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Efficiency of some plant extracts and/or algal extracts in controlling the root rot of sugar-beet caused by *Sclerotium rolfsii*

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6. SUMMARY

Sugar beet plants are often attacked by several pathogens such as fungi, bacteria and viruses which cause great losses in yield.

Sclerotium rolfsii Sacc. (Teleomprph: *Athelia rolfsii* (Curzi) Tu & Kimbrough) a soil-borne plant pathogenic fungi incident of southern sclerotium rot in sugar beet.

Algae are one of the chief biological agents that have been studied for the control of fungi plant pathogens**.** Various strains of cyanobacteria are known to produce intracellular and extracellular metabolites with diverse biological activities such as antibacterial, antifungal and antiviral activity.

Plants are very rich sources of bioactive chemical such as phenolic, polyphenols, quinones, flavonoids, flavonols, cumarins, lectins and polypeptides. Some plants yield fraction of essential oils, which have inhibitory effects on microorganisms. They are gaining popularity and drawing the attention of researches globally due to their volatile, biodegradable, eco-friendly, economical and safety characteristics and are easily variable in local environment

Algal and plant extract are effective and alternative sources of fungi toxic chemicals showing considerable methods for disease control.

Therefore, our study was aimed to:

1- Selection of some effective plant extracts and algal species which have antimicrobial activities.

2- Preparation of extracts from these plants and algae in different solvents and testing their antimicrobial activities on the fungus in vitro.

3- Application of the obtained extracts on the infected sugar beet plants grown in greenhouse.

The present work was carried out at Gemmeiza Agricultural Research Station, Agricultural Research Center (ARC) to control Sclerotium root-rot of sugar beet caused by *Sclerotium rolfsii* under laboratory and field conditions.

Trials were carried out to control the disease by using algae and plant extracts as control agents.

Four algal strains (two green and two blue green algae) were tested. They are *Chlorella vulgaris* and *Scenedesm usobliquus* (green algae) and *Nostoc muscorum* and *Anabaena variables* (blue green algae). Algae extraction was carried out with different solvents (ethanol 100 %, methanol 80 % and acetone 100 %)

Five plant extracts (Roselle - Pomegranate - Black pepper - Rosemary - Chamomile) were screened for their inhibitory effect on the growth of one major root pathogen *S. rolfsii* in the lab.

The impacts of the plant extracts were tested for their efficacy against *S. rolfsii* under the Lab conditions. Four concentrations (0.01, 0.1, 1.0, 10.0 ppm) from Moncut fungicide and six concentrations of each extract (1000, 2000, 3000, 4000, 5000 and 6000 ppm) had been used to test antifungal activities against *S. rolfsii*. Stock solutions of each extract were prepared after dilution in solvent (ethanol, methanol and acetone).

Disease severity was calculated for the rotted roots according to the scale of 1-10 grades.

At harvesting time, vegetative growth characters of sugar beet were recorded in different treatments and control including:, Leaf area, Root length, Root weight and Root diameter. In addition, yield / plot kg and analysis of the total soluble solids (TSS) and sucrose content were estimated.

Total carbohydrate, total soluble sugars, non-soluble carbohydrate, Chlorophyll contents (a, b and Carotenoid), Peroxidase and polyphenoloxidase and Phenolic compounds were determined in the extraction of sugar beet leaves. Also, GC-MS analysis was performed to identify the components of the most effective plant extracts and algal extract.

The obtained results can be summarized as following:

A- Laboratory experiments

1) Algal extracts were screened for their effect on sugar beet root rot under artificial infection with *S. rolfsii* under Laboratory conditions.

- Nostoc and Anabaena reflected some response and reaction against *S. rolfsii* and able to affect the growth causing inhibition of *S. rolfsii* growth compared with the other algal extracts. However the different algal growth differed in their efficiency to reduce fungal growth.
- Results showed high specificity interaction among solvents and algal where, methanol was a good solvent for Nostoc and aceton was strong solvent for anabaena.
- Concerning the solvent reaction, different solvents exhibited wide variation with different algae, where Methanol was the most effective solvents, it recorded almost 50 % inhibition of *S. rolfsii*. While, Acetone reflected efficiency from 30-50 %.
- Nostoc demonstrated a Synergistic effect i.e. Moncut mixed with Nostoc, at the ratio (1:1) and (3:1) showed (SR) values of 1.7 and 1.9 respectively. Data also revealed that, the observed of IC_{50} for these mixtures were lesser than those of expected one. On the other hand, Anabaena and Moncut combination reflected antagonistic effect at

low ratio (1:1) but recorded Synergistic ones at high ratio (3:1) with synergistic ratio (SR) value 1.5.

