**Effect of some plant extracts on *Rhizoctonia solani* fungus causal disease of watermelon seed decay and seedling rot**

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**Abstract:** This study was conducted in the laboratories of Plant Protection Department, Agriculture College, during season 2019-2020, to determine isolate the fungi that cause the phenomenon of watermelon seedling's, evaluation of efficiency of three plant extracts (turmeric, eucalyptus leaf, and chamomile), on inhibition of radial growth of the fungus. The results of the study showed that the fungus *Rhizoctonia solani* was the fungus that causes seed rot and seedling death in the studied plant samples, concentration 5 chamomile gave the least radial growth, which was 4.350 cm, as for the concentration 15, chamomile gave the highest rate of radial growth, reaching 8.017 cm, there were no significant differences between the cold and hot extract of the chamomile plant. The cold aqueous extract of turmeric outperformed compared to the hot extract in the inhibition rate, as it was 5.442 and 6.108 cm, respectively, the inhibition of the radial growth of the pathogen increases with the increase in the concentration of the extract, as the concentration 15 gave the lowest radial growth rate of 2.350 cm, while the highest radial growth was in concentrate 5, at 8,217 cm. The hot aqueous extract of eucalyptus leaves outperformed the cold extract in the rate of growth inhibition, reaching 5.758 and 5.183 cm, respectively, growth inhibition of the pathogen increases with increasing concentration, as the least growth was at concentration 15, reaching 1.450 cm, while the highest radial growth was in concentration 5, reaching 7.217 cm.

**Keywords:** plant extracts, *Rhizoctonia solani* fungus, watermelon, seed and seedling rot

**Introduction**

Watermelon is a summer vegetable, cultivation was spread in most regions of Iraq and is considered an important vegetable, it is grown in open fields and greenhouses, the cultivated area in Iraq reached 19179 acres (Central Statistical Organization, 2017). The expansion of watermelon cultivation and large areas in the recent period, led to the emergence of many plant diseases, which the main problems, causes a significant reduction in production, includes the fungus *Rhizoctonia solani*, a very virulent nurses, characterized by infecting plants in all stages of growth, attacks seeds in the soil and causes rot, attacks seedlings before and after emergence and causes root rot (Agrios, 2007).

Many of the methods used to control pathogens, however, does not affect most of the pathogens, especially the pathogen *R. solani*, it is characterized by its formation of resistant phases (stone bodies) that can survive on plant debris or in the soil for several years, it also has a wide family range as well as a high restoration capacity (Hwang *et al.*, 2007).

Chemical control has proven successful in controlling the pathogen, however, the side effects were due to the widespread use of pesticides and mineral fertilizers, negatively effect of human and animal health and non-target soil regeneration, the researchers were find other ways, that less influential in the environment, recent studies have resorted to using plant extracts to reduce pathogen damage and environmental pollution (Kaewchai *et al*. 2009; Yassin *et al.*, 2013).

Therefore, this study aimed to isolation of the fungus that causes seed rot and seedling death, uses turmeric, eucalyptus leaves and chamomile plants to determine the influence on the pathogenic fungus, evaluating the efficacy of concentrations of hot and cold aqueous extract (5, 10 and 15)% on the growth of fungus in the laboratory (the extract is mixed with the PDA).

**Materials and Methods**

**Plant samples collection:**

Plant samples were collected and placed in plastic bags (eucalyptus plant), as for the chamomile and turmeric plants, they were collected from the herbal stores in Al-Samawah and transferred to the laboratory, washed with water to remove the dust, it was air dried in the laboratory for three days, until the weight stabilized, cut the plants into small pieces, grinded with an electric grinder, the powder was stored in small plastic boxes with the information recorded in the boxes.

