °c with agitation daily until fungal growing.

Pots experiment:-

Mix soil, 1 peat moss: 1 soil (v: v)in jute sac of 5 kg size were watered and autoclaved twice at 121°c and 1.5kg/ cm^2 for one hour in two successive days. The sterile mix soil was distributed in pots of 2 kg size and inoculated with *Metarhizium*sp.inoculum at 1%. The pots were covered with plastic sacs for 3 days and inoculated with *P. aphanidermatum* inoculum 75ml/ pot and recovered with plastic sacs for another 3 days. Pots inoculated with *P. aphanidermatum* only were used as a control. The inoculated pots were cultivated with cucumber seed, 10 seeds/ pot, and distributed in the greenhouse in complete randomize design (CRD) as following;

1) Seeds in sterilized soil.
2) Seeds in sterilized soil inoculated with the pathogen (75 ml/ pot).
3) Seeds in sterilized soil inoculated with *Metarhizium sp.* (1%).
4) Seeds in sterilized soil inoculated with *Metarhizium sp.* + pathogen.

Five replications of each treatment were used. The percent of healthy seedling was calculated after 7 and 21 days of germination. Samples from stems, cotyledons, first, and second true leaves were cut to small pieces, sterilized in 2% sodium hypochlorite and cultivated on PDA at 25 ±2 °c to determine the associated fungi.

Samples (0.5 gm) of seedling were weakly taken (4 weak), for peroxidase determination as described by Whitaker and Berhard (1972). The plants were oven dried in a punchy paper sac at 60 °c and the dry weights of roots and foliage were estimated.

3. Results

Effect of *Metarhiziumsp.* on seedling growth on WA:-

Results indicated that inoculation WA medium with 5 mm diameter disc of *Metarhizium* sp. growth on PDA medium 7 days old, induced high significant increase in lateral roots and root length of cucumber seedling, grown from cucumber seeds cultivated on this medium after 4 days of cultivation, 13.2 lateral roots/ seedling and 9.3cm root length, compared with 7.8 lateral roots/ seedling and 4.3 cm root length in control respectively, table (1).

Table 1: Effect of *Metarhiziumsp.* on the number of lateral roots and root length of cucumber seedling on WA after 4 days of cultivation

Treatment	No. of later roots	Root length(cm)
Seedling on WA only	7.8	4.3
Seedling on WA inoculated with *Metarhizium*sp*	13.2	9.3
LSD(P=0.01)	4.35	3.55

* A disc, 5mm dimeter, of *Metarhizium* sp. growth on PDA, 7 days old, was used for WA inoculation.

It was found that the hairs formed on the main root were more intense in seedling grown on WA inoculated with *Metarhizium* sp. compared with those formed on seedling roots grown on non-inoculated WA after 4 and 7 days of germination (Fig 1 and 2).

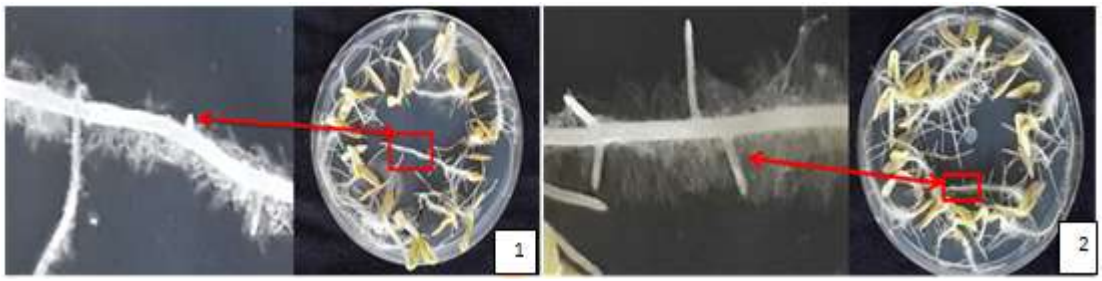

Figure 1: Effeact of hair root formation when cucumber seedling grown on WA only (1), compared with those formed on seedling roots grown inoculated WA with *Metarhizium*sp, (2) after 4 days of culture under incubator condition

