The Use of Fermented Typha Domingensis Residues by Pleurotus Ostreatus as A bio-Fortification to Control Root Disease on Cucumber Caused by Rhizoctonia Solani

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Abstract

Cucumber is one of important crops and susceptible to root disease caused by Rhizoctonia solani. The study aimed to evaluate the efficiency of two isolates of P. ostreatus (Ah and Ak) and soil treatment with several rates of Typha domingensis residues fermented by P. ostreatus to control R. solani that causes root diseases on cucumbers. In vitro trails, R. solani inhibited significantly by isolate (Ah) as well as redial mycelial growth and the percentage of cucumber seeds germination. In field trails, the number of germinated seedlings was highest at fermented T. domingensis 59.81 compare to control treatment which was 59.81. Disease severity (DS) of root damage was recorded in R. solani and R. solani + Fermented T. domingensis treatments and reached 70.4 and 64.27 respectively.

Keyword: Rhizoctonia solani, Pleurotus ostreatus, Typha domingensis.

1. Introduction

Cucumber (Cucumis sativus L.) belongs to the Cucurbitaceae family which is one of important summer vegetables crops. Its cultivation spreads in most regions of Iraq, both in fields and in green houses throughout the year, and the area that grown by cucumber in Iraq reached 23820 hectare [1]. The expansion of cucumber cultivation in large areas all over of Iraq has led to the emergence many plant diseases that infecting this crop, which considered one of main problems that cause a significant decrease in quantity and quality. Rhizoctonia solani is one of these diseases that infect plants at all growth stages as a highly virulent pathogen, it causes seed decay, pre and post emergence damping off, root decay for a wide host of crops [2].

Many methods have been used to reduce the effects of pathogens, but they do not affect for most pathogens, especially R. solani, which have it formation of sclerotia that can survive in soil or plant residues for several years, in addition its has wide host range and has high saprophytism to organic matter [3]. Pleurotus ostreatus (Oyster mushroom) is one of Basidiomycete that produces many active chemical compounds [4], and its has antimicrobial, antifungal and toxins, mycelium contains immune system stimulants [5]. Oyster mushrooms are characterized by their high growth potential and the rapid formation of mycelium on a variety of culture media in different environmental conditions [6]. It also contains salts, vitamins and other beneficial nutrients [7]. One of modern agriculture is the adoption of organic farming as an alternative to chemical fertilizers [8] as it maintain soil fertility like plant residues or their extract [9, 10], increase the natural biological activity in soils and avoid pollution [11]. The aim of the study is to evaluate the efficiency of two isolates of P. ostreatus and soil treatment with several rates of Typha domingensis residues fermented by P. ostreatus to control R. solani that causes root diseases on cucumbers.

2. Materials and Methods

Experiments were conducted in laboratories of College of Agriculture/AlMuthanna University, and second research station.
2.1 *P. ostreatus* isolates

Two pure isolates (Ah and Ak) of *P. ostreatus* were obtained from Mycology laboratory in the Plant Protection Department, College of Agriculture, University of Kufa.

2.2 Collect of vegetative parts of *Typha domingensis*

The vegetative parts of *Typha domingensis* were collect from river banks and fileds in Al-Suwayr district - Al-Muthanna governorate, Iraq, then placed in nylon bags and bringed to the laboratory, washed with tap water to get rid of suspended dust and mud, left for 30 minutes, then cut into small pieces, dried in the laboratory at room temperature with continuous stirrin, the samples were grinded by an electric grinder to get dry powder and then placed in paper bags at laboratory temperature until used.

2.3 Influence of fermented *T. domingensis* by *P. ostreatus* on inhibition percentage and radial mycelial growth of *R. solani* in vitro

After prepare *T. domingensis* as dry powder then sterilized by autoclave, then inoculated by *P. ostreatus* for both isolates (Ah and Ak) separately incubated at 25±2ºC for 21 days, Prepared six rates by added (0, 0.5, 1, 1.5, 2.0 and 2.5) gm to 100 ml potato dextrose agar (PDA) medium separately. The plates were poured according to the proportions added to PDA medium then inoculted with 0.5 cm disc of *R. solani* and incubated for three days at 25±2ºC every treatment represented by three replicates. Then parameters inhibition percentage and radial mycelial diameter were measured.

