Biological control of fig root rot disease

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Abstract

The study aimed to isolate and diagnose pathogens of fig root rot disease in addition to evaluation effect of the biological control agents *Trichoderma harzianum* and *Penicillium cyclpium* in control of the pathogens of fig root rot. It was found fungi accompanying the roots of fig plantlets. *Fusarium solani* was the most frequent pathogenic fungus, followed by *Rhizoctonia solani*. The results showed a high effectiveness of biological agents in inhibiting the growth of pathogenic fungi, as the percentage of inhibition of the fungus *T. harzianum* against the fungus F. solani reached 100%. The percentage of inhibition against the pathogenic fungus R.solani was 89.99%. As for the bioagent *P. cyclopium*, the results displayed its high antagonistic ability against the two pathogenic fungi. As well as, the test results revealed the antagonistic ability of the microorganism EM-1 formula against the pathogenic fungi *F.solani* and R.solani that cause the root rot disease of fig plantlets.

Keywords: *Penicillium cyclopium, Trichoderma harzianum*, Fig, Root rot diseases.

Introduction

Fig fruits are characterized by their high mineral content about four times of iron and copper compared to fresh and dry vegetables and fruits [1]. They are also rich in vitamin E and antioxidants such as phenols that abound in dark-colored varieties, as well as vitamin C necessary for iron absorption [2]. The statistics of the International Food and Agriculture Organization of the United Nations indicated that the area planted with figs in Iraq for the year 2017 amounted to 463 hectares with a production capacity of 3349 tons [3]., The number of fig trees in Iraq have reached 412,859 trees, and the average productivity of one tree reached 22.58 kg / tree, and the total production is 9.322 tons and its relative importance to production has reached 1.09, and the rate of change in production has reached 0.62 . Figs are like other plants that are exposed to many agricultural pests, such as insects and pathogens, which cause diseases of, roots rot, wilt and others. The progression and success of root rot pathogens depends on the availability of the conditions of the epidemiological triangle, [4,5]. Root rot diseases caused by some fungi are among the most important and widespread endemic diseases in the soil, and the amount of loss resulting from infection is related to a greater degree to the density of the pathogenic fungus inoculum

present in the soil and the presence of environmental factors, sensitive hosts and planting date [6] and [7]. Because of the large number of diseases that affect plants in general, farmers have taken the method of control by using chemicals to avoid infection by plant pathogens. However, we cannot consider them as a strategic solution, as their uses have led to many environmental and health problems for humans and animals. Besides the development of new pathogenic strains that are resistant to the effect of these pesticides [8,9,10]. Recently, attention has been focused on biological control factors through the application of microorganisms that are not pathogenic to plants and inhibit the pathogenic organisms in the cultivated soil without affecting the remaining groups of microorganisms. These organisms have different mechanisms, such as competition for nutrients and direct parasitism on pathogens, as well as the production of secondary metabolites such as mycotoxins and antibiotics, and induction of systemic resistance in plants [11,12]. Among those microorganisms used in biological control are *Trichoderma* spp for its ability to improve root growth and development, resistance to various environmental conditions, and an increase in crop yield and nutrient uptake [13] and [14]. Additionally, the biological preparation EM-1 was used as one of the best agents used in preparing bokashi biofertilizer, which was used against fungal and bacterial pathogens due to the microorganisms contained in this preparation that compete with the pathogen and produce secondary metabolites, growth regulators and antifungal materials during its growth. It has a role in stimulating the systemic resistance of plants against many pathogens [15,16]. The results of adding the biological preparation EM-1 showed a high inhibitory activity and reduced the rate and severity of infection to the pathogens of seed rot and plantlet death of eggplant caused by the pathogenic fungi *R. solani* and *F. solani* [17]. Due to the lack of studies related to the application of biological control agents that keep pace with modern agricultural directives in combating the root rot disease of the fig plant and the importance of this disease. The study aimed to isolate and diagnose some pathogens of fig root rot disease Moreover, the study planned to evaluate the effectiveness of biological factors *Trichoderma*, *Penicillium cyclpium* and EM1 against the pathogens of fig root rot under laboratory conditions.