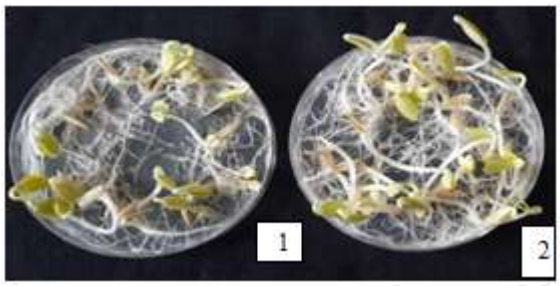

Figure 2: Cucumber seedling grown on WA only (1), compared with seedling grown on inoculated WA with *Metarhiziumsp*. (2) after 7 days of culture under incubator condition.

Results of fungal isolation from seedling on WA showed the presence of *Metarhizium* sp. in all parts of seedling (roots, steam and leave) cultivated on PDA, as well as from the plants after 30 days of cultivation, which indicated to endophyte relation nature between the fungus anda plant.

Inhibition activity of *Metarhizium sp.* against*P. aphanidermatum*growth on PDA:-

High inhibition effect was exerted by *Metarhizium sp.* against *P.aphanidermatum* growth as proved by high reduction of*P. aphanidermatum* mycelium growth (restricted to one-third of the plate) on PDA compared with the control. No inhibition zone between the two fungi was observed and the myceliums of the two fungi were inter-grown. This indicates the mechanism of inhibition is due to competition for nutrients or may be to the direct parasitism.

The **activity of *Metarhizium sp.* against *P. aphanidermatum* in pots under green conditions:-**

The cultivation of cucumber seeds in soil amended with of *Metarhizium sp.* and inoculated with *P. aphanidermatum* induced a significant increase in healthy seedlings after 7 and 21 days of cultivation compared with those in control. The percentages of healthy seedling were attained to 86.7 and 66.7% after 7 and 21 days respectively, compared with 50 and 40% in control (pathogenonly) respectively.

The increase of healthy seedling was found associated with the increase in the plant growth parameters after 30 days of cultivation. The dry weight of root and foliar systems that attained to 0.56 and 0.8 g/ plant for plants in pots inoculated with *Metarhizium sp.* and *P. aphanidermatum* respectively, compared with 0.31 and 0.45g/ plant respectively for the plants grown in pots inoculated with *P. aphanidermatum* only (table 2).

Table 2: Effect of soil inoculation with *Metarhizium sp.* and contaminated with *P. aphanidermatum* on dry weights of cucumber roots and foliage after 30 days, and on the percentage of healthy seedling after 7 and 21 days of cultivation under green house conditions

Treatments	Dry weight (g/ plant)		% healthy plants after	
	root	Foliage	7 days	21 days
Sterile soil(control)	0.70	0.95	100	100
Sterile Soil inoculated with py	0.31	0.45	50	40
Sterile Soil inoculated with me	0.67	1.02	86.7	100
Sterile Soil inoculated with both me+ py	0.56	0.80	86.7	66.7
L.S.D (p=0.01)	0.082	0.145	26.23	15.82

py=*P. aphanidermatum* (75 ml of mycelium suspension/pot).me= *Metarhizium sp.*(1% w:w).

Peroxidase Activity

Result showed that the peroxidase activity stay low in plant grown in soil inoculated with *Metarhizium sp.*and *P. aphanidermatum* during first, second and third weeks that attained to 59.69, 6.24, 2.76 units/ g of the fresh cucumber leaves respectively compared with those grown in soil contaminated with the pathogen only, 131.26, 9.22, 4.78 units/ g fresh weight (fig.3), which indicate that *Metarhizium sp*. is not inducer of systemic resistance in the plants.