2.4 In vitro germination of cucumber seeds

Same methods and treatments were used as mentioned above to prepare PDA with same rate of fermented *T. domingensis* by *P. ostreatus* and inoculate with *R. solani*, 10 seeds of cucumber were sown for each plate. All treatment of *P. ostreatus* isolates and rates of fermented *T. domingensis* mix with medium consisted of three replicates. All plates incubted at 25±2ºC for seven days and percentage of germination was calculated as following equation :

\[
\% \text{ Germination} = \left( \frac{\text{No. of germinated seeds}}{\text{Total number of seeds}} \right) \times 100
\]

2.5 Field experiments

Field experiment was carried out in greenhouses at the second research station of the College of Agriculture/University of Al-Muthanna in spring season 2021, cucumber seeds were planted in plastic pots (diameter 26 cm), which contained 10 kg of soil, and

2.6 g residue/kg of soil was added to treatments which contain fermented *T. domingensis* with *P. ostreatus* inoculum as previous experiments.

*R. solani* grown on millet seeds for 21 days to prepare inoculum and added 5 g/kg soil to the pot that contain pathogen treatments.

Treatments of field experiment were Control, *R. solani*, fermented *T. domingensis*, *R. solani* + *T. domingensis*.

(A) Number of germinated seedlings

Cucumber seedlings germinated were calculated in each experiment after 10 days of sowing.

(B) Disease severity (DS) of root damage

Plant disease severity (DS) estimated as the severity percentage of disease damage on root of cucumber seedlings . Severity of symptoms on infected plants was estimated on a scale from 0 to 4 according to percentage of root rotting and damage with colorsis in extreme progression :

\[
0 = 0\%, \ 1 = 1 \ to \ 33\%, \ 2 = 34 \ to \ 66\%, \ 3 = 67 \ to \ 100\%, \ and \ 4 = \text{dead plant} \ [12].
\]
2.7 Statistical Analysis

In vitro, experiments were arranged in completely randomized design (three replication) data were subjected to analysis of variance and mean values were compared by Least significant difference LSD test ($p < 0.01$). Field experiments conducted with the one-way ANOVA and LSD test ($p < 0.01$).

3. Results

3.1 Inhibition percentage of Rhizoctonia solani

Inhibition percentage of $R$. solani was 0% at rate of Typha domingensis 0 gm/100 ml PDA for both of $P$. ostreatus isolates and control and inhibition increase by add rates of Typha to tested medium from 0.5, 1, 1.5, 2, 2.5 gm/100 ml PDA. Superiority of inhibition percentage of $R$. solani at rate 2.5 gm/100 ml PDA was 32.28% followed by rate 2 gm/100 ml PDA was 25.22%. Isolate $P$. ostreatus (Ah) it was superior statistically compare with $P$. ostreatus (Ak) inhibition percentage of $R$. solani were 24.96 and 16.79 respectively, while control treatment was 7.06%. Results of inhibition percentage of $R$. solani for interaction between $P$. ostreatus isolates and rates of $T$. domingensis present in table (1) was in treatment $P$. ostreatus (Ah) at 2.5 gm/100 ml PDA highest and superiority of inhibition of $R$. solani was 47.45% followed by rates 2 gm/100 ml PDA for same isolate 37.25% and reduce inhibition to $R$. solani by reduction rate of $T$. domingensis in the medium. $P$. ostreatus (Ak) isolate was less inhibiting for $R$. solani than $P$. ostreatus (Ah) isolate its was at rates of $T$. domingensis 1.5, 2.0 and 2.5 the percentage of inhibition were 22.74, 26.66 and 32.54% respectively.

| Isolates         | Rates of $T$. domingensis | Isolates = 1.887 | Interaction = 4.622 |
|------------------|---------------------------|------------------|---------------------|
| P. ostreatus Ah  | 0 14.11 24.31 26.66 37.25 47.45 24.96 |                   |                     |
| P. ostreatus Ak  | 0 6.66 12.15 22.74 26.66 32.54 16.79 |                   |                     |
| Control          | 0 0.78 5.09 7.84 11.76 16.86 7.06 |                   |                     |
| Mean             | 0.00 7.18 13.85 19.08 25.22 32.28 |                   |                     |
| LSD0.01          | Rate of $T$.domingensis = 2.668 |                   |                     |
|                  | Interaction = 4.622         |                   |                     |

