Antagonism of Trichoderma harzianum and Clonostachys rosea against fungi associated with grapevine decline in Kurdistan region - Iraq

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Abstract:

This study was carried out to test in vitro the efficiency of Clonostachys rosea and Trichoderma harzianum as bicontrol agents against fungi isolated from declining grapevine trees in Iraq. In vitro assays showed a good antagonistic activity of C. rosea against the colony growth of Phaeoacremonium aleophilum and Cylindrocarpon destructans; the values of x coefficient were 0.90 of each with significance differences comparing with antagonism behavior against B. parva (x=1.43). T.harzianum showed highly response against the growth of all tested fungi with x coefficient ranged between 0.50 - 0.65; which was higher than that of C.rosea. The test revealed significant growth inhibition of C.rosea against P.aleophilum, which were 46.60%, 55.17%, 74.33% in the concentrations 5%, 10%, and 15% respectively. No inhibition appeared in the growth of both Botryosphaeria parva and Macrophomina phaseolina .The filtrate of T. harzianum decreased the growth of P. aleophilum to 19% and 19.83% with the filtrate concentrations 10% and 15% respectively. B.parvawas also affected by the concentration 10% and 15% of T harzianum, the inhibition reached to 12.66% and 12.82% respectively. The inhibition in the growth of M. phaseolina ranged between 20 – 28%, but in the C. destrcuctans have recorded the lowest inhibition percentages in the concentration 5% (5.25%) and 10% (6.91%).

Keywords: *Antagonism, biological control, grapevine decline, fungi, Iraq.*

Introduction:

Fungi such as *Botryosphaeria parva, Cylindrocarpon destructans, Macrophomina phaseolina* and *Phaeoacremonium aleophilum* have been found associated with declining grapevine trees. These fungi can cause extensive internal decay with discoloration of the plant tissue (Halleen *et al*.2006; Martin and Cobos, 2007; Savocchia *et al*.2007; Lunque *et al*.2009). In Iraq, *B.parva, C.destructans,*and *P.aleophilum* were reported as main causes of grapevine decline (Haleem *et al*., 2011; 2012; Haleem *et al.* 2013a, b).

Clonostachys rosea (Link: Fr) Schroers, Samuels, Seifert & Games is the anamorph of *Bionectria ochroleuca* (Schw.) Shroers& Samuels. It is a member of family Bionectriaceae and it was previously described as *Gliocladium roseum* (Schroers et al. 1999). *C. rosea* is a common soil fungus that occurs in a broad range of habitats (Sutton et al. 1997). It is a antagonist to several phytopathogens. It decreases the symptoms of *Plasmodiophora brassicae* in canola roots (Lahlali& Peng 2013), *Pythium tracheiphylum* in cabbage (Moller et al. 2003) and of *Botrytis cinerea* in several hosts (Sutton et al. 1997; Cota et al. 2008; Nobre et al. 2005).

The bioactivity of *C. rosea* in reduction Post-emergence infection and increasing seedling survival indicates that this fungus has the capability of protection seedling from the beginning of development of pre-emergence stage, producing a protective effect on germinated seeds. A biocontrol agent by its use of two forms of antagonism: competition on space and nutrition and, more importantly, parasitism of hypha. Considering that both the pathogen and the antagonist presented the similar growth rate, this form of antagonism collapsed pathogenic hyphae, removing it from the substrate and previously colonized tissues (Sutton et al., 1997). Some *Clonostachys* strains act as non-pathogenic endophytes, colonizing host tissues without causing alteration in the plant and thereby eliminating the potential development and sporulation of pathogenic fungal agent (Sutton et al, 2002).Currently, biocontrol products based on *C. rosea* are available in Europe. Prestop (Verdera Oy) which is based on *C. rosea f. catenulate* and GlioMix (Verdera Oy) which is produced from a mix of *Clonostachys* fungi (Jensen *et al*. 2007).

