Isolation and Diagnosis of Pathogenic Fungi Associated With Zucchini Cucurbita Pepo Roots and Their Bio-Control

Muhsen Abd Ali¹, Nazar Rashid Merzah² and Ali Faraj Jubair³

¹University of Karbala, College of Agriculture, Iraq.
²Ministry of Agriculture, Plant Protection Directorate, Iraq.
³University of Al-Muthanna, College of Agriculture, Iraq.

Abstract

This study aimed to isolate and diagnose the main pathogens of zucchini root rot, test their pathogenicity, and evaluate their control with some biological agents under laboratory conditions. The results of diagnostic isolation from the roots of the zucchini crop that showed symptoms of the disease showed the presence and dominance of two isolates of Fusarium oxysporum (FO1, FO2), three isolates of Fusarium solani (FS1, FS2, FS3) and three isolates of Rhizoctonia solani (RS1, RS2, RS3) and two isolates of Pythium spp (PY1, PY2), which was present in a ratio of 65-25% in most of the samples collected from the fields of the Faculty of Agriculture / University of Karbala. F. solani had the highest frequency, at 60.00%, and had the highest occurrence in sample 3, which was 65.00%, followed by R. solani and F. oxysporum with 48.33 and 40.00% occurrence, respectively. The pathogenicity test of fungal isolates on red radish seeds showed that all isolates significantly reduced radish seeds germination under laboratory conditions. The results showed the effect of pathogenic fungal isolates on germination and growth of zucchini plants in protected cultivation, which led to a significant reduction in germination percentage, fresh weight of the plant, plant height and a high rate of disease severity. FS1 and RS1 isolates completely prevented seed germination, followed by FS2 and FO2 isolates with equal germination rate of 22.22%, compared to control treatment with 100% germination rate. FS2 isolate had the highest infection severity rate of 97%, followed by FO2 isolation with 94% compared to no infection in control treatment. The minimum plant length was 0.267 cm in the FS2 isolation treatment, followed by the FS3 isolate, with a plant length of 0.567 cm, compared to 2.553 cm in the control treatment. FO1 isolation led to the highest reduction in plant fresh weight by 81%, followed by isolate FS2 with 78% compared to control treatment. The test of the inhibitory ability of the biological control agents showed the presence of high antagonism of Trichoderma harzianum, Chaetomium sp and Sordaria sp against pathogenic fungal isolates. T. harzianum was the highest antagonistic against all the pathogenic fungal isolates, recording grade 1 antagonism according to Bell et al. (1982) after seven days of dual culture. Also, Chaetomium sp and Sordaria sp showed antagonistic ability of 1 and 2 degrees against pathogenic fungal isolates under study.

Keywords: Cucurbita pepo, Bio-control, Zucchini, Pathogenic fungi.

1. Introduction

Cucurbita pepo is one of the main vegetable crops in the usual diet for humans. It contains important nutrients and is easy to digest for the elderly and children. It also has medicinal benefits in lowering body temperature, reducing thirst, stimulating the liver, and getting rid of free ions in the human body [1]. Zucchini cultivation in various parts of the world is threatened by many pathogens endemic to the soil. These fungi include Rhizoctonia solani, Phytophthora spp., Fusarium solani, Fusarium oxysporum f.sp curcuminum, and Pythium spp. Which adapt to a long residence time in the soil by forming resistant structures to outperform the inappropriate ones. Among the most important symptoms caused by these fungi are seed rot, seedlings death, root rot, ulcers on the stems, spots on leaves and their yellowing, and deterioration of plants in general, which may lead to their death by severe infections [2]. These fungi cause large yield losses ranging between 30 - 80% and may reach 100% under certain conditions [3]. The heavy and continuous use of fungicides increased the chances of the emergence and development of resistance trait and increased environmental pollution and health risks, which prompted the trend to search for safe alternatives to agricultural chemicals in general and pesticides in particular. In this aspect, studies...
focused on biological control of plant pathogens using biological agents such as *Trichoderma sp.*, which proved antagonistic efficacy against many pathogenic fungi on different plant hosts [4]. Researches had also indicated the success of the fungi *Sordaria* and *Chaetomium* as an anti-pathogen agent in the soil on the one hand and stimulating plant growth on the other [5,6]. Therefore, the aim of this study was to determine the main causes of zucchini root rot through isolation and diagnosis, confirm their pathogenicity, and control them using fungal biological agents under laboratory conditions.