**Table (1) Plants used in the study and places of collection.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Plant sample** | **Scientific name** | **Family** | **Place and date of plant collection** |
| **turmeric** | *Curcurma* sp | Zingiberaceae | Local markets on 10/11/2019 |
| **eucalyptus leaves** | Eucalyptus spp. | Myrtaceae | Al- Maali area on 10/11/2019 |
| **chamomile** | *Matricaria chamomilla* | Asteraceae | Local markets on 10/11/2019 |

**Isolation and purification of pathogenic fungi *Rhizoctonia solani*:**

Watermelon seedlings symptoms brought into the laboratory, cut into small pieces up to 1-1.5 cm, washed with water for 6 hours, surface sterilized with a 2% NaCo3 solution for 2 mins, with sterile distilled water 3 times to remove the sterile effect, and dried with filter paper to get rid of the water, distributed in Petri dishes containing the middle of P.D.A., they were incubated at 25 °C for three days and the mushrooms were classified according to the taxonomic characteristics mentioned in El‐Gadi and Elkington (1977).

**Preparing plant extracts**

**Cold Aqueous Extract:**

Parekh and Chanda (2007) method were used as follows, took 20 g of dry matter powder for turmeric, eucalyptus and chamomile leaves separately. put in a 500 ml glass beaker, add 200 ml of distilled water, placed in a shaking incubator at 37 °C for 24 hours, filter with gauze in glass tubes and centrifuged in a central centrifuge at 5,000 rpm for 10 minutes, filter the scent with 0.22 μm perforated filter paper, the filtrate was evaporated in the oven at a temperature of 40 °C until an almost dry powder was obtained, the powder was placed in an opaque and sealed tube, and kept in the freezer at a temperature of -18 ° C until use.

**Hot aqueous extract:**

El-Fallal and El-Kattan (1977) method were used as follows, took 20g of dry matter powder for turmeric, eucalyptus and chamomile leaves separately, put it in a 500 ml glass beaker, add 200 ml of boiled distilled water, placed in a shaking incubator at 28 ° C for 30 minutes, the mixture was filtered with gauze, then discarded with a central centrifuge at 3000 rpm for 10 minute, dried by oven at a temperature of 40 °C, the extract powder was stored in darked sealed tube at freez at -18° C to use.

**Result and Discussion**

**Radial growth of *Rizoctonia solani***

Table (2) indicates that no significant differences cold and hot aqueous extracts, as they were 7.13 and 7.14 cm, respectively, as concentration 5 gave the lowest radial growth with significant differences compared to the control treatment, which amounted to 4.35 cm, while the highest radial growth rate was in concentrate 15, reaching 8.02 cm, as for the interaction, the results showed that the cold chamomile extract at a concentration of 5 gave the lowest radial growth rate, reaching 4.13 cm.

The plant extracts had a significant effect on the activity of *R. solani*, Bianchi *et al*. (1997) indicated that the aqueous extract of garlic, Allium sativum, had an effective role in inhibiting the growth of R. solani, Hadizadeh *et al*. (2009) explained that bitter melon extract completely halted (100%) the growth of fungi *R. solani* and *A. alternate*, Mohammed *et al*. (2006) indicated the ability of the aqueous extract of melon to inhibit the growth of fungi, including *A. niger* and *Candida albicans*.

**Table (2) Effect of cold and hot aqueous extract of chamomile on radial growth (cm) of *R. solani* fungus in Petri dishes.**

|  |  |  |
| --- | --- | --- |
| **Extract** | **Concentrate** | **Mean** |
| **0** | **5** | **10** | **15** |
| **Hot extract** | 9.00 | 4.57 | 6.67 | 8.33 | 7.14 |
| **Cold extract** | 9.00 | 4.13 | 7.67 | 7.70 | 7.13 |
| **Mean** | 9.00 | 4.35 | 7.17 | 8.02 |  |
| **L.S.D0.05** | **Extract** | **Concentrate** | **Interaction** |
| 0.316 | 0.446 | 0.632 |

**Radial growth of *Rizoctonia solani***

Table (3) indicates that a significant differences between the cold and hot aqueous extracts, reached 5.44 and 6.11 cm, respectively, the inhibition of radial growth of the pathogen *R. solani* increases with increasing the concentration of the extract, the lowest radial growth was at concentration 15, reaching 2,35 cm, with significant differences compared to the control treatment, while the highest radial growth was in concentration 5, reaching 8.22 cm, as for the interaction, the results showed that the cold tumeric extract with a concentration of 15 gave the highest inhibition of the radial growth rate of the pathogenic fungi, reaching 1.87 cm.