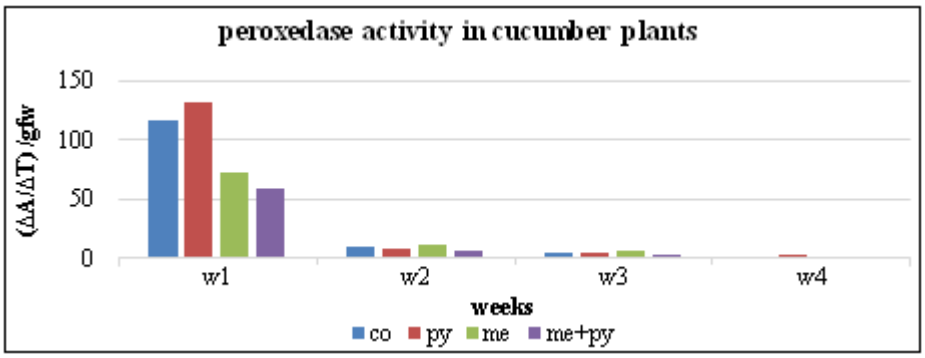

Figure 3: Effect of soil inoculated with *Metarhizium* sp. (me) and contaminated with *P. aphanidermatum* (py) on peroxidase activity in the cucumber plants during 4 weeks (w1, w2, w3 and w4) of cultivation under greenhouse conditions.co=control.

4. Discussion

The results obtained from this study demonstrate clearly that *Metarhizium sp.* exerted inhibition activity against *P. aphanidermatum*on culture media. The absence of inhibition zone between with *Metarhizium* sp. and *P. aphanidermatum* growth on PDA as well as the intergrown between the two fungi indicates that the mechanism of *Metarhizium sp.* effect may be due to the competition for place and nutrients and/ or to the direct parasitism on the pathogen and production of hydrolytic enzymes. It was reported that many genera of the insect pathogenic fungi in plant rhizosphere as well as invaded plant tissues internally (Endophyte) (Bult et al., 2001; Hu and ST. Léger, 2000; Kabaluk and Ericcson, 2007; Ownley et al., 2008; Vega, 2008).

The cultivation of the seeds in soil inoculated with *Metarhizium sp.* and contaminated with *P. aphanidermatum* has significantly increased healthy seedlings compare with seedlings emerged in soil contaminated with *P. aphanidermatum* only. The increasing of healthy seedling may be due to the inhibition of *P. aphanidermatum* as previously mentioned. It was reported that *Metarhizium sp.* is found mainly in the rhizosphere where the root exudate act as a source of nutrients for its growth and produce protease enzyme as an important factor in insect biocontrol. Similar mechanism effect may be exerted on plant pathogenic fungi by *Metarhizium sp.*(Pozo et al., 2003).

The stimulation of seed germination was found associated with the promotion of plant growth parameters (root lengths, number of lateral roots and plant dry weights). The promotions of plant growth by *Metarhizium sp.* maybe direct as a result of production secondary metabolites including phytohormonsthat stimulate seed germination and root formation leading to promote nutrients uptake. It was reported that *Metarhizium* sp. invade plant root tissues inducing root hairs and lateral roots formation that increase nutrients uptake and promote plant growth (Felton et al., 2009 ; Wu et al., 2010; Ramanpreet and Michael, 2013).*Metarhizium*sp. may act as a mediator for transmitting the nutrients from rhizosphere to plant as was reported by Shoreshet al., (2010) that some rhizosphere fungi act as the bridge for passing nutrients from the soil to plant root.

From the results of this study, we concluded that the fungus *Metarhizium sp.* may be adopted as a factor in the management of damping-off disease in cucumber cultivation.