2. Materials and Methods

2.1 Isolation, diagnosis, and preservation of zucchini root rot pathogens

Samples were collected from the fields of the College of Agriculture / University of Karbala from infected zucchini plants that show symptoms of root rot, stem lesions, leaf spots, yellowing, and deterioration of the plants in general [7,4]. Infected plants were uprooted and cut to a height of 5 cm above the crown area. The roots and bases of the stems of the plants were washed with running water for 30 minutes and cut into small pieces 0.5 cm long. Samples were superficially sterilized with sodium hypochlorite (0.5% chlorine) for 3 minutes, after which they were washed with sterile distilled water twice for 2 minutes and dried with sterile filter paper. The cuttings were cultured in 9 cm Petri dishes (four replicated for each sample) containing sterile Potato Dextrose Agar (PDA) with Tetracycline added at a concentration of 200 mg/L. The plates were incubated at 25 ± 1°C for 4 days, then the fungi were purified by the Hyphal tip method and cultured in the center of a petri dish containing the PDA and incubated for 4 days. The fungi were identified to the species level depending on the phenotypic characteristics, which include the color of the fungal colony, the branching nature of the young mycelium, the shape and size of the conidiophore, spores formed, and the ability to form sclerotia [8,9,10,11].

2.2 Pathogenicity tests of isolated fungi using radish seeds on water agar medium

Pathogenicity of *Fusarium oxysporum* (FO1, FO2), *Fusarium solani* isolates (FS1, FS2, FS3), *Rhizoctonia solani* (RS1, RS2, RS3) and *Pythium spp* (PY1, PY2) isolates under study were tested according to [12]. Nine cm Petri dishes containing 20 ml of (Water Agar) WA medium were prepared and inoculated with the aforementioned fungi with a 0.5 cm diameter disc from the edge of the fungi colonies at 5 days of age. The dishes were incubated at 25°C for three days, after which the plates were planted with local red radish seeds surface sterilized with 10% sodium hypochlorite. The seeds were planted circularly near the edge of the fungus growth, at a rate of 15 seeds/plate, 3 plates per isolate in addition to the control treatment without the fungus. The plates were incubated at 25°C and the results were taken after 7 days by calculating the percentage of germination where:

\[
\text{Germination} \% = \frac{\text{No. of germinated seeds}}{\text{Total No. of seeds}} \times 100
\]

And the percentage of inhibition/stimulation:

\[
\text{Inhibition} \% = \frac{\text{No. of germinated seeds in the control} - \text{No. of germinated seeds in a treatment}}{\text{No. of germinated seeds in the control}} \times 100
\]

2.3 Pathogenicity of fungus isolates on zucchini seeds and plants, under greenhouse conditions

This experiment was conducted in the biological control laboratory belongs to the Department of Plant Protection at the College of Agriculture on 1/3/2017 and the treatments were distributed according to a complete randomized design (C.R.D), inocula of *F. oxysporum* (FO1, FO2), *F. solani* (FS1, FS2, FS3), *R. solani* (RS1, RS2, RS3) and *Pythium spp* (PY1, PY2) which reduced radish seed germination in vitro were prepared. Dishes for developing fungus isolates were prepared and incubated for 7 days. Local millet seeds were used as they were washed and cleaned to remove impurities, and then soaked in water for 6 hours. Then the excess water was discarded and the seeds were distributed in 250 ml glass flasks and sterilized in an autoclave, then left to cool to room temperature, after which they were inoculated with fungus isolates at a rate of 4 tablets of 0.5 cm and mixed well. The flasks were incubated for two weeks with shaking and stirring every five days to ensure aeration and distribution of the inoculum.

For the planting, sterile plastic pots were used, which were filled with autoclave sterile soil mixture at 121 °C and a pressure of 1.5 kg/m2 for 20 minutes. The fungus inoculum was added to the potted soil at a rate of 0.01 (w/w) with 3 replications for each treatment in addition to the control treatment. The pots were planted with zucchini seeds, a local variety, with 3 seeds each and irrigated as needed. The percentage of germination was calculated and the severity of the disease was calculated after four weeks according to a 5 degrees disease index, where 0= No symptoms, 1= presence of small rotten spots on the roots, 2= lesions presence on the stem base and the roots, 3= the roots and stem base are completely rotten, 4= Plant is dead. The percentage of infection severity was also calculated [13].