Materials and Methods

Isolation and identification of fungi accompanying the roots of fig plantlets infected with root rot disease

The process of isolation was carried out from each of the samples collected from the infected roots of fig plantlets. The plantlet was gathered from some regions of middle provinces of Iraq (Table 1), which showed symptoms of infection on the day following the survey process. Then it was sterilized by immersing it in a solution of sodium hypochlorate (minor) 1% free chlorine for two minutes, then washed with sterile distilled water for a period ranging between 2-3 minutes, then the water was removed using sterile filter paper and 4 of the pieces were then transferred by sterile forceps to Petri dishes. Plates contain a medium culture of potato dextrose agar

amended with Tetracycline antibiotic at a concentration of 250 mg/liter after sterilization of the medium with an autoclave device, the dishes were incubated at a temperature of 25±1 °C for three days [18]. The different fungal isolates were purified and examined under the compound microscope, and the genera and species were diagnosed with the help of Dr. Ahed Abd Ali, and based on the approved taxonomic keys [19,20,21,22]. The percentage of appearance of the studied fungi was calculated according to the following equation:

%** repeat the fungus in the sample = (The number of fungus appeared in dishes\Total number of pieces used in the sample)x 100.

Detection of pathogenic isolates of *Fusarim solani and Rhizoctonia solani* using cabbage seeds

The pathogenicity of three isolates of *F. solani* and three isolates of *R. solani* was tested according to the method of [23], Petri dishes with a diameter of nine cm were prepared and sterilized with an osmosis device for 20 minutes and supplemented with the antibiotic Tetracycline at a concentration of 250 mg/L, and after solidification of the medium The plates were inoculated in the center with a 0.5 cm drop disc taken from near the edges of the colony of *F. solani and R. solani*. At five days of age, the plates were incubated at a temperature of 25 ± 1 °C for three days after which the seeds were sown (pre-tested for germination) sterilized surface with sodium hypochlorite solution (1% free chlorine) and in a circular motion near the edge of the used plate and at a rate of 10 seeds per plate. Three plates were used for each isolate used as replicates, in addition to the comparison treatment without pathogenic fungi. The plates were placed in the incubator at a temperature of 25 ± 1 °C. Then the results were taken after Seven days, calculating the percentage of germination and according to the following equation:

% Germination = (Number of seeds grown / Total number of seeds) ×100.

Examination the antagonistic ability of *Trichoderma harzianum and Pencillium cyclopiu* against *Fusarium solani* and *Rhizoctonia solani*

The biological fungi *Trichoderma harzianum* and *Pencillium cyclopium* were obtained from the Plant Pathology Laboratory / Mussaib Technical College. The antagonistic ability was tested against selected isolates of *F. solani* and *R. solani* by double culture method, where a 9 cm diameter Petri dish was divided into two equal parts, The first part of the dish was inoculated with the pathogenic fungus, both individually. 0.5 cm disc was taken from the fungus culture at the age of seven days, while the other part of the dish was inoculated with a 0.5 cm disc at the age of seven days from the cultures of the biological control fungi used. The experiment was carried out with three replications. Placing the dishes in the incubator at a temperature of 25±1 °C for a week, then estimating the antagonistic ability according to the scale of [24], which consists of 5 degrees, and the biological agent is considered antago-

nistically effective when showing a degree of antagonism equal to 2 or less with isolates of pathogenic mushrooms.

Test the antagonistic ability of EM1 against pathogenic fungi on PDA medium

The biological preparation EM1 was obtained from University of Al-Qadisiyah / College of Agriculture in a plastic bottle of 1 liter. 90:5:5 (water : EM1: molasses:) Dissolve the molasses well with warm sterile water, then add EM1 solution to it and mix well, and then put it in a warm and dark place away from sunlight for two weeks, during which time the package was opened from the time to another in order for the gas formed to escape until a layer of precipitate is formed at the end of the package [25]. The antagonistic susceptibility of the previously prepared microorganism EM1 preparation against the pathogenic fungi *F.solani* and *R.solan* was tested on PDA culture media by adding the preparation (EM1) grown on the medium using 3 concentrations of 10, 20, 30%, respectively, through Add 10, 20, 30 ml of the EM1 preparation to beakers containing 70, 80, and 90 ml of cooled PDA culture medium, stirring well to ensure a homogeneous distribution of the mixture, then pour it into Petri dishes with a diameter of 9 cm. After the medium solidifies, a 0.5 cm diameter disc is placed. From a culture of pathogenic fungi *F.solani* and *R. solani* growing on PDA culture media and with an age of seven days in the center of the dish. Each treatment was repeated 3 times, leaving 3 dishes without adding the preparation as a comparison. For each fungus, the dishes were incubated at a temperature of 25 + _1 C for 7 days [26]. The growth rate of the pathogenic fungus and the percentage of inhibition were calculated according to the following equation:

% Inhibition = [(R – r)/ R] × 100.