2) **Antifungal activity of plant extracts against *S. rolfsii* by using solvent acetone extraction:**

- All used plant extract affected the growth and inhibited *S. rolfsii* growth compared to control, in general the extracts differed in their efficiency to reduced the fungal growth and the effects were increased gradually by increasing the concentration from 1000 to 6000 ppm.
- The plant extract of Black pepper was the most effective against S. rolfsii, with an inhibition percentage of 83.62 % followed by Chamomile and Roselle with inhibition percentages of 75.11 and 59.13 respectively.
- All mixtures demonstrated an antagonistic effect i.e. Moncut mixed with Roselle, Pomegranate, Rosemary, Black pepper and Chamomile with (SR) values of 0.058, 0.055, 0.052, 0.036 and 0.033 respectively.
- The methyl extract of *Piper nigrum* acetone was superior to the other extracts and could affect drastically the growth of S. rolfsii compared with the other extracts. Black pepper was the most effective one against the fungus with (89.17%) while Rosemary the lowest in this respect (33.95%).
- The synergy of Moncut fungicide combined with plant extract at the ratio (1:1). All mixtures demonstrated antagonistic effect i.e. Roselle, Black pepper , Chamomile , Rosemary and pomegranate with synergistic ratio (SR) values 0.065, 0.031, 0.029, 0.022 and 0.020 respectively. In case of mixtures the observed values of IC_{50} were lesser than those of expected one, for pomegranate and rosemary (1.190 and 1.070 ppm) respectively.

- In case, 3:1, the synergy of Moncut and plant extract demonstrated an antagonistic effect, where the observed values of IC_{50} for these mixture were lesser that those of expected one.

3) Effect of the tested plant extracts against *S. rolfsii* by using methanol as solvent:

- All the tested plant extracts at different concentration inhibited the growth of *S. rolfsii*. Black pepper was the most effective one against the fungus with inhibitory percentage of 89.17% while; Rosemary was the lowest in this respect (33.95%).
- All mixtures demonstrated antagonistic effect i.e. Roselle, Black pepper, Chamomile, Rosemary and pomegranate with synergistic ratio (SR) values 0.065, 0.031, 0.029, 0.022 and 0.020 respectively. In the case of mixtures the observed values of IC_{50} were lesser than those of the expected one.
- In case, of 3:1 extract: Moncut data revealed that, the mixture of Moncut and plant extract demonstrated an antagonistic effect, hence the observed values of IC_{50} for these mixture were lesser that those of expected one.

4) Impact of tested plant extracts against *S. rolfsii* by using ethanol as solvent:

- The results show that treatment with the different plant extracts reduced the liner growth compared with control.
- Black pepper was the most effective extract against *S.rolfsii*, with an inhibitions percentage of 66.86% followed by Roselle and Chamomile with inhibitions percentages of 50.93 and 43.64 respectively.
- The mixture of Moncut with the tested plant extract at 1:1 showed an antagonistic effect i.e. Moncut mixed with Black pepper, Pomegranate, Rosemary, Chamomile and Roselle with synergistic

ratio (SR) values of 0.072,0.043,0.038,0.033 and 0.032 respectively. The observed values of IC50 for these mixtures were lesser than those of expected one.

- In the case, 3:1 extract : Moncut showed an antagonistic effect i.e., Moncut mixed with Roselle, Rosemary, Pomegranate, Black pepper and Chamomile with synergistic ratio (SR) 0.089, 0.790, 0.660, 0.262 and 0.130 respectively. The observed values of IC_{50} for mixtures were lesser than the expected one (1.00).

