

Evaluated efficiency of *Trichoderma viridi* and Sewak (Arak) Powder to Control The Root Rot Fungi of *Pistacia khinjik* Seedlings

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Abstract

The current study showed the effect of *Trichoderma viridi* on growth inhibition of *Pistacia khinjik* seedling root rot fungi which usually used in biological control with pathogens and using four concentrations of sewak powder 0 , 10 , 20 , 30 gm/l represented control treatment .

Isolation results showed that the four fungi *Fusarium chlamydosporium* had 50% in frequency ratio then the fungus *Rhizoctonia solani* and *Macrophomina phaseolina* had 16.6% then *Fusarium culmorum* which had 8.3 % .

The results of biological control of tested fungi by *Trichoderma viridi* showed that all fungi were affected by biological resistance in different degrees , the degrees of inhibition was the first and second degree so those two degrees make the fungi with high parasitic abilities against pistasio root rot fungi .

The results of bioassay of sewak powder in several concentrations showed that in corporeal differences in the efficiency of sewak powder effect on root rot fungi control as compared with biological control for the same fungi where the fungi affected in different ratios, *F. chlamydosporium* inhibited a concentration 10gm/l of sewak powder which was maximum inhibition ratio 77% , *Rhizoctonia solani* , *F. culmorum* , *M. phaseolina* showed inhibition ratios 72 , 49 , 28 % respectively , *Rhizoctonia solani* showed a height ratio at two concentrations 20 and 30 gm / l , inhibition ratio were 40 and 55 % , while the other fungi had no effect even with high concentrations of sewak powder .

R. solani was more fungi affected with concentration stepped comparative with others fungi .

Key words : bio control agent , root rot , sewak , *Trichoderma* , *Pistacia* , Arak .

المستخلص

شملت الدراسة تقييم فعالية فطر المقاومة ومسحوق شجيرة الاراك (السواك) وبأربعة تراكيز (صفر و 10 و 20 و 30) غم/لتر في تثبيط نمو فطريات مرض تعفن جذور حبة الخضراء نوع *Pistacia khinjik* والمستخدم عادة في المقاومة الحيوية للفطريات الممرضة ، تبين من نتائج العزل من شتلات حبة خضراء مصابة بتعفن جذور ظهور الفطر *Fusarium chlamydosporium* بنسبة عزل 50 % تلاه الفطرين *Rhizoctonia solani* و *Macrophomina phaseolina* بنسبة عزل 6 و 16 % ثم الفطر *Fusarium culmorum* بلغت 3 و 8 % .

اظهرت نتائج المكافحة الحيوية للفطريات المختبرة بالفطر *Trichoderma viridi* بأن جميع الفطريات تآثرت بالمقاوم الحيوي وبدرجات متفاوتة حيث اظهرت درجة تثبيط من الدرجة الاولى والثانية وكلا الدرجتين تعدان الفطر من الفطريات ذات القدرة التطفلية العالية ضد فطريات تعفن جذور حبة الخضراء ، كذلك تبين من نتائج الاختبار الحيوي لمسحوق الاراك ولعدة بتراكيز وجود تباين في كفاءة تأثير مسحوق الاراك في مكافحة فطريات تعفن جذور حبة الخضراء مقارنة مع المكافحة الحيوية لنفس الفطريات اذ تآثرت الفطريات بنسب مختلفة واطهر الفطر *F. chlamydosporium* اعلى نسبة تثبيط بمسحوق السواك عند التركيز الاول من المسحوق (10 غم / لتر) حيث بلغت 77% تلاه الفطريات *M. phaseolina* و *F. culmorum* و *Rhizoctonia*