The effectiveness of the effect of aquatic plant extracts on controlling the growth of *R. solani* fungus, attributed to the presence of saponins the cause of the effect (Papadopoulou, 1999), the variation in the inhibitory activity of plant extracts in the growth of *R. solani*, reflects of the variance in the active substances and the quantity affecting the inhibitory efficacy, which differs in different extraction methods (Park *et al.*, 2009), these effects may be due to the reduction in carbohydrates and protein content, as well as it reduce the effectiveness of Catalase enzyme, leads to increased toxicity and thus reduced the growth rate of the fungus (Hadizadeh et al. 2009).

**Table (3) Effect of cold and hot aqueous extract of turmeric on radial growth (cm) of *R. solani* fungus in Petri dishes.**

|  |  |  |
| --- | --- | --- |
| **Extract** | **Concentrate** | **Mean** |
| **0** | **5** | **10** | **15** |
| **Hot extract** | 9.00 | 7.93 | 4.67 | 2.83 | 6.11 |
| **Cold extract** | 9.00 | 8.50 | 2.40 | 1.87 | 5.44 |
| **Mean** | 9.00 | 8.22 | 3.53 | 2.35 |  |
| **L.S.D0.05** | **Extract** | **Concentrate** | **Interaction** |
| 0.264 | 0.373 | 0.528 |

**Effect of hot and cold aqueous extract of eucalyptus leaves on radial growth of *Rizoctonia solani***

 Table (4) showed that there were significant differences between the cold and hot aqueous extracts of eucalyptus leaves, reaching 5.76 and 5.18 cm, respectively, radial growth inhibition of *R. solani* was increased by increasing the concentration of the extract, the lowest radial growth was at concentration 15, reaching 1.45 cm, with significant differences compared to the control treatment, while the highest radial growth was in concentration 5, reaching 7.22 cm, as for the interaction, the results showed that the extract of hot eucalyptus leaves at concentration 15 gave the highest inhibition of the radial growth rate of the pathogen, which reached 1.07 cm.

The plant extracts had a significant effect on the activity of R. solani

Bianchi *et al.* (1997) indicated that the aqueous extract of the garlic plant *Allium sativum* had an effective role in inhibiting the growth of *R. solani*, Hadizadeh *et al*. (2009) indicated that bitter melon fruit extract completely stopped 100 % the growth of *R. solani* and *A. alternate*, Mohammed *et al.* (2006) indicated the ability of the aqueous extract of melon to inhibit the growth of fungi, including *A. niger* and *Candida albicans*.

**Table (4) Effect of cold and hot aqueous extract of eucalyptus leaves on radial growth (cm) of *R. solani* fungus in Petri dishes.**

|  |  |  |
| --- | --- | --- |
| **Extract** | **Concentrate** | **Mean** |
| **0** | **5** | **10** | **15** |
| **Hot extract** | 9.00 | 7.57 | 3.10 | 1.07 | 5.18 |
| **Cold extract** | 9.00 | 6.87 | 5.33 | 1.83 | 5.76 |
| **Mean** | 9.00 | 7.22 | 4.22 | 1.45 |  |
| **L.S.D0.05** | **Extract** | **Concentrate** | **Interaction** |
| 0.240 | 0.339 | 0.481 |

**Conclusions**

The fungus that causes seed rot and seedling death in the studied plant samples, concentration 5 chamomile gave the least radial growth, the concentration 15, chamomile gave the highest rate of radial growth, there were no significant differences between the cold and hot extract of the chamomile plant. The cold aqueous extract of turmeric outperformed compared to the hot extract in the inhibition rate, the inhibition of the radial growth of the pathogen increases with the increase in the concentration of the extract, as the concentration 15 gave the lowest radial growth rate, while the highest radial growth was in concentrate 5. The hot aqueous extract of eucalyptus leaves outperformed the cold extract in the rate of growth inhibition, growth inhibition of the pathogen increases with increasing concentration, as the least growth was at concentration 15, while the highest radial growth was in concentration 5.

