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Effect of Plant Extracts and Biological control agents on Rhizoctonia solani Kuhn

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Abstract
This study aims to examined antagonistic ability of some Plant Extracts and bio agents against of *Rhizoctonia solani*. The aqueous Extracts of plants used in study (Common Hornwort, Sage and Thorn apple) had against effect on the growth of pathogen in PSA medium. The Common Hornwort and Sage at rate 15% was superior inhibits fungal growth, it was 100.0 %. The results showed that alcoholic extract of plants (in concentrate 10 and 15%) was also effective and caused highly activity to inhibit radial growth of pathogen. In addition to the concentrates 1 and 5% were inhibited at rates 40.8-69.6% and 63.0-83.3%. Thorn apple (Datura) extract was superior on Sage then Common Hornwort respectively. In the other hand the results appeared that Effective Microorganisms (EM-1) reduced growth of fungus more than organic fertilizer, Sea Bloom 29 which was 100.0% at both rates 10 and 15% whereas, the concentration 1 and 5% have inhibition influence which was 48.5 and 76.3% respectively. These results showed for the first time that ability of using the plant extracts and bioagents as alternative methods to control of the most serious plant pathogen and reduce using of chemicals and their problems.

Key words: Plant Extracts, Rhizoctonia solani, Common Hornwort, Sage, Thorn apple.

1. Introduction

The fungus *Rhizoctonia solani* kuhn, which is one of the most important pathogens on many economic crops, causing significant damage and losses in production both in terms of quantity and quality [1-4]. The fungus attacks the plant at all stages of its growth, causing seed rot, seedling death, and root rot [5-8]. The primary symptoms of the disease appear in the form of light brown spots along the main and secondary roots. As the infection progresses, all the roots become rotten and dark brown [9-11]. The use of chemical methods to control plant diseases has led to many environmental and health problems and disturbed the natural balance of life [12, 13]. In addition, many of them have lost their effective control effect due to the development of new strains of pathogens tolerant of these chemicals [14-16]. Researchers have increased interest in using plant extracts to combat many plant pathogenic fungi, as these extracts contain effective secondary metabolic compounds and possess environmentally desirable characteristics such as their rapid decomposition, low toxicity, and high specialization [17]. Many of them have been used in the control of *R. solani* [5, 18, 19, 20]. In the midst of the search for alternatives to chemicals in agriculture, research has shown the efficacy of the biological combination of Effective Microorganisms (EM-1) in controlling many diseases and improving growth and production of Plant [21-23]. In view of the importance of this pathogen in Iraq and the attempt to control disease by using biological agents and plant extracts, this study was conducted with the aim of trying to control the pathogen with some plant extracts and biological control agents.

2. Materials and working methods

2.1. Preparation of aqueous and alcoholic extracts of Common Hornwort, Sage and Thorn apple

Three plants were selected (Table 1) to study their effect against the pathogen *R. solani* (a pure isolate was obtained from the Laboratory of Plant Diseases- Technical College of Al-Mussaib). They included Common Hornwort *Ceratophylum demereni*, Sage *Salvia officinalis* and Thorn apple *Datura stramonium*. Common Hornwort plants were collected from rivers, washed well to remove the impurities and mud from them while the Sage were obtained from the markets and the Thorn apple plant was collected from some fields at the Technical Institute / Al-Mussaib, after which the plant samples were dried in the open air under the sun's rays, by brushing them in the form of thin layers over wide surfaces of cloth and exposing them to the rays of the sun. For an appropriate period of time with continuous stirring of samples to prevent them from rotting and to speed up their drying [24]. The plant specimens were crushed with a grinder containing a fine-mesh sieve. The powder of each plant was placed in polyethylene bags with the name of the plant and the weight of the form written on it and kept in the refrigerator until use. The extraction was done using Shekhawat and Prasada method [25] with some