3.2 $R$. solani radial growth

Radial mycelial growth of $R$. solani of rates of $T$. domingensis that add to medium have variant response for all rates, less radial mycelial growth at 2.5 gm/100 ml PDA was 5.29 cm followed 2.0 gm/100 ml PDA was 6.07 cm and radial growth increases of $R$. solani by decrease the adding rate of $T$. domingensis medium until was full radial growth at 0 gm/100 ml PDA was 8.50 cm. Isolate of $P$. ostreatus (Ah) was superior significantly compare with $P$. ostreatus (Ak) radial growth of $R$. solani was 6.38 and 7.07 cm respectively while control treatment was 7.31 cm. Results of radial mycelial growth of $R$. solani for interaction between $P$. ostreatus isolates and rates of $T$. domingensis present in table (2) treatment $P$. ostreatus (Ah) at 2.5 gm/100 ml PDA less significantly radial growth for pathogen was 4.47 cm followed by rate 2.5 gm/100 ml PDA for $P$. ostreatus (Ak) isolate and control and rate 2.0 gm/100 ml PDA of $T$. domingensis at isolate $P$. ostreatus (Ah) radial growth were 5.73, 5.67 and 5.33 cm respectively, while at rate 0 gm/100 ml PDA for both isolates and control was 8.50 cm respectively.

| Isolates         | Rates of $T$. domingensis | Isolates = 0.174 | Interaction = 0.427 |
|------------------|---------------------------|------------------|---------------------|
| $P$. ostreatus Ah | 8.50 7.30 6.43 6.23 5.33 4.47 6.38 |                   |                     |
| $P$. ostreatus Ak | 8.50 7.93 7.47 6.57 6.23 5.73 7.07 |                   |                     |
| Control          | 8.50 8.17 7.67 7.23 6.63 5.67 7.31 |                   |                     |
| Mean             | 8.50 7.80 7.19 6.68 6.07 5.29 |                   |                     |
| LSD0.01          | Rate of $T$.domingensis = 0.246 |                   |                     |
|                  | Interaction = 0.427         |                   |                     |
3.3 Percentage of germination for cucumber seed in vitro

Percentage of germination for cucumber seed in vitro generally impacted by *R. solani* which was affected most treatment for each factor of the experiment. *P. ostreatus* (Ah) isolate achieve highest germination was 32.78% superior of *P. ostreatus* (Ak) isolate and control treatments it was 8.15 and 0.00% respectively. Rates of *T. domingensis* at 0.0, 0.5 and 1.0 gm/100 ml PDA were least germination of cucumber seed was 0.0% respectively, and at rates 1.5, 2.0 and 2.5 there is increase in percentage of germination were 16.3, 21.3 and 44.3% respectively, the last rate was superior of all rates of *T. domingensis*. Results of percentage of germination of cucumber seeds for the interaction between *P. ostreatus* isolates and rates of *T. domingensis* presented in table (3) was in treatment *P. ostreatus* (Ah) at 2.5 gm/100 ml PDA highest significantly germination was 83.90% followed by rates 1.5 and 2.0gm/100 ml PDA for same isolate were 48.90 and 63.93% respectively while the rest treatments for isolate (Ah) were 0.0%. *P. ostreatus* (Ak) isolate was less in vitro germination for cucumber seeds just in one treatment at 2.5 gm/100 ml PDA it was 48.90%.

Table 3. In vitro germination [%] of cucumber seeds in response of *R. Solani* and interaction with rates of *T. domingensis* and *P. ostreatus* isolates

| Isolates         | Rates of *T. domingensis* | 0   | 0.5 | 1   | 1.5  | 2   | 2.5  | Mean  |
|------------------|----------------------------|-----|-----|-----|------|-----|------|-------|
| *P. ostreatus* Ah |                            | 0.00| 0.00| 0.00| 48.90| 63.90| 83.90| 32.78 |
| *P. ostreatus* Ak |                            | 0.00| 0.00| 0.00| 0.00 | 0.00 | 48.90| 8.15  |
| Control          |                            | 0.00| 0.00| 0.00| 0.00 | 0.00 | 0.00 | 0.00  |
| Mean             |                            | 0.00| 0.00| 0.00| 16.28| 21.31| 44.26|       |

*LSD*<sub>0.01</sub> = Rate of *T. domingensis* = 4.537
Interaction = 7.859

* Numbers in the table are angular transformed

3.3 Field experiments

(A) Number of germinated seedlings

Results of cucumber seedlings germinated was highest at fermented *T. domingensis* and control treatments were 59.81 and 54.07 respectively, its superior of rest treatment, followed by treatment of *R. solani* + Fermented *T. domingensis* was 39.68. Least of cucumber seedling emergence was 25.04 at *R. solani* treatment Figure 1.