solani وبنسب تثبيط (72 و 49 و 28 %) على التوالي كذلك اظهر الفطر *Rhizoctonia solani* اعلى نسبة تثبيط (40 %) بفعل مسحوق السواك عند التركيز الثاني من مسحوق السواك (20غم / لتر) في حين لم تظهر بقية الفطريات تاثرا بمسحوق السواك عند نفس التركيز ، واطهر الفطر *Rhizoctonia solani* اعلى نسبة تثبيط بفعل مسحوق السواك عند معاملته بالتركيز الثالث (30 غم / لتر) حيث بلغت 55% ، في حين لم تظهر بقية الفطريات أي تاثر بمسحوق السواك حتى في التراكيز العالية من مسحوق السواك كما تميز الفطر *Rhizoctonia solani* بكونه اكثر الفطريات تأثراً بتدرج التركيز مقارنة بالفطريات الاخرى المختبرة .

Introduction

Trichoderma viride is a very promising agent that can be used against soil borne plant parasitic fungi (18) . The fungal pathogens play a major role in the development of diseases on many important fields and horticultural crops; resulting in severe plant yield losses. Intensified use of fungicides has resulted in accumulation of toxic compounds potentially hazardous to human and environment also in the build up of resistance of the pathogens. In order to tackle these national and global problems, effective alternatives to chemical control are being employed. Biological control by an antagonism is a potential, non-chemical and ecofriendly approach for managing plant diseases (9) . *Trichoderma* is one of the common fungal biocontrol agents being used world wide for suitable management of various foliar and soil-borne plant pathogens (14) . Biocontrol agents like *Trichoderma* are acclaimed as effective, ecofriendly and cheap, nullifying the ill effects of chemicals. Therefore, of late, these biocontrol agents are identified to act against an array of important soil borne plant pathogens causing serious diseases of crops (18) .

Present study was carried out to evaluate grains such as rice, wheat, pulses and maize at different temperatures, and naturally available solid media such as carrot, ladyfinger, jack seeds, rice husk, and saw dust for the biomass production of *T. viride* . For mass multiplication of bio agent through liquid state fermentation technology an enormous quantity of spore biomass is needed. Various substrates like sugarcane baggase, fruit juice waste vegetable waste, rotten wheat grains etc. are being used for mass multiplication of *Trichoderma viride* with various degrees of success (15) . The forest trees affect for numerous of fungus diseases one of them is the root rot diseases , fungi of root rot forest trees seedlings attach the seedling causing root rot in it , disease symptoms involved discoloration of leaves and change to brown color , symptoms showed at needle leaves of *Pinus brutia* from down to up , while root system have a black color , death of root hairs and side roots (20) . The fungi causing root rot whose isolated from Ninawa nurseries were *Rhizoctonia bataticola*, *Fusarium solani* , *Macrophomina Phaseolina* .

The noble prophet rubric was tripotanted to assurance of Sewak importance , there were more than hundred prophet hadeths (8) of Mohammed prophet (Sala allho al-yhi wa salam) stimulated about sewak using and assurance in numerous speeches whose at prophet hadeth books .

Researchers were mentioned about the antibacterial and antifungus of Sewak (Arak roots) , Bioassay of sewak extracts and Sewak powder on fungi showed that the

Sewak was antifungal for seed pathogens alcoholic extract of Sewak had high inhibition on *Fusarium* (5) .

Material and methods :

A field survey made on *Pistacia khinjik* seedling root rot disease at Forestry Dept. Nursery / College of Agriculture and Forestry / University of Mosul / Iraq by calculating disease infection percentage depending on disease symptoms , this survey was at autumn / 2013 , the samples was taken randomly by choosing pistasio seedlings which infected by root rot diseases , and its ages was 1-2 years then the infected samples translated to laboratory for fungi isolation .