[13]
Infection severity $\% = \frac{(\text{No.of plants in degree 0-0}) + (\text{No.of plants in degree 1-1}) + \ldots + (\text{No.of plants in degree 4-4})}{\text{No.of plants in degree 4-4}} \times 100$

2.4 Antagonism test of biological control agents against the studied fungal isolates under laboratory conditions

dual using the culture method, T. harzianum, Chaetomium sp and Sordaria sp (So) were tested for their antagonism to isolates of F. oxysporum (FO1, FO2), F. solani (FS1, FS2, FS3) and R. solani (RS1, RS2, RS3) and Pythium spp (PY1, PY2) that were isolated from infected zucchini roots.

The Potato sucrose Agar (PSA) culture medium was prepared after autoclaving at 121 ° C and a pressure of 1.5 kg/m2 for 20 minutes. The medium was distributed to 9 cm Petri dishes, 15-20 cm3 each and left until solidification. The plates were inoculated with a 5 mm disc 7 days old of the fungal growth of both the biological agent and the pathogen at with a 4 cm separation between the two discs. The plates were inoculated with fungal isolates only in the control. The experiment was carried out with 3 replicates for each isolate and results were taken after 7 days of incubation at 25+2°C. The colonies’ diameters were measured after 7 days of incubation. Antagonism level was estimated according to a 5 degree scale [14] where 1= the antignonstic fungal covering the entire plate, 2= the antagonistic covering 3/4 of the plate area, 3= 50% of the plate area is covered by the antignonstic or by the pathogenic fungus, 4= the pathogenic fungus covering 3/4 of the plate and 5= the pathogenic fungus covering the entire plate. The biological control agent is considered antagonistically effective at a degree 2 or less of antagonism against the pathogen.

\[
\text{Inhibition}\% = \frac{\text{Average diameter of control colony} - \text{Average diameter of a treatment colony}}{\text{Average diameter of control colony}} \times 100
\]

3. Results and Discussion

3.1 Isolation and diagnosis of the causes of zucchini root rot disease

The results of isolation from zucchini roots with symptoms of root rot, stem lesions, leaf spots, yellowing, wilting and deterioration of plants showed the presence and dominance of the fungi F. solani and F. oxysporum and R. solani and Pythium sp. whose growths appeared in the culture medium PSA in most samples, with a high presence of 65-25%. The phenotypic characteristics of F.solani were determined to form white to gray fungi, and F.oxysporum differed in colony pink color. Although the fungi are microscopically similar in the formation of three types of spores, namely microconidia, macroconidia and Chlamydospores, which appeared singly or in pairs in small lateral branches, and sometimes in the middle of the mycelium (Fig.1). According to the taxonomic key by [9] the fungus was identified to be F.solani. The isolates of the R.solani type were distinguished by the presence of branching near the terminal septum of cells in the modern mycelium, high branching, the formation of barriers near the point of origin of the branch, and the presence of septa, which are among the basic constant characteristics that characterize this species. colony color ranged between light brown to dark, the difference in width and length of cells was observed with different isolation, and that these characteristics were identical to the formerly mentioned parameter by [11]. Whereas, Pythium sp was distinguished by its formation of dense, profuse, non-dividing fungal spore capsules. This is due to the different environmental conditions favorable for the growth of fungi, the type of soil, the variation of the fungi in the formation of reproductive units and according to their density and size, as well as the influence of the type of crops [15].

Table 1. Presence percent of fungi associated with the zucchini plant roots.

| Fungi         | Presence of fungi samples % |
|---------------|-----------------------------|
| F. solani     | 60.00                       |
| R. solani     | 48.33                       |
| F. oxysporum  | 40.00                       |
| Pythium sp    | 19.75                       |
3.2 Test the pathogenicity of fungi isolated by using radish seeds on water agar

The results of Table (2) Figure (2) showed that all fungal isolates had a significant effect in reducing percentage of radish seeds germination under laboratory conditions. The isolates FS1, FS3, RS1, RS2 and PY1 were the most pathogenic in affecting seed germination. These isolates prevented seed germination completely resulting in 0% of seed germination, followed by FS2 and FO1 isolates, with germination rates of 3.78% and 6.60% respectively.