(B) Disease severity (DS) of root damage

Some cucumber seedling in pots of *R. solani* treatments infected and damaged roots, these appear at treatments of *R. solani* and *R. solani* + Fermented *T. domingensis* which were 70.4 and 64.27 respectively and treatments of control and fermented *T. domingensis* disease severity were 0.00 for both Figure 2.

![Figure 1. Germinated seedlings of cucumber.](image)
4. Discussion

Cucumber is one of the most important cucurbitacea crops in most countries including Iraq, cucumber expose for many disease especially root rot and damping-off in top of most virulent disease that caused by soil borne pathogen \textit{R. solani} which considered a limiting factor to the production of cucumber, and its pathogenicity to cucumber seeds in this study in all treatments contain \textit{R. solani} in vitro and field experiment was proven [13]. Pathogenesis by \textit{R. solani} is characterized by extreme damage or death of plant tissues before or immediately after penetration, and colonization is primarily a process of mycelium growth into infected tissue [14].

Results of treatments contain \textit{P. ostreatus} reducing and inhibited the growth of \textit{R. solani} that occurred due to some reasons, because oyster mushroom produce many metabolite phytochemical compounds one of its polyphenols that have an antioxidant and antifungal for some fungi [15], these compounds prevent and inhibited \textit{R. solani} in all treatments, Cowan [16] found that more highly oxidized phenols are more antimicrobial. \textit{T. domingensis residue} have many phytochemical compounds its produce phenol that have a toxicity to pathogens microorganisms like enzyme inhibition by the oxidized compounds, Gutierrez [17] reported these enzymes maybe binding or reaction with sulfhydryl groups or through more metabolite non-specific reaction with the proteins. Phenolic compounds had a C3 side chain at a lower level of oxidation and containing no oxygen are known essential oils and classified as antimicrobial.

Many researchers reported that \textit{P. ostreatus} and its substrate can control plant diseases, \textit{P. ostreatus} able to inhibit growth of \textit{R. solani in vitro} and control root necrosis disease in tomato seedling [18]. Istifadah and Sianipar [19] reported substrate of Shiitake mushroom that contain mycelial of \textit{Lentinula edodes} have antibacterial activity when soaking potato with aqueous extract. In other study aqueous extract for substrate of \textit{P. ostreatus} and \textit{L. edodes} have ability to effect of \textit{R. solani} and decrease their virulent for seedling of tomatoes [18].

Conclusions

\textit{P. ostreatus} (Ah) isolate was superior significantly of most experiment parameters in inhibition percentage and radial mycelial growth of \textit{R. solani} and enhanced germination for cucumber seed, Rate 2.5 gm/100 ml PDA of \textit{T.domingensis} in all experiment parameters. Soil treatment with fermented \textit{T.domingensis} enhanced growth and percentage of germination of cucumber and reduce disease severity of \textit{R. solani}.

References

[1] Central Statical Organisation, 2020. Information Technology Directorate of Agricultural Statistics.Ministry of Planning, Republic of Iraq.
[2] Howard, F.S. and D.H. Gent. 2007. Damping -off and seedling blight. Pages 1-4. Available on internet: https://bugwoodcloud.org/bugwoodwiki/DampingOff SeedlingBlight-EggplantPepperTomato.pdf
[3] Ritchie, F., Bain, R. and Mcquilken, M. 2013. Survival of sclerotia of \textit{Rhizoctonia solani} AG3PT and effect of soil-borne inoculum density on disease development on potato. J. Phytopathol, 161: 180-189. https://doi.org/10.1111/jph.12052
[4] Babu, P.D., and Subhasree, R. S. 2008. The sacred mushroom “Reishi”-a review. American-Eurasian Journal of Botany, 1(3), 107-110.

[5] Refaie, F.M., Esmat, A.Y., Daba, A.S., and Taha, S. M. 2009. Characterization of polysaccharopeptides from Pleurotus ostreatus mycelium: assessment of toxicity and immunomodulation in vivo. MicologiaAplicada International, 21(2), 67-75.