B- Greenhouse experiment

1) Algal extracts were screened for their effect on sugar beet root rot under artificial infection with *S. rolfsii* under greenhouse conditions

The obtained results could be summarized as following:

- All evaluated algal extracts were significantly effective in reduction of disease incidence.
- The lowest disease incidence was 7.11%. Generally, Nostoc extract in methanol, mixed with Moncut as seed soaking recorded the lowest disease incidence as Moncut fungicide alone
- Disease severity showed that all evaluated algal extracts were significantly effective compared with control pathogen. However, methanol extract of Nostoc soaking or mixed with Moncut as coating was highly effective in improving of healthy roots. Generally, Nostoc extract was superior to the other treatments in decreasing the disease severity percent of root rot caused by *S. rolfsii*, while the highly disease severity was recorded of Anabaena algae extract as seed soaking mix Moncut (5.24 %).

- Using Nostoc extract as seed coating with Moncut and Nostoc seed soaking with methanol solvent gave the highest yield / plot (16.66 and 15.66 Kg) respectively compared with control pathogen coating seeds (13.83 Kg).
- TSS and sucrose % of sugar beet root juice were significantly influenced by using the different algae culture filtrates.
- The highest increase in leaf area was recorded by using Nostoc mixed with Moncut as seed coating followed by Anabaena mixed with Moncut as seed soaking treatment (3000.217 cm^2 and 2900.330 cm^2) respectively.
- All treatments improved the root length compared with control infected.
- All treatments caused high increase in root diameter compared with control DMSO as seed soaking and control pathogen as soaking treatment. Anabaena / acetone as seed soaking treatment and Anabaena mixed with Moncut as seed coating increased the root diameter (46.433 cm and 43.567cm) respectively.
- As regards to root weight the results show that all treatment improved root weight (Kg) compared with control infected.
- At seedling stage, Nostoc / Methanol seed soaking and Anabaena mixed with Moncut as seed coating gave the highest percentage of total carbohydrate (7.983 % and 7.540 %) and soluble (5.116 % and 3.031 %) respectively, While insoluble carbohydrate, Anabaena mixed with Moncut as seed soaking and coating showed the best treatment (5.438 and 4.509).
- Total and insoluble carbohydrate of pre-harvest stage showed that highest percentage (7.098 % and 6.852 %) respectively. while in the case soluble differed in this respect.

- At seedling stage, the maximum amount of Chl. a was recorded in case of Anabaena mixed with Moncut as seed soaking and Nostoc mixed with moncut as seed coating (15.129 and 14.185 mg/g) respectively. Maximum amount of Chl. b was recorded in Anabaena mixed with Moncut as seed soaking and Nostoc mixed Moncut as seed soaking (6.819 and 5.410 mg/g) respectively.
- At pre-harvest stage, the maximum amount of chlorophyll contents was recorded by Nostoc / methanol as seed coating and Anabaena mixed moncut as seed soaking.
- In seedling stage, Nostoc / methanol as seed soaking and Nostoc mixed moncut as seed coating recorded the highest activity of peroxidase enzymes. On the other hand, in case pr-harvest Moncut as seed coating and Nostoc/methanol as seed coating increased peroxidase activity.
- In general all treatments showed high activity of peroxidase and polyphenoloxidase (seedling or pre- harvest) compared with control infected.
- In the case of seedling, using of Nostoc extract mixed with moncut as seed soaking showed the highest amount of total phenols followed by Nostoc / methanol as seed coating. Anabaena mixed moncut fungicide as seed coating was the lowest in this respect.
    - On the other hand, in the case of pre-harvest, the data showed that, treatment with Anabaena mixed moncut as seed soaking or seed coating showed the highest amount of total and free phenols.

2) Plant extracts were screened for their effect on sugar beet root rot under artificial infection with *S. rolfsii* under greenhouse conditions

- Pomegranate was the most effective in reduction of disease incidence percentage compared with the other treatments.

- Pomegranate and Black paper exhibited the lowest disease severity in this respect compared with control with pathogen .
- Pomegranate and Black paper recorded the highest yield / plot compared with the infected control.
- The yield/plot was increased due to treatments as coating or soaking seeds in any of the plant extract. Acetone pomegranate extract followed by pomegranate / ethanol caused the highest increase in the yield /plot compared with the other treatments.
- Concerning the estimated TSS and sucrose content in beet roots, Black pepper extract showed the highest increase in TSS and sucrose content 19.569 and 13.551 % respectively, compared with the infected control. While the aceton extract of black pepper recorded 18.363% and sucrose percentage of 10.925%.
- The highest increase in leaf area was recorded by plant extract pomegranate compared with control infected.
- All treatments improved the root length compared with the infected control. Black pepper extract increased root length significantly.
- All treatments improved root weight (Kg) compared with infected control. Generally pomegranate extract showed the highest increase in root weight.
- At seedling stage, treatment with Black pepper and Pomegranate extracts showed the highest percentage of total carbohydrates, Soluble and insoluble. While, in case of pre-harvest, total carbohydrate showed that plant extract black pepper and pomegranate caused marked increase in the percentage of total carbohydrate. For soluble carbohydrate of the control healthy followed by control with solvent and pomegranate showed the highest percentage compared with control infected.