| No. | Treatment (fungal isolate) | Seed germination % |
|-----|---------------------------|--------------------|
| 1   | FS1                       | 0.00               |
| 2   | FS2                       | 3.78               |
| 3   | FS3                       | 0.00               |
| 4   | FO1                       | 6.60               |
| 5   | FO2                       | 13.33              |
| 6   | RS1                       | 0.00               |
| 7   | RS2                       | 0.00               |
| 8   | RS3                       | 48.44              |
| 9   | PY1                       | 0.00               |
| 10  | PY2                       | 55.55              |
| 11  | Control                   | 86.00              |

L.S.D<sub>0.05</sub> 2.46

Figure 2. Effect of pathogenic fungi isolates on red radish seeds germination where the isolates are: 1- FO2, 2- PY1, 3- FS1, FS2, and FS3, 4- RS1 as the germination rate was compared to the control on the left of each isolate.
As for the rest of the isolates, their germination rate ranged from 13.33 to 55.55%, compared to the germination rate of 86.00% with the control treatment. This is due to the ability of these isolates to secrete various compounds of enzymes, toxins and secondary metabolites by which they attack the seeds, cause rotting and prevent their germination [16,17, 18]. These results are also consistent with the results of previous studies on the effect of fungal isolates *F. oxysporum, R solani,* and *Pythium sp* on seed germination of many plant hosts [19,20].

3.3. Pathogenicity of fungal isolates on zucchini plants under greenhouse conditions

The results (Table 3 and Fig. 3 and 4) showed that all treatments had a significant effect in reducing zucchini seeds germination and seedlings fresh weight. Most of the treatments significantly affected the percentage of disease severity and plant height. The isolates FS1 and RS1 were the highest pathogenic and were equal in preventing seed germination, severity of infestation, plant height and fresh weight with values that were 0%, 100%, 0% and 0%, respectively, followed by FS2 and FO2 isolates that performed the same germination rates of 22.22% compared to 100% in the control treatment. As for the remaining isolates, germination rates were 33.33-100%. FS2 isolation also resulted in the severity of injury to 97%, followed by isolation FO2, with a severity of injury 94%, compared to 0% in the control treatment.

### Table 3. Pathogenicity tests of fungal isolates on zucchini plants under greenhouse conditions.

| Treatments | No. of germinated seeds (Average) | Germination % | Infection severity % | Plant length | Shoot fresh weight |
|------------|----------------------------------|---------------|----------------------|--------------|--------------------|
| FS1        | 0.00                             | 0.00          | 100.00               | 0.00         | 0.00               |
| FS2        | 22.22                            | 22.20         | 97.00                | 0.267        | 7.00               |
| FS3        | 44.44                            | 44.66         | 85.67                | 0.567        | 13.17              |
| FO1        | 33.33                            | 33.33         | 91.00                | 1.040        | 6.00               |
| FO2        | 22.22                            | 22.33         | 94.00                | 0.823        | 10.80              |
| RS1        | 0.00                             | 0.00          | 100.00               | 0.00         | 0.00               |
| RS2        | 44.44                            | 44.66         | 88.67                | 0.717        | 11.33              |
| RS3        | 55.55                            | 55.55         | 85.67                | 1.513        | 14.33              |
| PY1        | 100.00                           | 100.00        | 33.33                | 1.927        | 23.67              |
| PY2        | 88.88                            | 88.89         | 41.33                | 2.117        | 28.50              |
| Control    | 100.00                           | 100.00        | 0.00                 | 2.553        | 32.33              |
| L.S.D. 0.05|                                  | 02.57         | 6.34                 | 0.32         | 2.18               |

The minimum plant length was recorded in treatments FS1 and RS1 (0.267 cm), followed by FS2 and FS3, with a plant length of 0.567 cm, compared to plant length 2.553 cm in the control. The isolate FO1 led to the lowest shoot fresh weight (6 g) followed by the isolation RS1 (7 g), compared to the control treatment with the highest mean of shoot fresh weight (32.33 g). The variation in the percentage of germination is due to the variation of fungal isolates in their enzymatic activity and the secretion of other substances that affect the viability and germination of the seeds [21]. These results agree with many researchers that fungal isolates differ in their pathogenicity on different host plant [22,23]. The results of high infestation severity and low growth parameters represented by fresh weight and plant height are consistent with [24] for the ability of *F. oxysporum* to infect zucchini crop with high infection severity and reduce plant length, plant fresh and dry weight. The fungus *R. solani* is one of the fastest pathogens that kill the infected host. Laboratory experiments have shown that this fungus possesses a group of enzymes that help break down cell walls such as Pectinase, methyll Pectin esterase, Cellulase and Phosphatase [1, 25].