Where, r is the radius of the fungal colony against the bioagents and R is the radius of the fungal colony without the bioagents.

Results and Discussion

Isolation and identification of fungi accompanying the root rot of fig plantlets

It was found through microscopic examinations of the fungal growths that appeared through planting the infected plant pieces on the PDA medium (Table 1)and the presence of types of fungi accompanying the roots of fig plantlets. The fungus *F. solani* was the most frequent type of pathogenic fungus, as its rate of appearance reached between 56.5% and the highest percentage of its appearance reached 34.5%. It was followed by the fungus *R.solani* with an appearance rate of 30.7%, and the highest percentage of its appearance was 47%. The results of the diagnosis showed the presence of many fungi accompanying the roots of fig plantlets, such as *Penicillium* spp. *T. harzianum, Aspergillus nigeria, Alternaria alternate, Sclerotia sclerotium, Helminthosporium* sp, *Botrytis* sp. and *Cladosporium tenuissimum*. These results are in agreement with what [27] found that *F. solani and R. solani* were among

the most frequent pathogenic fungi found in samples of pepper plantlets infected with root rot disease.

Table (1): Fungi accompanying the roots of infected fig plantlets, their locations and their frequency in samples

Fungus	No. Samples	Fungi Appearance rate (%)	
		High ratio of appearance	Appearance rate (%)
Fusarium solani	9-1	56.5	34.3
Pencillium cyclopium	7 ،5 ،6 ،1	15.2	5.2
Rhizoctonia solani Kuhn	9 -1	47	30.7
Alternaria alternate	6,4,3	28.5	9.4
Stemphylium sp	1،4	5.7	3.4
Aspergillus niger	7,5,4,2,1	13.8	3.1
Botrytis sp	2،6	5.6	3.7
Cladosporium tenuissimum	1	2.5	2.1
Helminthosporium sp	1،2،6	5.2	2.6
Sclerotia sclerotium	1،3 ،4	3.5	2.5
Trichoderma harzianum Rifai	1،7,5	13.8	2.3
Fusarium oxysporum	1،4	1.4	1.10

Examination the pathogenicity of isolates of the pathogenic fungi *Fusarium solani* and *Rhizoctoni solani* using cabbage seeds on Water Agar medium

The results of (Table 2) showed that all tested fungi isolates induced a significant reduction in the percentage of germination of cabbage seeds compared to the control treatment in which the percentage of seed germination reached 96.67%. %. Two isolates of *Fusarium solani* Fus1, Fus6, recorded a percentage of 16.67% germination, while the percentages of germination for the rest of the isolates ranged between 40.00, 23.33%, and the results indicated that all tested isolates of the fungus *R. solani* . The isolates varied among themselves in reducing the percentage of germination, where the isolate (Rh1) outperformed which reached 10.00%, followed by isolate Rh6 which had a germination rate of 13.33% while the percentages of germination in the rest of the isolates ranged between 36.67, 23.33%.

Table (2): Detection of pathogenic isolates of *Fusarium solani and Rhizoctoni solani* using cabbage seeds on PDA medium

No.	Region	Isolates	No. germinated seeds	Germination (%)
control			9	96.67
1-	Karbala / Hussainiya	Fus1	2	16.67
2-	Karbala / Hussainiya	Fus2	4	40.00
3-	Karbala / Khan Al-Nass	Fus3	2	23.33
4-	Babylon / Al-Kifl	Fus4	3	26.67
5-	Babylon / Al Siahi	Fus5	3	26.67
6-	Babylon / Bani Muslim	Fus6	1	13.33
7-	Baghdad- Al-Grayat	Fus7	2	23.33
8-	Baghdad- Al-tajeiat	Fus8	2	16.67
9-	Baghdad-Al-Jazeera	Fus9	4	36.67
10-	Karbala / Hussainiya	Rh1	1	10.00
11-	Karbala / Hussainiya	Rh2	4	36.67
12-	Karbala / Khan Al-Nass	Rh3	2	23.33
13-	Babylon / Al-Kifl	Rh4	3	26.67
14-	Babylon / Al Siahi	Rh5	3	26.67
15-	Babylon / Bani Muslim	Rh6	1	13.33
16-	Baghdad- Al-Grayat	Rh7	3	26.67
17-	Baghdad- Al-tajeiat	Rh8	3	26.67
18-	Baghdad-Al-Jazeera	Rh9	3	33.33
L.S.D$_{0.05}$			-	3.50