Isolation and diagnosis of pathogenic fungi :

Isolation from Pistasio (*Pistacia khinjik*) seedlings by root rot disease were planting in Nylon bags in transplanting beds in Forestry Dept. / College of Agriculture and Forestry / Univ. of Mosul , Isolation were done after translating to laboratory , samples were put under current water for four hours to clean seedlings from soils then the samples of seedlings root drying and cutting into small pieces (1 cm length) , surface sterile had by 1% sodium hypochloride (19) for four minutes then dried by filter papers , samples of roots pieces translated to sterilize petri dishes containing potato dextrose agar (PDA) medium and supplied with antibiotic (50mg/l of streptomycin sulfate concentration) , the root pieces put for each petri-dish were placed in petri-dish with 4 pieces / petri-dish as a means , each petri-dish were placed incubated at 25+- 5 C , there were three replications , Results were taken by calculate the number of fungi colonies / petri dish , then the values changed to percentage ratio for isolated fungi , identification of fungi were made for fungi to genus order according to International and identification keys by (10) and untell species order according to on taxonomies (1,2,7,11,13,17,21,23,25,26).

Control of root rot :

1- Biological control :

Biological control was studied for biological agent (*T. viridi*) against the pathogenic fungi (*F. chlamyosporium* , *R. solani* , *F. culmorum* , *M. phaseolina*) by dual culture technique by dividing each Petri-dish (9.0 cm diameter) which contained potato dextrose agar to two equal parts by marker pencil , the first half part inoculated with a disk of colony (0.5 cm in diameter) at a center point , these colonies were taken from edge of modern parasite colony fungi at four days old , sterile cork borer were used for this work , the second part of petri dish was inoculated by 0.5 cm in diameter of bio control factor (*T. viridi*) which were taken from edge modern colony , the treatments were replicated for three times with the three tested fungi , control treatments were inoculated by the two parts of petri dish and these were made separately for the two tested remembrance fungi , petri dishes were incubated at 25+- 2 C , degrees of parasitism were calculated by (11) which was contain fungi with five degrees (table 1) present through the degree 1,2 have high parasite ability .

Table (1) Bell meter for parasitism ability of *T. viridi* fungus .

Degree	Description
1	Antagonistic fungi covered all petri dish
2	Antagonistic fungi cover 2/3 of petri dish
3	Antagonistic fungi and parasite each of the cover 1/2 of petri dish
4	Parasite fungi cover 2/3 of petri dish
5	Parasite fungi cover all petri dish

* The fungi (Bio control agent) taken from Dr. Khaled Hasan Taha / Ass. Prof. of plant pathology / plant Protection/College of Agri. and Forestry .

2 – Control by Arak powder :

Bioassay of Arak powder :

Primary material preparation:

Pieces of Sewak were supplied and had taken from Sewak which imported from Sudia Arabia Kingdom and were present in our markets , the nylon covers of sewak was lifted then the Sewak was cut into small pieces , dried under laboratory for 24hrs. Then the pieces put in electri drill , Samples of Sewak powder (25gm) was taken to supply three containing samples of Sewak powder weights, the weights were 1 , 2 , 3 gm with concentrations 1% .2%, 3% , these concs. was mixed directly with PDA medium , then medium was sterilized in autoclave , these concentrations mixed directly with PDA medium separately , then sterilized in autoclave pressure , The media put in sterilize Petri dishes and incubated each petri dish by disk of fungi colony which had 0.5cm in diameter of tested fungi separately , The replications were made to each conc. and for all fungi also separately , the fungi incubated at 25 -27C , Results recorded after 4 days by calculated the means of two orthogonal diameters then , Inhibition percentage was calculating with this law(13) :

$$\text{Inhibition \%} = \frac{\text{Control growth mean} - \text{treatment growth mean}}{\text{Control growth mean}} \times 100$$

The experiment was carried as a completely randomized Design (CRD) . Data were analyzed by SAS Program and mains were compared using Duncan multiple Range Test (D.M.R.) (6) .

Results and conclusion :

Field survey:

A field survey results from pistasio root rot disease showed that the infection percentage was 50% during the autumn 2013 .