![Figure 3](image-url)
3.4 Antagonistic (inhibitory) ability of biological control agents against fungal isolates under laboratory conditions

The results showed a high antagonistic ability *T. harzianum*, *Chaetomium* sp and *Sordaria* sp against *F. oxysporum* (FO1, FO2), *F. solani* isolates (FS1, FS2, FS3), and *R. solani* isolates (RS1, RS2, RS3), and *Pythium* spp. (PY1, PY2) isolates. According to the scale by Bell et al. (1982), *T. harzianum* showed high antagonism against all pathogenic fungal isolates as resulted in degree 1 antagonism after seven days of dual culture. *Chaetomium* sp and *Sordaria* sp were also effective by giving 1 and 2 degree of antagonism against the pathogenic isolates.

This is in agreement with the findings of [18] on the efficiency of *Trichoderma* spp. In stimulating the growth of zucchini plants and protecting them from infection with the pathogen *F. solani* that causes rotting of zucchini fruits under field conditions.

The high antagonistic potential of *T. harzianum* may be due to the direct parasitism of the mycelium of the pathogen, wrapping around and breaking down cell walls by enzymes Chitinase or B-1,3 gluconase [26,27]. These results also agree with the findings of [5] of the ability of three isolates of *Sordaria* sp. to inhibit *Pythium aphanidermatum* and *Dematophora necatrix* during field experiments in Japan. [28] reported the efficacy of *T. harzianum*, *Chaetomium globosum* and endomycorrhizae in stimulating and strengthening plant growth when used as seed treatment with wettable powder or granules prior to planting for many field crops.

Conclusion

It was found through this study that the main causes of root rot and death of zucchini seedlings are *F. oxysporum*, *F. solani*, and *R. solani*. *Pythium* spp. Showed less damage to zucchini plants than other fungi due to unfavorable environmental conditions. Also, some of the fungal isolates in this study showed high pathogenicity and completely prevented seeds germination. The biological control agents used in this study showed high efficiency in inhibiting the pathogenic fungal isolates on the PSA culture medium.
References

[1] Murphy, J.B., McFerran, J. and Good M. J. (1984). The effect of genotype and ethephon on Rhizoctonia soil rot of processing tomatoes. Horticulture. Science. 19, 676-677.

[2] Al-Hmoud, G. and A. Al-Momany. (2015). Effect of Four Mycorrhizal Products on Fusarium Root Rot on Different Vegetable Crops. Journal Plant Pathology Microbial. 6:2-5.

[3] Rampersad, S. N. (2009). First Report of Fusarium solani Fruit Rot of Pumpkin (Cucurbita pepo) in Trinidad. The American Phytopathological Society. 93, (5), 547.

[4] Nahed, Z.H. (2007). Control of Pythium Damping-off of Squash (Cucurbita pepo) by Seed Treatment with Crop Straw and Soil by the Biocontrol Agent Trichoderma harzianum. Plant Pathology Journal. 6(1), 95-98.

[5] Watanabe, T. (1991). Evaluation of Sordaria spp. as bio-control agents against soil borne plant diseases caused by Pythium aphanidermatum and Dematophora necatrix. Annals of the Phytopathological Society of Japan. 57(5), 680-687.

[6] Soytong, K., Kanokmadhakul, S., Kukongviriyapa, V. and Isee, M. (2001). Application of Chaetomium species (Ketomium®) as a new broad spectrum fungicide for plant disease control: A review article. Fungal Diversity. 7, 1-15.

[7] Erwin, D. C., and Ribeiro, O. K. (1996). Phytophthora Diseases Worldwide American Phytopathological Society, St. Paul, MN.

[8] Parmater, J. R. and H. S. Whitney. (1970). Taxonomy and nomenclature of the imperfect stage In: Rhizoctonia solani Biology and Pathology. (ed.) J. R. Parmater. University of California Barkely, Los Angeles. 7 – 19.