modification, where a certain volume of each powder from the used plants was taken and placed in a 1 liter glass flask and distilled water was added (70% ethyl alcohol in the case of an alcoholic extract) in a ratio of 1: 2 (Volume / volume) The flask was closed and placed in a Julabo Sw20 electric shaker for 24 hours at room temperature (24 + 1 ° C). The solution was filtered with a clean cloth to get rid of large particles, then the extract was filtered through Whatman No.1 filter paper placed in a Buechner funnel with a vacuum. The total filtrate obtained from the extraction was concentrated in a water bath at a temperature of 50 ° C to get rid of the water, and a thick liquid was obtained. The extracts were weighed and stored in marked and sealed glass bottles and placed in the refrigerator until use.

2.2. Inhibitory activity of the aqueous and alcoholic extracts of Common Hornwort, Sage and Thorn apple in the growth of Rhizoctonia solani in PSA

The effect of plant extracts on the growth of *R. solani* was tested by food poisoning method [26]. The concentrations (1, 5, 10, 15%) of the extract of the plants prepared for the test were prepared with sterile and cooled PSA medium respectively, while the medium was left without adding the extract as a control, the media was poured into sterile dishes (9 cm in diameter) and after the medium hardened The plates were inoculated in the center with a disc of diameter (0.5 cm) from the culture medium containing the growths of the pathogen *R. solani* at 5 days old. The experiment was carried out using a completely random design, and three plates were used for each treatment as replicates. The plates were incubated at a temperature of 25 + 1 ° C, and after the diameter of the fungal culture in the control treatment (without extract) to reached to the edge of the plate, the results were taken by calculating the rate of measurement of two perpendicular diameters from the growth of each colony and the percentage of inhibition was calculated according to the following equation:

Inhibition (%)=[(Comparative colony diameter rate - the average transaction colony)\ Comparative colony diameter rate] x 100.

Table 1. plants used to study their effect against pathogen *R. solani*.

Common name	Scientific Name	Used parts	Site of collection
Hornwort	*Ceratophylum demereni*	Leaves and stems	Mashroa Al-Mussaib river
Sage	*Salvia officinalis*	Leaves	Local markets
thorn apple	*Datura stramonium*	Leaves, stem	Al-Mussaib Technical institute

2.3. Evaluation of the inhibitory effect of EM-1 and Sea Bloom29 on the growth of Rhizoctonia solani under laboratory conditions.

The EM-1 bioactive preparation was activated by mixing the stock material with molasses and warm sterile distilled water in a ratio of 5: 5: 95, respectively [21]. Dissolve the molasses well with warm water, then add the stock solution and mix well and put in a small plastic 5-liter barrel sealed with a plastic cover to prevent air contact with the solution and put in the incubator at a temperature of 37 + 1 m for 10 days with opening the barrel 2-3 times to leak gas When a layer of sediment is formed at the bottom of the barrel. The effect of EM-1 and Sea Bloom (obtained from the College of Agriculture, University of Baghdad) on the growth of *R. solani* was tested by food poisoning method. The same concentrations mentioned in the previous paragraph were prepared by adding a certain volume of each solution to a specific volume of the culture medium, where 1, 5, 10, and 15 ml of the solution were added to 99, 95, 90, 85 ml of sterile and cooled PSA medium, Then the medium was shaken to distribute the solution homogeneously in the medium and poured into sterile dishes (9 cm in diameter). Three dishes were used for each treatment in addition to the control treatment, which contained only the PSA medium. As for the chemical pesticide Beltanol, a concentration of 0.5 ml / liter of sterilized and cooled PSA was added. The dishes were incubated at a temperature of 25 + 1 ° C. After the fungal culture was reached to the edge of the plate in control treatment, the results were taken as followed in the previous paragraph.