[6] Kong, W.S. 2004. Descriptions of commercially important Pleurotus species. In: Mushroom world (Ed.). Oyster mushroom cultivation. Part II. Oyster mushrooms. Seoul: Heineart Incorporation, 2004.p.54-61. (Mushroom growers’ handbook, 1).

[7] Furlani, R.P.Z. and H.T., Godoy. 2008. Contents of folates in edible mushrooms commercialised in the city of Campinas, São Paulo, Brazil, Cienc. Tecnol.Aliment., Campinas, 27(2): 278-280.

[8] Elia, A.P., Santamaria and F., Serio. 1998. Nitrogen nitration yield and quality of Spinach. J. Science. Food Agriculture, (76) : 341 –346.

[9] Al-Mohamadawy, A. J., Al-Haidery, A. A. and Aljawasim, B. D. 2020. Efficiency of bio-evaporation of Arugula (Eruca sativa) leaves to control root rot disease on cucumber caused by Pythium intermediate in Greenhouse. IOP Conf. Ser.: Mater. Sci. Eng. 928, 062016.

[10] Jubair, A.F., Al-Haidery, A.A. and Al-Haidary, F.H. 2020. Effect of ginger and cinnamomum extracts in protect orange fruits from infection Penicillium digitatum the pathogen of green mould disease. Plant archives, 20 (1): 886-890.

[11] Rundgren, S.A and N.Gunner.2003. They principles of agriculture properties induced by admixtures of manures from various domestic animals. Soil Sci.,118:53- 59.

[12] Mohammed, M.A., Salman, S.R., (2017), Structural and surface roughness effects on sensing properties of ZnO doping with Al thin films deposited by spray pyrolysis technique, Journal of Engineering and Applied Sciences, 12 (Specialissue6), pp. 7912-7918.

[13] Yousef, S.A., El-Metwally, M.M., Gabr, S.A and El-Ghadir,A.H. 2013. New Strategy for managing damping-off and root rot disease of cucumber caused by Rhizoctonia solani by seed soaking in formula of antioxidant with micronutrients. J. Plant PatholMicrob 4: 196 doi:10.4172/2157-7471.1000196

[14] Weinhold, A.R., Sinclair. J.B. 1996. Rhizoctonia solani: penetration, colonization, and host response. In: Sneh B, Jabaji-Hare S, Neate S, Dijst G, (eds.) Rhizoctonia species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease control. Dordrecht, the Netherlands: Kluwer Academic Publishers 163-74.

[15] Shirmila, J.G., and Radhamany, P. M. 2012. Identification and determination of antioxidant constituents of bioluminescent mushroom. Asian Pac. J. Trop. Biomed.2, S386-S391 doi: 10.1016/S2221-1691(12)60194-4

[16] Mohammed, M.A., Abdulridha, W.M., Abd, A.N., (2018), Thickness effect on some physical properties of the Ag thin films prepared by thermal evaporation technique, Journal of Global Pharma Technologythis , 10(3), pp. 613–619.

[17] Gutierrez J., Barry-Ryan C., Bourke P. 2008. The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients.Int. J. Food Microbiol. 124 (1): 91–97.

[18] Herawati, L., and Istifadah, N. 2018. The potential of spent substrate of oyster (Pleurotus ostreatus) dan Shiitake (Lentinula edodes) Mushrooms to Control Damping-off Disease (Rhizoctonia solani) in tomato. CROPSAVER-Journal of Plant Protection, 1(2), 93-97.

[19] Istifadah, N. and R.P., Sianipar. 2015. Potensi limbah media jamur untuk menekan layu bakteri (Ralstonia solanacearum) pada tanaman kentang. Jurnal Agrikultura, 26, 84-89.

[20] Yusidah, I. and Istifadah, N. 2018. The abilities of spent mushroom substrate to suppress basal rot disease (Fusarium oxysporum f.sp cepae) in shallot.International Journal of Biosciences, 13 (1):440-448

[21] Roy, S., Barman, S., Chakraborty, U. and Chakraborty, B. 2015. Evaluation of spent mushroom substrate as biofertilizer for growth improvement of Capsicum annuum L. Journal of Applied Biology & Biotechnology, 3 (03): 022-027.