[9] Booth, C. (1977). Fusarium. Laboratory guide to the identification of the major species. Commonwealth Mycological Institute, Kew, Survey, England, 58 pp.

[10] Leslie, J. F. and B.A. Summerell. (2006). The Fusarium laboratory manual. 388 PP.

[11] Sneh, B., S. Jabaji-Hare, S. Neate and G. Dijst. (1996). Rhizoctonia species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease control. Kluwer Academic Publishers, London. 576pp.

[12] Bolkan, H.A. and D.F. Butler. (1974). Studies on heterokaryosis and virulence of Rhizoctonia solani. Phytopathology. 64, 513-522.

[13] Mckinney, H.H. (1923). Influence of soil temperature and moisture on infection of wheat seedling by Helminthosporium sativum. Journal Agriculture Research. 26,195-217.

[14] Bell, D. K.; H. D. Well, and G. R. Markham. (1982). In vitro antagonism of Trichoderma Spp. against six fungi, Plant pathogens. Phytopathology. 72, 379-382.

[15] Agrios, G. N. (2005). Plant Pathology. 5th Ed. Elsevier Inc. USA. 998pp.

[16] Al-Mousawi, Mohsen Abd Ali. (2012). Determination of the causes of disease root and stem rot of cowpea and their control using some chemical and biological induction agents. Master Thesis, faculty of Agriculture. University of Baghdad. Iraq.

[17] Rothrock, C. S. (2015). Role of Seedling Diseases and the Efficacy of Fungicide Seed Treatments in Stand Establishment of Cotton. University of Arkansas, USA.

[18] Hatem N. Arabi; Jawad A. Kamal. The role of single inoculation and dubie and triple bacterial interaction Azosphirillum brasilianse and VAM Glomus mossea and phosphate rock in availability NPK In the Rhizosphere Cu. Science. 19, 676-677.

[19] Hassoun, Ibrahim Khalil. (2005). Biological and chemical control of potato stem lesion pathogen Rhizoctonia solani Kuhn's. Ph.D thesis, faculty of Agriculture, University of Baghdad. Iraq.

[20] Al-Hamiri, Yasser Nasser. (2014). Some integration methods in controlling Fusarium oxysporum lycopersici, and in diagnosing its strains. Ph.D thesis, faculty of Agriculture, University of Baghdad. Iraq.

[21] Al-Jiashy, M.M., Al-Haidery, A.A. and Al-Taher, F.M. 2020. Effect of wheat seed treatment with sporulation suspension of three isolates of Trichoderma harzianum and its interaction with half of NPK fertilizer recommendation on some growth characteristics of wheat crop. Plant archives 20 (1) : 1259-1264.

[22] Altnik, H. H. (2005). First report of fusarium wilt of eggplant caused by Fusarium oxysporum f. sp. melongenae in Turkey. Plant Pathology, 54(4), 577-577.

[23] Alwand Tahir Dizayee. The Effect of Boron, Humic Acid and Interference on Water Productivity and Yield of Cauliflower (Brassica Oleracea). Al-Qadisiyah Journal For Agriculture Sciences. 9, 2019, 193-205. doi: 10.33794/qjas.2019.167058

[24] Al-Hmoud, G. and A. Al-Momany. (2015). Effect of Four Mycorrhizal Products on Fusarium Root Rot on Different Vegetable Crops. Journal Plant Pathology Microbial. 6:2-5.

[25] Dillard, H.R. (1987). Characterization of isolates of Rhizoctonia solani from lima bean grown in New York State. Phytopathology. 77, 748-751.

[26] Kubicek, C.P.; Mach, R.L.; Peter baner, C.K. and Lorito, M. (2001). Trichoderma from genes to biocontrol. J. of Plant Pathol., 83; 11-23.

[27] Elaf Ali Makttoof; Jabbar Kadhim Kassim; Kahraman Hussein; Habeeb Aikhuziaa. "A relationship Between Phosphorous Image and some chemical properties in the Middle and the South of Iraq”. Al-Qadisiyah Journal For Agriculture Sciences. 10, 1, 2020, 304-312. doi: 10.33794/qjas.2020.168459

[28] Kaewchai, S., Soytong, K. and Hyde, K.D. (2009). Mycofungicides and fungal biofertilizers. Fungal Diversity 38, 25-50.