Trichoderma spp. are effective biocontrol agent against several soil-borne fungal plant pathogens (Howell,2003; Benitez *et al*. 2004;Maher *et al*.2014) and some its species are also known for their capability to enhance systemic resistance to disease plant (Harman *et al.* 2004). The antagonistic mechanism by *Trichoderma* can occur by means of several

methods such as nutrient competition, antibiotic production, and mycoparasitism (Schirmbock *et al.* 1994; Maher *et al*.2014). Mycoparasitism has been proposed as the major antagonistic mechanism displayed by *Trichoderma* spp. (Kubiecek et al. 2001; Vinale *et al*. 2008). After host recognition, *Trichoderma* spp. attaches to the host hyphae via coiling, and penetrate the cell wall by secreting cell wall-degrading enzymes (Viterbo *et al*. 2002). Mycoparasites producing cell wall degradation enzymes which permit them to bore holes in to its fungi host and extract nutrients for their own growth. Most phytopathogenic fungi have cell wall that contain chitin as a structural backbone arranged in regularly ordered layers and β-1,3-glucan as filling materials arranged in an amorphic manner. Chitinases and β -1,3-glucanases have been found to be directly involved in the mycoparasitism interaction between *Trichoderma* spp. and their host (Kubicek *et al*. 2001; Geraldine at al. 2013; Steindorff *et al*. 2014).

The objective of this study was to evaluate the *in vitro* antagonistic activity of *T. harzianum* and *C.rosea* against fungi associated with grapevine decline in Iraq.

MATERIALS AND METHODS

Antagonistic fungi and pathogens

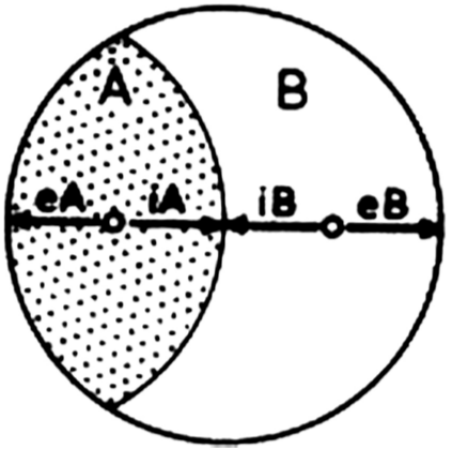
Clonostachys rosea was isolated from grapevine shoot samples collected from Salahdin province, middle Iraq (Abdullah *et al.*2015). A local isolate of *T.harzianum* was obtained from mycology bank, Department of plant protection, University of Duhok, Kurdistan Region, Iraq.

Isolates of *B.parva, C.destructans, M.phaseolina* and *P.aleophilum* were isolated from shoot or roots of grapevine plants showing decline during previous studies (Haleem *et al*.2011; 2012; 2013a, b).

1. In vitro antagonistic activity

Mycelial disks (5 mm in diameter) of each of *B.parva ,C. destructans, P.aleophilum* and *M.phaseolina* were separately placed on one edge of Petri dishes containing potato dextrose agar (PDA) medium and mycelial disks (5 mm in diameter) of each of bioagents *T. harzianum* and *C.rosea* were placed on the opposite side of Petri dishes . The Petri dishes were incubated at 25ºC in darkness and antagonistic activities were evaluated after 15 days based on radial growth of pathogen according to Jouan *et al.* (1964), using the following equation:-

X = iA/iB × eB/eA



i = Inner radius
e = Outer radius
A = Test Fungus
B = Antagonistic fungus
X < 1 = antagonism
X = 1 no influence
X > 1 = no antagonism

In this formula the coefficient X is the value of the quotient of inner radius (i) and outer radius (e) of the test-fungus (A) and the antagonistic fungus (B).

In case of x = 1, no influence has been expressed between the two tested fungi; when x < 1, the antagonism is stronger when the coefficient x value is lower or close to 0 (zero); when x > 1, the tested isolates prove no antagonism against the checked pathogens.

2. In vitro toxicity activity

Mycelial disks (5 mm diam.) of each bioagents grew on ¼-strength PDA medium were cut and separately inoculated into 100 ml flasks containing potato dextrose broth and incubated in rotary shaker incubator (120 rpm)at 25 ± 3ºC for10 days. The cultures were then filtered through 0.22 mm Millipore filters (Millex GP filter unit, Cormghwohill Co., Ireland) and 15 ml of this filtrate were added into sterile Erlenmeyer flasks containing PDA medium cooled at 45ºC and then poured into Petri dishes. After medium solidifying, mycelial disks(5 mm diam.) of each of *B. parva, C.destructanse, P. aleophilum* and *M. phaseolina* derived from actively growing colonies were placed on the center of medium plates and incubated at 25 ± 3ºC (Dennis and Webster,1971). Control was Petri dishes containing PDA medium with the mycelia disk of pathogenic fungi only. Three filtrates concentrations 5%, 10% and 15%) were also prepared. The Petri dishes were incubated in the

same temperature and dark conditions. Radial growths of fungi were measured after 7 and 14 days. Inhibitory percentages were calculated following formula:

n= (a-b)/a×100; a is the colony area for untreated control and b is the colony area for treated fungus.