Isolation :

Isolation from pistasio seedlings infected by root rot diseases were planted in Forestry nursery , the results of isolation as illueterated in Table 2 showed that the fungus of *F.chlamydosporium* had 50% of isolation percentage then the fungus *R.solani* , *F.culmorum* and *M.phaseolina* had (16.6)% , while *F. chlamydosporium* was more isolated fungi which caused root rot of pistasio seedlings where it showed maximum isolation ratio then the others fungi were showed less isolation ratio .

The studies about the previous fungus assure that these fungi had high parasitism ability against the seedlings of forest trees . There were appliance species of *Fusarium* and species of *Rhizoctonia* in addition to *M. phaseolina* were isolated from Zawita pine (*Pinus brutia*) , Cupressus and Casuarina seedlings th at were grown in Ninavah and Hammam alaleel nurseries (20) .

Table 2 :Isolation percentage from pistasio roots seedlings which affected by root rot disease

Fungi	Isolation (%)
<i>Fusarium chlamydosporum</i>	50
<i>Rhizoctonia solan</i>	16.6
<i>Macrophomina phasiolina</i>	16.6
<i>Fusarium culmorum</i>	8.3

1 –Bioassay control test :

T. viridi used as a biological agent control and this depended on (11) .

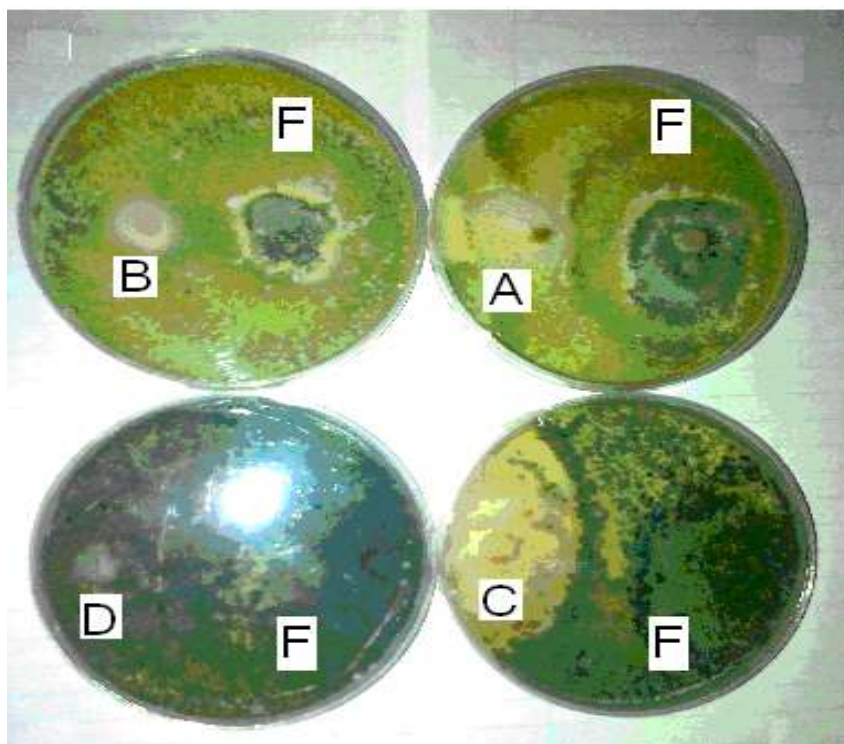
The results of bioassay control test of pistasio root rot fungi illustrated in table 3 . The biological agent fungi showed a high parasitism and inhibition ability for all fungi and as in table (3) and the number of ability was from the first degree (1). for *F. culmorum* and *M. phaseolina* the growth means were (15 , 5) mm . Then the fungus *F.chlamydosporum* and *R. solani* showed number 2 of inhibition degree whom growth means were 25mm and 27 mm as the controller fungi growth effects.

The results were compared with previous scale that the two degrees (1 , 2) were the controller fungi *T. viridi* one of the fungi that had high parasitism ability so this fungus is possible to be used in bio control against parasitic fungi .