Each number in the table represents an average of three replicates, Fus = *Fusarium solani* , Rh = *Rhizoctonia solani* , The number near the isolate symbol represents the isolate number

The reason for the variation of isolates in influencing the percentage of germination of cabbage seeds may be due to the difference in isolates in their ability to secrete pectin- and cellulose-dissolving enzymes, phosphatas pectinase, lyasepectin and cellulase in the early stages of infection, and these enzymes play a role in penetrating the host and in its pathogenicity to the fungus, or due to genetic variation. Among the fungi isolates collected from different regions. From the results of this test, the two isolates that reduce the germination of cabbage seeds, which are Fus6, Rh1 isolates, were selected for subsequent tests.

Examination the antagonistic ability of *Pencillium cyclopium and Trichoderma harzianum* against the pathogenic fungi *Fusarium solani* and *Rhizoctonia solani*

The results of (Table 3) test the antagonistic ability of the biological control fungi *P.cyclopium* and *T.harzianum* against the pathogenic fungi *F. solani* and *R. solani* showed a high effectiveness of biological factors in inhibiting the growth of pathogenic fungi, as the percentage of inhibition of the fungus *T. harzianum* against the fungus *F. solani* reached 100% The percentage of inhibition against the pathogenic fungus *R.solani* was 89.99% successively after seven days of double culture compared with the treatment of pathogenic fungi alone, which had a inhibition rate of 0.00%. The antagonistic ability of *T.harzianum* biological control fungus is due to the various mechanisms By which it affects the pathogenic fungus by direct parasitism by wrapping the mycelium of *T. harzianum* around the pathogenic fungus hyphae and penetrating it and absorbing the cellular contents or through the food and place and the secretion of antibiotics and some enzymes that degrade the walls of the cells of pathogenic fungi such as Protase, glucanase and 1,3-B and others [28]. As for the fungus *P. cyclopium*, the results showed its high antagonistic ability against the pathogenic fungi *F.solani and R. solani* on the PDA culture medium. It worked on those isolates with rates of 87.37, 92.22 for each of them sequentially after seven days of double cultivation compared to the treatment of pathogenic fungi. alone, which amounted to 0.00%. This is due to the ability of the fungus P.cyclopium to produce antibiotics, including Viomellein, Penecillic acid, and Vioxanthin, which have an effective role in inhibiting the growth of pathogenic fungi on PDA culture medium, in addition to many secondary compounds that have a high ability to inhibit the growth of many pathogenic fungi. of the plant [29].

Table (3): Antagonistic ability of *Pencillium cycloplum* and *Trichoderma harzianum* against pathogenic fungi *Fusarium solani* and *Rhizoctonia solani* .

Fungus	Colony diameter.cm	Inhibition (%)
Fus	9.00	0.00
Fus+ Th	0.00	100.0
Fus+Pi	1.08	87.37
Rh	9.00	0.00
Rh+ Th	0.90	89.99
Rh+ Pi	0.70	92.22
L.S.D 0.05	0.18	1.85

Each number in the table represents an average of three replicates, Fus = *Fusarium solani* , Rh = *Rhizoctonia solani*. Th= *Trichoderma harzianum*, Pi= *Pencillium cycloplum*

Antagonistic ability of Effective Microorganism EM-1 preparation against pathogenic fungi