3. Results and discussion

3.1. The effect of the aqueous extracts of Common Hornwort, Sage and Thorn apple on the growth of R. solani in the PSA medium

The results (Figures 1 and 2) showed the difference in the efficiency of the aqueous extract of the plants used in inhibiting the growth of *R. solani* according to the used concentrations, as the concentration of 15% of the extract of the Common Hornwort

and Sage achieved the highest reduction in the growth rate of the pathogen fungus by 0.0 cm and the rate of inhibition of 100% while it appeared A slight growth in Thorn apple extract at the same concentration reached 1.2 cm, with an inhibition rate of 78.3%, with a significant difference from the control treatment, in which the growth rate of the fungus was 9.0 cm and the inhibition rate was zero. It was followed by a concentration of 10%, as the fungus growth rate was 1.8, 1.3 and 2.7 cm for Common Hornwort, Sage and Thorn apple extract, respectively, with a significant superiority for a Sage extract over a Thorn apple extract, as it achieved a high inhibition rate of 85.2%, while the concentrations 1 and 5% achieved significant inhibition ratios except for concentration 1 % of Thorn apple that grew the fungus well and did not differ from the control treatment. The results of the current study showed, for the first time in the world, that the aqueous extract of the Common Hornwort plant, with the concentrations mentioned above, has an inhibitory effect on the pathogen *R. solani*, which is one of the most dangerous fungi living in the soil and the most pathogenic for the plant. The results are consistent with what Fareed et al [18] have shown regarding the effectiveness of extracts of some plants, including the Common Hornwort plant, in inhibiting the growth of a number of bacterial and fungal pathogen species. And with Ismail [27] results, which proved that Sage extract with concentrations of 250-1000ppm was the best among the plants tested in inhibiting the growth of the pathogen *Fusarium oxysporum* that causes *Fusarium* wilt in the medium, with an inhibition rate ranging between 17.2 - 40.8%. And with what San Aye and Matsumoto [28] found that the aqueous and alcoholic extract of Sage and some other plants inhibit the ability of some pathogenic fungi, including the pathogen *R.solani* Jalander and Gachande [17] demonstrated the effectiveness of the aqueous extract of the Thorn apple plant in inhibiting the growth of some pathogenic fungi. This inhibitory activity of Thorn apple is due to the fact that it contains many antifungal compounds such as tigloidine (3B-tigloyloxytropane), tropine (3a-hydroxy tropane), apoatropine, hyoscyamine and others [29].

3.2. The effect of the alcohol extract of some plants and the chemical pesticide Beltanol on the growth of R. solani

The results (Fig. 3 and 4) showed the effectiveness of the alcoholic extract of the tested plants in inhibiting the growth of the pathogen *R. solani*. Inhibition was 92.6% compared to the control treatment in which the fungus growth was 9cm and the inhibition rate was 0%. Concentrations 1 and 5% achieved good inhibition rates ranging between 40.8- 69.6% and 63- 83.3%, respectively, with a significant superiority for the Thorn apple plant extract, followed by the Sage plant extract and then the Common Hornwort plant extract. On the other hand, the results showed the efficiency of the treatment of the chemical fungicide Beltanol with a concentration of 0.05% in completely inhibiting the pathogenic fungus, which is one of the most efficient chemical pesticides in controlling the pathogen, as it completely prevented the growth of the fungus with an inhibition rate of 100%. The results are consistent with what was found by many studies of the efficacy of the pesticide Beltanol in controlling the disease fungus [30-32], which is attributed to it being an effective pesticide against a wide range of pathogenic fungi and this efficacy is due to the formation of chelating compounds with copper in the host's tissues. This facilitates its way into the pathogen cells, and then it is liberated and kills the pathogen [33]. The inhibitory effect of the Common Hornwort plant extract may be attributed to a change in the properties of the growing medium because the extract contains some inhibitory substances, and due to the lack of studies on the subject, we recommend conducting studies on the subject and identifying the active substances.