Statistical analysis

The factorial experiment was set in a Completely Randomized Design (CRD) in three replications with three plate/ replicate. Data were analyzed using Multiple Range Duncan Test.

RESULTS AND DISCUSSION

1) In vitro antagonistic activity

The *in vitro* assays showed mainly a good antagonistic activity of *C. rosea* against the colony growth of *P.aleophilum* and *C.destructans*; the values of x coefficient were 0.90 of each with significance differences comparing with antagonism behavior against *B. parva* (x=1.43) (Table1). *T.harzianum* colonized a large area of the culture medium in the plates as result of the speed of their mycelial growth and showed highly response against the growth of all tested fungi with x coefficient ranged between 0.50 - 0.65; which was higher than that of *C.rosea*. Furthermore, it was observed that *Trichoderma* strain sporulated abundantly when growing over the pathogenic colony; thereby indicate that it can be highly competitive for space and nutrient.

Bell *et al.* (1982) indicated the differences in the responses of antagonism by *Trichoderma* against different genera of pathogens due to several genes of both the antagonist and pathogen involved in regulating the different levels of antagonism.

Gajera *et al.* (2012) reported that the highest chitinase activity was significantly recorded by*T. koningi* MTCC 796 followed by *T. harzianum* NABII Th 1 in degrading the cell wall of *M. phaseolina*. These results was also in confirmation with the finding of Ramezani (2001) who documented that *T. harzianum* significantly inhibited the growth of *M. phaseolina.*

Table (1): The antagonistic activity of T.harzianum and C.rosea against pathogenic fungi based on x coefficient

Bioagents / *Fungi*	*C.rosea*	*T.harzianum*
B. parva	1.43 a*	0.50 d
C. destructans	0.90 bc	0.60 cd
P.aleophilum	0.90 bc	0.65 cd
M.phaseolina	1.13 ab	0.64 cd

*Means followed by different letters are significantly different based on Duncan's Multible Range test (p=0.05).

1. In vitro toxicity activity

The interaction between the filtrate of two bioagents and mycelial growth of pathogenic fungi after 14 days of incubation regardless their concentration (Fig.1) showed highly inhibitory effect of *C.rosea* filtrate on the mycelial growth of *P.aleaphilum* and *C.destructans* by 58.70%, 26.07% respectively with significance differences from other treatments. While *T.harzianum*, inhibits the mycelial growth of *M.phaseolina*, *P.aleophilum* to 23.47%, 22.28% and 21.43% respectively. The capability of *C. rosea* for mycoparasitism of hyphae, sclerotia, and other fruiting bodies in different types of fungi is well known (Sutton *et al.,* 1997). This species also produces fungal inhibitors and cell wall degrading enzymes, induce the loss of turgor and cause lyses of pathogenic hyphae (Papavizes, 1985; Sutton *et al*., 1997). *C. rosea* showed ability for production chitinase and glucanase enzymes as well as secondary metabolites (peptides) (Xue 2003; Pisi *et al*. 2006; Roberti *et al*. 2008; Chatterton & Punja 2009; Rodríguez *et al*. 2011). It was also reported to grow endophytically in cucumber (Chatterton *et al.* 2008); and to induce expression defense genes in wheat and canola (Roberti *et al*. 2008; Lahlali& Peng 2013).