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**Author Contributions:**

Conceptualization and methodology, Marwah Mohammed hanaf; investigation, Saad Manee Enad Al-Jabry; resources and writing—original draft preparation, Zina abdulhussein Jawad; writing—review and editing, Saad Manee Enad Al-Jabry; visualization, Zina abdulhussein Jawad and Saad Manee Enad Al-Jabry; supervision and project administration, Marwah Mohammed hanaf. All authors have read and agreed to the published version of the manuscript.

**Conflicts of Interest**

The authors declare no conflict of interest.

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**Data Availability**

Data presented in this study will be available on a fair request to the corresponding author.

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**Ethics Approval**

Not applicable to this research.

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**References**

Agrios GN 2007. *Plant Pathology* . 4th Ed. Academic press 606 pp, New York .U.S.A.

Al-Rawi KM and Khalaf-Allah AM 2000. *Design and analysis of agricultural experiments*. Directorate for Book House of Publishing and Pressing. University of Mosul. Ministry of Higher Education and Scientific Research. Iraq. 488pp.

Bianchi A, Alessandra Z, D’Aulerio AZ and Bellesia F 1997. Ultra structural Studies of the Effects of *Allium sativum*on Phytopathogenic fungi in vitro. *Plant Disease*, **81(11)**: 1-6.

Central Statistical Organization 2017. *Agricultural Statistics Directorate*. Ministry of Planning and Development Cooperation. Baghdad, Iraq .

El-Fallal AA and El-Kattan MH 1977. Effect of plant Extracts on the Mycelia Growth of some cultivated Mushrooms. *Egypt. J*. *Microbial* **32(1)**: 41- 48.

I.C.S.O., Iraqi Central Statistic Organization (2016). Annual Statistical Group, Ministry of Planning-Iraq.

El‐Gadi  A and Elkington TT 1977. Numerical Taxonomic Studies on Species in  *Allium subgenus Rhizideum*. *New Phytologist*, **79(1)**: 183-201.

Hadizadeh I, Peivastegan B and Kolahi M. 2009. Antifungal activity of nettle (*Urtica dioica* L.), colocynth (*Citrullus colocynthis* L. Schrad), oleander (*Nerium oleander* L.) and konar (*Ziziphus spina-christi* L.) extracts on plants pathogenic fungi. Pakistan Journal of Biological Sciences: PJBS, **12(1)**: 58-63.

Hwang SF, Gossen BD, Conner RL, Chang KF, Turnbull GD, Lopetinsky K and Howard RJ 2007. Management strategies to reduce losses caused by Rhizoctonia seedling blight of field pea. *Can. J. Plant Sci*., **87**: 145-155.

Kaewchai S, Soytong K and Hyde KD 2009. Mycofungicides and fungal biofertilizers. *Fungal diversity*, **38**: 25-50.

Mohamed IAI, Bauiomy MAM and Ibrahim ASA 2006. Efficacy of different natural products as safe management of guar damping-off disease in Egypt. *Egypt. J. Phytopathol*, **34(3)**: 1-15

Batta, Y.A. (2016). Invert emulsion method of preparation and application as proper formulation of entomopathogenic fungi. Methods, X; 119-127.

Papadopoulos AP 2003. Growing greenhouse seedless cucumbers in soil and in soiless media .(Publication) greenhouse and humic acids on the growth, yield and chemical parameters of strawberries*. J. Medic. Plants Res*, **5**: 2304-230.

Parekh J and Chanda S 2007. In vitro antimicrobial activity Photochemical Analysis of some India medical plant. *Turk.J. Biol*. **31**: 53-58.

Park JY, Jin J, Lee YW, Kang S and Lee YH 2009. Rice blast fungus *(Magnaporth oryzae)* infects Arabidopsis via a mechanism distinct from that required for in the infection of rice. *plant physiology*, **5(1)**:474-486.

Yassin MA, Moslem MA, El-Samawaty AMA and El-Shikh MS 2013. Effectiveness of *Allium* *sativum* in Controlling Sorghum Grain Molding Fungi. *J. Pure Appl. Microbiol.*, **5(1)**: 101-108.