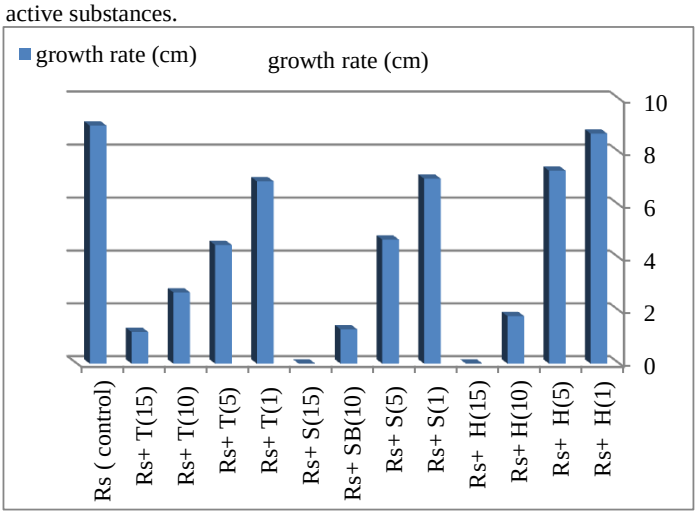

Figure 1. The effect of the aqueous extract of Common Hornwort, Sage and Thorn apple on the growth of *R. solani* in the medium, LSD (*p*0.05)= 0.62.

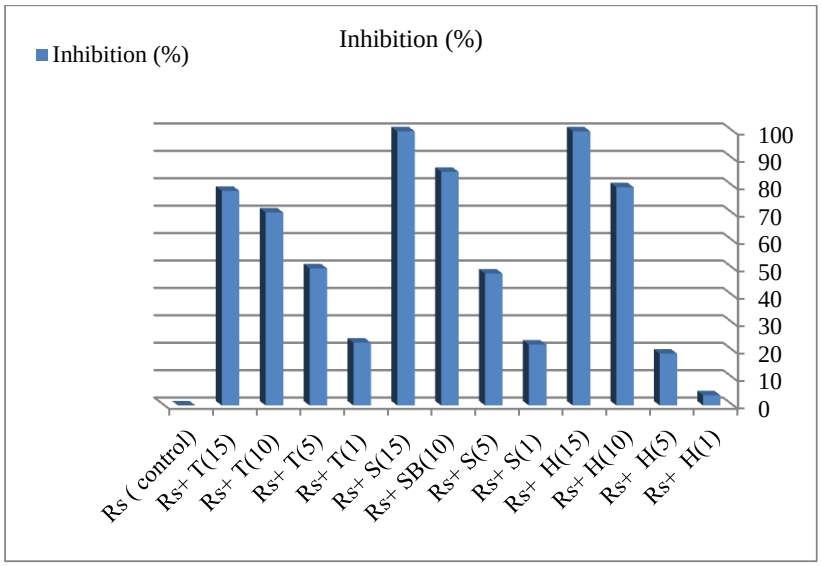

Figure 2. Percentage of inhibition in the growth of the pathogen *R. solani* in the medium with the aqueous extract of Common Hornwort, Sage and Thorn apple, LSD (*p*0.05)=6.87