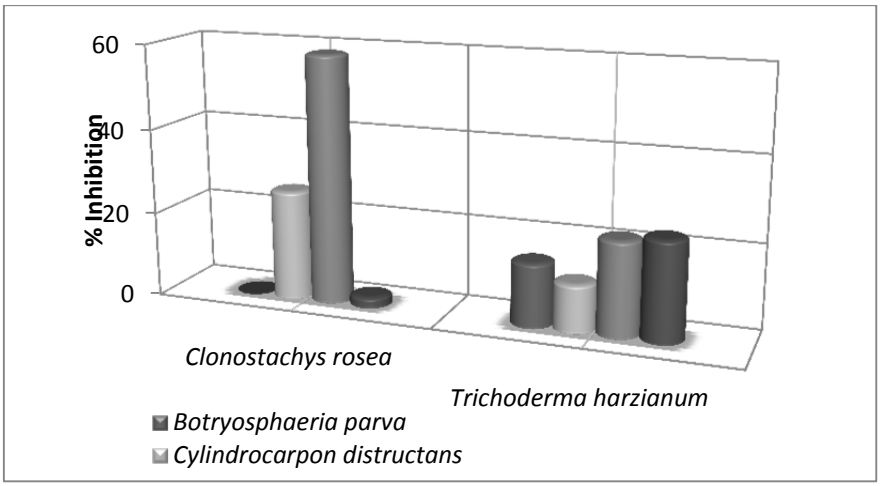


Fig. (1): The interaction between the filtrate of two bioagents *T.harzianum* and *C.rosea* against pathogenic fungi regardless their concentration.

Table (2) showed the effect of different concentrations of bioagents filtrate on inhibition of the growth of pathogenic fungi. The results indicated significant growth inhibition of *C.rosea* against *P.aleophilum*, which were 46.60%, 55.17%, 74.33% in the concentrations 5%,10%, and 15% respectively. This bioagent also inhibit the growth of *C. destructans* to the 32.63% in the concentration 15%. No inhibition appeared in the growth of both *B.parva* and *M.phaseolina* particularly with the concentrations of 5% and 10%.

The filtrate concentration of *T. harzianum* was also affected on the *in vitro* growth of pathogenic fungi. It decreased the growth of *P. aleophilum* to 19% and 19.83% with the filtrate concentrations 10% and 15% respectively. *B.parva* was also affected by the concentration 10% and 15% of *T harzianum*, the inhibition reached to 12.66% and 12.82% respectively. The inhibition in the growth of *M.phaseolina* ranged between 20 – 28%, but in the *C. destrcuctans* has recorded the lowest inhibition percentages in the concentration 5% (5.25%) and 10% (6.91%).

Maximum growth reductions in all studied pathogenic fungi were observed with increasing concentrate. Similar effect have been observed by Anita *et al.* (2012) when studying the effect of secondary metabolites secreted by *T. atroviride* at different concentrations and found rapid decreasing observed in the linear growth of the pathogen with increase concentration of the metabolites. Effect of filtrate concentrations of *T. harzianum* on mycelial growth of test pathogenic fungi was revealed that the aqueous extracts of *T. harzianum* reduced the mycelial growth of the most target fungal pathogens. Dennis and Webster (1971) and Jinantara (1995) showed that culture filtrate of *Trichoderma* contain inhibitors against many microorganisms. The antibiotics produced by *T.harzianum* were pyridone, anthraquinones, butenolides, isonitrin D and F, trichorzianines and furanone (Claydon *et al.* 1987; Ordentlich *et al*., 1992). Doi and Mori (1994) successfully proved the antifungal potential of culture filtrates of two *Trichoderma* spp., on wood decay fungi. Papavizas (1982) demonstrated that the culture filtrates of various *T. harzianum* strains suppressed growth of the white rot pathogen**.**

Table (2): The antagonism of different concentrate of Bioagents filtrates of T.harzianum and C.rosea against pathogenic fungi.

Pathogenic Fungi	Bioagents concentrates					
	*C.rosea*filtrate			*T.harzianum*filtrate		
	5 %	10%	15%	5 %	10%	15%
B.parva	0.00g*	0.00g	0.00g	7fg	12.66fg	12.82fg
C.destructans	23.88c-e	21.69d-f	32.63c	5.25fg	6.91fg	21.70d-f
P.aleophilum	46.60b	55.17b	74.33a	18ef	19d-f	19.83d-f
M.phaseolina	0.00g	0.00Gg	0.00g	20d-e	22.08d-f	28.35cd

* Means followed by different letters are significantly different based on Duncan's Multible Range test (p=0.05).