The test results of the antagonistic ability of the microorganism EM-1 preparation against the pathogenic fungi *F.solani* and *R.solani* that cause the root rot disease of fig plantlets (Table 4) showed a high antagonistic ability of the microorganism EM1 against the tested isolates of all pathogenic fungi, as the preparation completely prevented their growth. Where the percentage of inhibition was 100% and for all concentrations used 10, 20, 30, respectively. It was stated [16] stated that the mechanism of inhibition of EM1 is due to what it contains of microorganisms that are anti-pathogens, and the microorganisms are characterized by their production of fungi-inhibiting substances such as antibiotics that kill or inhibit the growth of pathogens [25]. The results with what was reached by a previous study [17] that the antagonism of the microorganism preparation EM1 against the pathogenic fungi *R.solani, F.solani,* and *M.phaseolina* that cause eggplant seedling death disease on PDA culture media at concentrations of 15, 10, 5%, respectively, has exceeded The concentration of 15% in the inhibition rates for pathogenic fungi ranged between 94.44-98.15% compared to the comparison treatment in which the percentage of inhibition appeared to be zero.

Table (4): Antagonistic susceptibility of microorganism EM1 against the pathogenic fungi *Rhizoctonia solani* and *Fusariun solani*.

Treatment	Concentration %	Colony diameter.cm	Inhibition (%)
Em-1 + Fs	0	9	0.00
	10	0	100.00
	20	0	100.00
	30	0	100.00
Em-1 + Rh	0	9	0.00
	10	0	100.00
	20	0	100.00
	30	0	100.00
L . S .D 0.05	-	0.3	3.33

Each number in the table represents an average of three replicates, Fus = *Fusarium solani* , Rh = *Rhizoctonia solani*. EM1 = Effective Microorganisms.

Fusarium solani and *Rhizoctonia solani* was the most frequent pathogenic fungus, which were the major causes of Fig root rot disease. The inhibitory activity of EM-1 and the bio-fungi *P.cyclopium*, *T.harzianum* against causes of Fig root rot disease.

References

1) Sadia, H.,M Ahmad,S., Sultana, A.Z.,AbdullahTeong, L., Zafar,L M. and A. Bano,. (2014). Nutrient and mineral assessment of edible wild fig and mulberry fruits. Fruits, 69(2), 159-166.
2) Javed, G.,S.A Majid, S.Khatoon, and A.,Ulfat. (2017). Biochemical and nutritional evaluation of Ficus palmata Forssk. fruits from different ecotype population. Bangladesh Journal of Botany, 46(4), 1277 1283.
3) FAO .(2017). UN Food and org/faoststen/#data/Qc. Agriculture Organization http://www.fao.org/faoststen/#data/Qc.
4) Cleary M, Sturrock R, Hodge J .(2011). Laminated Root Disease- Stand Establishment Decision Aid.Journal of Ecosystems and Managment, North America.
5) Shinohara, N., Woo, C., Yamamoto, N. et al. (2021). Comparison of DNA sequencing and morphological identification techniques to characterize environmental fungal communities. https://doi.org/10.1038/s41598-021- 81996-w Sci Rep 11, 2633.
6) Dar, W. A, M. G. Hassan, P. A. Sheikh, B. Summuna and S. A. Ganaie.(2018). Integrated Disease Management Capsule for Wilt/Root Rot Complex of Chili. Int. J. Curr.Microbiol.App. Sci. 7(1): 1253-1261.

7) Lahuf, A.A. (2019). First report of *Fusarium proliferatum* causing stem and root rot on lucky bamboo (Dracaena braunii) in Iraq, Hell. Plant Prot. J.12:1–5.

8) Al-Obaidy, O. M. A. (2019). Antimicrobial Activity of Some Trichoderma spp. against Different Kinds of Fungal and Bacterial 9-16. Isolates. JOURNAL OF EDUCATION AND SCIENCE, 28(1),9-16.

9) Lahuf, A.A., Alfarttoosi, H.A., Al-Sweedi, T.M. and Middlefell-Williams, J.E. (2019b) Evaluation of an integration between the nanosized zinc oxide and two cultivars for the control of damping-off disease in sunflower crop. Res Crops 20:174–179.

10) Wang, L., and Ji, P. (2021) Fitness and competitive ability of field isolates of Phytophthora capsici resistant sensitive. Plant Disease, PDIS-08.

11) Silva, M. R. B. L. D. (2019). Gliotoxin and Bis-methyl-glitoxin production by Trichoderma spp. as biocontrol agents running title: human risk potential metabolites (Doctoral dissertation) by using Trichoderma spp.