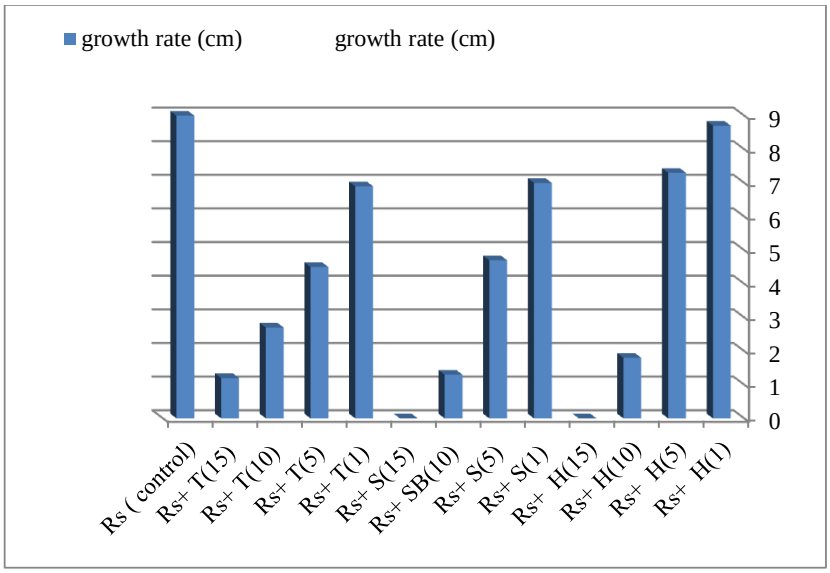

Figure 3. The effect of alcoholic extract of Common Hornwort, Sage and Thorn apple on the growth of *R. solani* fungi in the medium, LSD (*p*0.05)=0.51 .

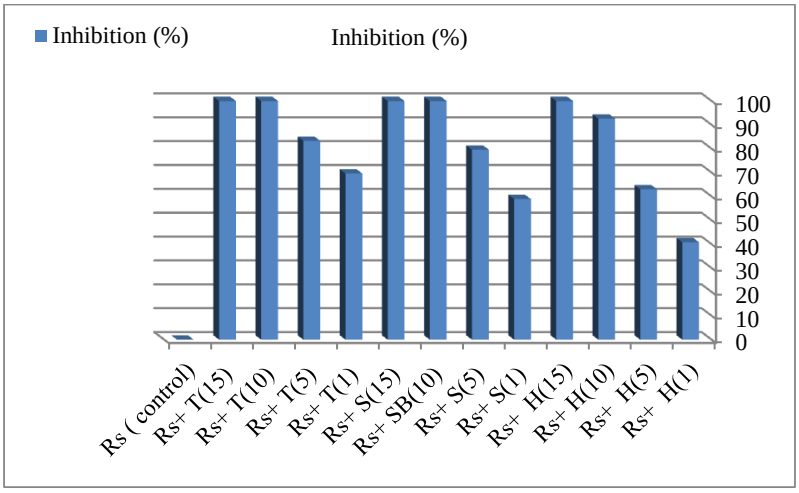

Figure 4. Percentage of inhibiting the growth of *R. solani* in the medium using the alcoholic extract of Common Hornwort, Sage and Thorn apple, LSD (*p*0.05)=5.64 .

Whereas studies have shown that the inhibitory action of Sage plant is due to its containment of many essential oils, Tannis, Oestrogens, Organic acids, Terpenhydroxides, Myrecine, Cymene and other substances that have an inhibitory action for microorganisms, including fungi [34].

3.3. The effect of EM-1 and Bloom 29 Sea on the growth of R. solani in PSA culture.

The results (Figures 5 and 6) showed a significant superiority of EM-1 product over Sea Bloom and the different concentrations used, thus achieving high inhibition ratios reaching a maximum of (100%) at concentrations of 10 and 15% respectively, which is recommended to use a concentration of 10% as there is no difference in the result Concentration to 15%, while concentration 1 and 5% brought about a clear reduction in the rate of fungal growth, causing inhibition rates of 48.5 and 76.3%, respectively. The results were consistent with what Castro et al. [36] found when testing the effect of EM-1 on many pathogenic fungi of the plant in a laboratory, where he observed inhibition of fungi and bacteria in the PDA medium with an addition of EM-1 at a concentration of 5% with different inhibition rates and that the most sensitive fungi was *Pythium* sp. It obtained 100% inhibition, while *R. solani* was inhibited by 50%.

The results also agreement with what the surgeon found [21] that the EM-1 product has good levels of inhibition of the pathogen *R. solani* amounted to 88.67% at concentration 10%, but the percentages of inhibition that the researcher reached do not agree with the percentages of inhibition in the current study, and this difference may be due to different working conditions and different fungal isolates, as the fungus isolates vary in their toxicity and speed of growth between plant hosts, different geographical areas. Sea Bloom29 bio fertilizer contains many nutrients from seaweed, growth regulators, and amino acids, and helps stimulate systemic resistance and increase plant growth.