- The increase in the activity of peroxidase and polyphenoloxidase within sugar beet as a result of treatments application (seedling and pre-harvest) was found to be the highest in comparison with the control. In case of seedling and pre-harvest plant extract black pepper and pomegranate recorded the highest peroxidase and polyphenoloxidase (peroxidase, 1.114, 1.061, 0.881 and 0.792) respectively, while polyphenoloxidase recorded (0.355, 0.312, 0.359 and 0.293) respectively compared with control infected.

- In case of seedling, pomegranate extract and black pepper extract recorded the highest amount of total phenols (11.926 and 11.860 mg/g fresh weight). Black pepper and Pomegranate have the highest amount of free phenols (8.939 and 8.519 mg/g fresh weight) respectively. While control healthy have the highest amount of conjugated phenols (7.126 mg/g fresh weight). In case of pre-harvest, Pomegranate exhibited the highest amount of total phenols (14.878 mg/g fresh weight) followed by Black pepper (14.458 mg/g fresh weight) compared with control infected (10.636 mg/g fresh weight).While Pomegranate extract induced synthesis of high amount of free phenols (8.510 mg/g fresh weight) compared with infected control (6.908 mg/g fresh weight). While, Pomegranate and Black pepper caused the synthesis of high amount of conjugated phenols (6.369 and 6.223 mg/g fresh weight).

- **GC-MS analysis of Nostoc extracts**

- The most effective antifungal activity of Nostoc extracts against *S. rolfsii* could be attributed to the highest and major content of 5,7,3',4'- Tetramethoxyisoflavone.

- In addition, the most common compounds in Nostoc extracts belongs to natural flavonoids such as 5, 7, 3', 4'-Tetramethoxyisoflavone and 7, 3', 4', 5'- Tetramethoxyflavone. Flavonoids are phenolic compounds composed of fifteen carbons that are found in plants.

GC-MS analysis of Black paper extract

- Black paper extract exhibited a unique and highly complex GC-MS profile analysis of main chemicals constituents, therefore, the highest ingredients with high Area Sum % will presented as follow: Glycitein 7-(6-O-acetyl-beta-D-glucoside) is a glycosyloxyisoflavone that is glycitin carrying an acetyl substituent at position 6 on the glucose moiety. It has a role as a plant metabolite. It is an acetate ester, a glycosyloxyisoflavone, a hydroxyisoflavone, a methoxyisoflavone, a monosaccharide derivative, a beta-D-glucoside and an O-acyl carbohydrate. It derives from a glycitin. 6''-O-Acetylglycitin already representing 26.55 % of Area Sum % and could be play a vital role in inhibition of *S. rolfsii*. Carvacrol representing 4.15 %, is a monoterpenic phenol produced by aromatic plants, carvacrol possess a variety of biological properties including antioxidant, antibacterial, antifungal. Piperine is the major plant alkaloid present in black pepper.

GC-MS analysis of Pomegranate extract

- Pomegranate extract exhibited a unique and highly complex GC-MS profile analysis of main chemicals constituents, therefore, the highest ingredients with high Area Sum % and based on peak area presented as follow: 7, 4'-Dimethoxy-3-hydroxyflavone was considered as the

major components in pomegranate extract whereas representing area sum 48 %, therefore the inhibition percentage against *S. rolfsii* could be attributed to this compound as aforementioned with Nostoc and Black papper, Dinicotonic acid represented high Area Sum % reached to 9.39 %. Thererfore, it may play a role against *S. rolfsii* through Pomegranate extract. Dinicotinic acid (pyridine-3, 5-dicarboxylic acid) is an organic compound that belongs to the heterocycles (more precisely, heteroaromatics). It belongs to the group of pyridine dicarboxylic acids and consists of a pyridine ring, which carries two carboxy groups in the 3- and 5-position.