12) Abdulmoohsin, R. G., Lahuf, A. A., Husain, Y. N. and Hameed, Z. L. (2019). Bioefficiency of some indigenous biocontrol agents against Rhizoctonia solani causing cowpea seed rot and preemergence damping-off. IOP Conf. Series: Earth and Environmental Science 388 012011.doi:10.1088/1755-1315/388/1/012011.

13) Lahuf, A. A., Kareem, A. A., AL-Sweedi, T. M. and Alfarttoosi, H. A. (2019a). Evaluation the potential of indigenous biocontrol agent Trichoderma harzianum and its interactive effect with nanosized ZnO particles against the sunflower damping-off pathogen, Rhizoctonia solani. IOP Conf. Series: Earth and Environmental Science 365: 012033. doi:10.1088/1755-1315/365/1/012033.

14) Ruangwong, O. U., Wonglom, P., Suwannarach, N., Kumla, J., Thaochan, N., Chomnunti, P., ... and Sunpapao, A. (2021). Volatile Organic Compound from Trichoderma asperelloides TSU1: Impact on Plant Pathogenic Fungi. Journal of Fungi, 7(3), 187.

15) Karimuna, L.; N. M. Rahni and Boer, D. (2016). The use of bokashi to enhance agricultural productivity of marginal soils in southeast Sulawesi, Indonesia. J. Tropical Crop Sci., 3(1): 1-6.

16) Al-jarah, N. S. A. (2011). The effect of the EM1 biomass and the mitochondrial field in the protection of cucumber plants from causative of rotting and seedling damping off. PhD thesis. College of Agriculture. University of Baghdad.

17) Al-Mamouri, Muhammad Kadhim. (2019). Evaluation of the effectiveness of some biological and chemical factors and bio-fertilizer (Bokashi) in resisting

some fungi that cause eggplant seedling death disease. Master Thesis, College of Technology / Al-Musaib, Al-Furat Al-Awsat University.

18) Lahuf, A. A., Jaafar, O. H., Al-mosoy, M. and Hameed, Z. L. (2018). First record of the crown rot fungus Fusarium equiseti affecting Triticum aestivum L. and Aptenia cordifolia in Iraq. Asian j. agric. biol. 6 (4): 543-548.

19) Parmeter, J. R. and H. S. Whitney. (1970). Taxonomy and nomencleature of the imperfect stage In: Rhizoctonia solani Biology and pathology. Parmeter, J. R. Univ. of California . 7–19.

20) Ellis, M.B. (1971). Dematiaceous Hyphomycetes. Commonwealth Mycological Institute Kew , survey England .1- 608.

21) Booth, C. (1977). Fusarium laboratory guide to the identification of the major species. Commonwealth Mycological Institute Kew, Surrey, England. 58 pp.

22) Summerell , B . A . and Leslie , J. F. (2006). The Fusarium Laborator manual. 388pp.

23) Bolkan, H. H., and E. E. Butler . (1974). Studies on heterokaryosis virulence of Rhizoctonia solani . Phytopathology 64: 513 – 522.

24) Bell, D. K., H. D. Well and G. R. Markham (1982). In vitro antagonism of Trichoderma species against six fungal plant Pathogens. PhytoPathology, 72 : 379 – 382 .

25) Higa, T. (1998). Effective microorganisms for more sustainable agriculture, environment and society. Proceeding of the 4th international. conf. on kyusei Nature farming Paris-France.

26) Fatima, Z., M. Saleemi, M. Zia, T. Sultan, M. Aslam, R. U. Rehman and M. F. Chaudhary (2009). Antifungal activity of plant growth- promoting rhizobacteria isolates against Rhizoctonia solani in wheat. African J. of Biotech., 8: 219-225.

27) Matloob, A. A. H., K. F. Alwan and S. H. Segar. (2019). Control of the causes of the damping off disease on pepper by some biological agents in Babylon province-Iraq. Journal of Biopesticides, 12 (2):215-223.

28) Harman, G. E. (2000). Myths and Dogmas of biocontrol change in perceptions derived from reseaech on Trichoderma harzianum strain T-22. Plant Disease, 84: 377-393.

29) Samson RA, Seifert KA, Kuijpers AF, Houbraken JA, Frisvad JC (2004) ″Phylogeneticanalysisof Penicillium subgenus Penicillium using partial beta-tubulin sequences″ (PDF). Studies in Mycology. 49:175-200.