Table 3 : Bio control of pistasio root rot dependent on (Bell et al , 1982) .

Fungi	Growth Means (mm)	Controller fungi	Growth Means (mm)	Inhibition degree
<i>Fusarium chlamydosporum</i>	25	<i>Trichoderma viridi</i>	65	2
<i>Fusarium culmorum</i>	15	=	75	1
<i>Rhizoctonia solani</i>	27	=	63	2
<i>Macrophomina phaseolina</i>	5	=	85	1

Previous studies showed that a high ability of *T. viridi* to inhibit numerous fungi *R. solani* (15) .



Picture 1 : Bioassay of *T. viridi* against the fungi :
A – *F. chlamydosporium* B- *F. culmorum* C- *R. solani* D- *M. phaseolina* F- *T. viridi*

2 - Control by using Sewak powder :

Four concentrations were used of Sewak powder with control treatment and this involved the conc. (0 , 10 , 20 and 30 gm/L) . The table (3) showed that the tested fungi were affected by different ratios , the fungus *F. chlamydosporium* showed a maximum in corporeal inhibition ratio with first conc. of sewak powder (10mg /L) which inhibition ratio was 77% . Then the fungi *M. phaseolina* , *F. culmorum* and *R. solani* had inhibition ratio (72 , 49 , 28 %) upon. These ratios were different between them in corporeal , the fungi *R. solani* appeared maximum in corporeal difference in inhibition ratio by Sewak powder at the second conc. of Sewak powder (20mg/ L) then *F. culmorum* which had in corporeal difference with control treatment but left over fungi did not appeared any inhibition by Sewak powder with the same conc , also there were no differences with control treatment , the fungi *R. solani* was showed maximum in corporeal inhibition ratio by Sewak powder treatment when treated with third conc. (20mg/L) which reached to 55% , then *F. culmorum* which had in corporeal difference with control treatment about another fungi had no in corporeal effect by Sewak powder treatment till it when it was used at high conc. of Sewak powder and also there was no in corporeal different with control treatment . Table (3) also showed that the fungi *R. solani* was the most fungus affected by concentration hierarchy where

had inhibition ratio between them and with control treatment also but the another fungi had incorporeal difference effect with conc. hierarchy , this perhaps the custom cause of the difference fungus control to chemical constants which Sewak contains . The previous studies (1) of Sewak chemical analysis showed that it was contain of glycosides and this perhaps have stimulation role for growth in spite of inhibition where the inhibition compounds had conjugated with sugars and inhibition effect after its separation from the sugar part and it is changed to free pole roots (aglucans) .

Table (4) Effect of different concentrations of Sewak powder in growth of fungi caused pistasio root rot disease

Fungi	Concentrations (gm / L)			
	10	20	30	0
	Inhibition growth %			
<i>R.solani</i>	28 f	40 e	55 c	0 h *
<i>F. culmorum</i>	49 d	9 g	9 g	0 h
<i>F. chlamyosporum</i>	77 a	0 h	0 h	0 h
<i>M. phaseolina</i>	73 b	0 h	0 h	0 h

* The numbers which have the same letters have no significant difference .

The researchers showed the the importance of Sewak which manufactured from Arak (*Salvadora persica*) roots in mouth sanitation from bacteria (*Streptococcus faecalis*) where is one of antibacterial as well as , it is antifungal against many of fungi especially the fungus *Candida albicans* (3) . These studies showed that the concentration 200mg / ml of Sewak extract inhibited to this fungi , the results of researches mentioned that the testes of Sewak extract and its activity against primary granular microbes *Echinococcus granulosus* which present sheep liver (4) , the inhibition activity of Sewak constants perhaps related to tannin , saponin and flavonoids that are presented (1) .

All these compounds were present in Sewak powder substance , and these substance perhaps dissolved in fungal media which make the inhibition active for fungi and the researches assure presence this compounds in Sewak especially after its chemical analysis .

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