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كارتێكرنا *Trichoderma harzianum* و *Clonostachysrosea* بەرامبەر ئەگەرێت تێكچوونا مێوێت تری ل هەرێما كوردستانێ – عیراق .

كورتیا لێكولینێ:

ئەڤ ڤەكولینە هاتە ئەنجامدان ب مەرەما دیاركرنا كارتێكرنا دوو گەروان *Clonostychysrosea* و *Trichodermaharzianum* دژی ئەگەرێت تێكچوونا مێوێت تری ل عیراقێ .

پشكنین ل لابوریێ دا دیاركر كو گەروێ *C. rosea* كارتێكرنەكا بەرز ل سەر هەردوو ئەگەرێن تێكچوونا مێوێت تری كر ئەوژیك*Phaeoacremoniumaleophilum* و *Cylindrocarpondestructans* وریژا X گەهشتە 0.90 بو هەر ئێكێ دگەل جیاوازیەكا بەرچاڤ دگەل *B. parva* (X=1.43).

T. harzianum كارتێكرنا بەرچاڤ دژی هەمی گەروێن هاتینە بكارئینان كر (X=0.50 – 0.65) وب ئەنجامێت باشتر بەرامبەر *C. rosea*.

ڤەكولینێ دیاركر كارتێكرنا را شحێ گەروێ *C. rosea* دژی ئەگەرێ تێكچوونا مێوێت تری *P. aleophilum* بریژا 46.60% و 55.17% و 74.33% دگەل بكارئیناناC.rosea بریژا 5%و 10% و 15% لدویف ئێك. هەروەسا كارتێكرنا ل سەر گەروێBotryosphaeriaparva و *Macrophomainaphaseolina* نەبوو راشحێ گەروێT.harzianum كارتێكرنا بەرچاڤ ل سەر مایسیلومێ گەروێ*P. aleophilum* هەبو بریژا 19% و 19.8% ل دەمێ هاتیە بكارئینان بریژا 10% و 15% ل دویف ئێك. هەروەسا كارتێكرن ل سەر *B.parva* هەبوو بریژا 12.66% و 12.82%ل دەمێ هاتیە بكارئینان بریژا 10% و 15%. كارتێكرن ل سەر مایسیلومێ*M. phasiolina* ل ناڤ بەینا 28% (20% - بوو.*C. destructans* كێمترین كارتێكرن ل سەر بوو ل ریژا 5% (5.25%) و10% (6.91%) .

المستخلص:

أجريت هذه الدراسة لاختبار كفاءة استخدام كل من الفطر *Clonostachys rosea* والفطر *Trichoderma harzianum* كعوامل حيويه ضد بعض الفطريات التي عزلت من أشجار العنب التي ظهرت عليها صفة التدهور. أوضحت النتائج ان الفطر *C. rosea* نشاط جيد ضد نمو مستعمرات الفطرين *Phaeoacremonium aleophilum* و*Cylindrocarpon destructans*وكانت قيمة معامل X تساوي 0.90 لكلا الفطرين مع اختلاف معنوي مع الفطر*Botryosphaeria parva* . أظهر الفطر*T. Harzianum* مقاومه عالية ضد نمو جميع الفطريات المختبرة وقيمة X تتراوح مابين

(X=0.50 – 0.65)والتي كانت اعلى من الفطر *C. rosea* . أظهر الاختبار انللفطر *C. rosea* تاثير معنوى ضد الفطر *P.aleophilum*وصلت 46.6% و 55.17% و 74.33% عند التراكيز 10% , 5% و 15% على التوالي. لم يظهر اي تثبيط ضد الفطرين *B. parva* و *Macrophomaina phaseolina* . راشح الفطر *T. harzianum* ثبط نمو الفطر *P.aleophilum* بنسبة 19%و 19.83% عند تركيز 10% و15%على التوالي ثبط الفطر *B. parva* بالتركيز 10% و 15% من الفطر *T. harzianum*وبنسبة تثبيط 12.66% و 12.8% على التوالي. اما بالنسبة لتثبيط نمو للفطر *M. phaseolina* تراوحت ما بين 20-28% . بينما في الفطر *C.destructans* سجل اقل نسبة تثبيط في التراكيز (5.25%) 5% و (6.91%) 10%