References

[1] Abbasi, P.A., and Lazarovits, G.2006.Seed treatment with phosphonate (AG3) suppresses Pythium damping-off of cucumber seedlings. Plant Dis.90:459-464.
[2] Agrios, G. N. 2005. Plant Pathology. 5thedition. Elesvier, Academic Press, pp. 996.
[3] Bakker, P. A.H.M.;.Doornbos, R. F.; Zamioudis, C.; Berendsen, R. L. and Pieterse, C. M.J. 2013. Induced Systemic Resistance and the Rhizosphere Microbiome. Plant Pathol. J. 29(2): 136-143.
[4] Bidochka, M. J., and C. L. Small. 2003. Phytogeography of Metarhizium, an insect pathogenic fungus. In Vega, F. M. and M. Blackwell. (eds) Insect Fungal Associations: Ecology and Evolution. Oxford University Press, New York.(In Ramanpreet and Michael 2013).
[5] Butt, T. M.; C. Jackson; and N. Magan. 2001. Fungi as biocontrol agents: Progress, problems and potential. CABI Publishing, Wallingford, UK.(InRamanpreet andMichael 2013).
[6] Felten, J.; A. Kohler; E. Morin; R. P. Bhalerao; K. Palme; F. Martin; F. A. Ditengou; and V. Legue. 2009. The ectomycorrhizal fungus Laccaria bicolor stimulates lateral root formation in Poplar and Arabidopsis through transport and signaling. Plant Physiology 151: 1991-2005.
[7] Hammerschmidt, R. 1999. Induced disease resistance: How do induced plants stop pathogen? Physiol. Mol. Plant pathology.55:77-84.
[8] Hu, G., and R. ST. Leger. 2002. Field studies using a recombinant mycoinsecticide (*Metarhizium anisopliae*) reveal that it is rhizosphere competent. Applied and Environmental Microbiology 68: 6383-6387.
[9] Kabaluk, J. T.; and J. D. Ericsson. 2007. *Metarhizium anisopliae* seed treatment increases yield of field corn when applied for wireworm control. Agronomy Journal 99: 1377-1381.
[10] Kang, C.S.; B. y. Goo; L.D.Gyu; and K.Y. Heon. 1996. Antifungal activities of *Metarhizium anisopliae* against *Fusarium oxysporum*, *Botrytis cinerea* and *Alternariasolani*. Korean journal of Mycology. 24; 49-55.

[11] Lifshitz, R., M. E. Stanghellini, and, R. Baker.1984. A new species of Pythium isolated from soil in Colorado. Mycotaxon 20, 373–379.
[12] Ownley, B. H.; M. R. Griffin; W. E. Klingeman; K. D. Gwinn; J. K. Moulton; and R.M. Pereira. 2008. *Beauveriabassiana*: endophytic colonization and plant disease control. Journal of Invertebrate Pathology 3: 267-270.
[13] Pozo, M. J.; J.-M. Baek; J. M. Garcia; and C. M. Kenerley. 2003. Functional analysis of tvsp 1, a serine protease-encoding gene in the biocontrol agent *Trichoderma virens*. Fungal Genetics and Biology. 41(3): 336-348.
[14] Ramanpreet K. S. and Michael J. B. 2013. Antagonism of the endophytic insect pathogenic fungus *Metarhizium robertsii* against the bean plant pathogen *Fusarium solani* f. sp. *phaseoli*. Canadian Journal of Plant Pathology.35 (3)288-293.
[15] Shoresh, M.; G. E. Harman; and F. Mastouri. 2010. Induced systemic resistance and plant responses to fungal biocontrol agents. Annual review of Phytopathology. 48: 21-43.
[16] ST.leger; R. J.; Wang, C.;and Fang, W. 2011. New perspectives on insect pathogens. Fungal Biology Reviews. 25: 84-88.
[17] Vanloon, L., P.Bakker and C.Pietorse. 1998. Systemic resistance induced by rhizosphere bacteria. Ann. Rev. Phytopathology. 36: 453-483.
[18] Vega, F. E. 2008. Insect pathology and fungal endophytes. Journal of Invertebrate Pathology. 98: 277-279.
[19] Walters, D.R.; D.Walsh;A.C. Newton; and G.O. Lyon. 2005. Induced resistance for plant diseasecontrol: Maximizing the efficacy of resistance elicitors.Phytopathology: 95; 1368-1373.
[20] Whitaker, J. R., and B. A.Berhard. 1972. Experiments for an introduction to enzymology.The Whiber Press. Davis. California
[21] Wu, L.;Y.Lv;Z.Meng;J. Chen, and, S. X.Guo. 2010. The promoting role of an isolate of dark-septate fungus on its host plant Saussure a involucrate Kar. Et Kir. Mycorrhiza 20: 127-135.