The effect of EM-1 biosynthesis is due to the components of this product, which is composed of a biological combination that includes many bacterial species, yeasts and selected fungi compatible with each other that are beneficial to plants and soil and that have efficiency in inhibiting the activity of many plant pathogens [37].

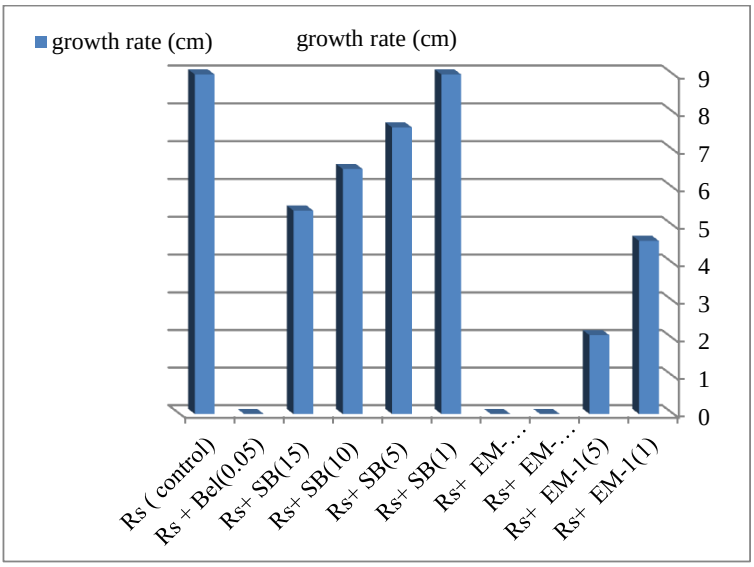

Figure 5. Inhibition of the growth of the pathogen *R. solani* in the culture environment by using the EM-1 microbiology and seaweed preparations LSD (*p*0.05)=0.52.

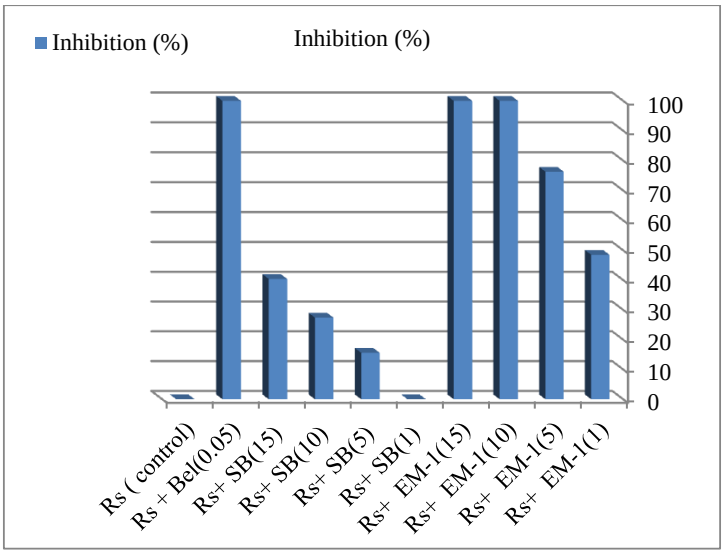

Figure 6. Percentage of inhibition of the growth of the pathogen *R. solani* in the medium using the bio preparation EM-1 and the Sea Bloom 29, LSD (*p*0.05)= 5.81 .

Conclusions

We can concluded from The results of the current study for the first time in the world, the possession of aqueous and alcoholic extract of Common Hornwort, Sage and Thorn apple have a inhibitory effect on the pathogen *R. solani*, which is one of the most dangerous fungi inhabit in the soil and the most pathogenic for the plant. We also conclude the biological effectiveness of EM-1 and Sea Bloom against pathogenic fungi, which gives a new distance for the use of these environmentally safe materials as an alternative to harmful chemical pesticides.

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