Biosynthesis of Ag nanoparticles by associated root rot disease fungi of sugar beet and its biocontrol by Trichoderma hamatum fungus

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ABSTRACT

Rhizoctonia solani, Sclerotium rolfsii, Fusarium oxysporum and other fungi were positive in extra
than intracellular production of Ag NPs with different in ability. The tested fungi could be reduced
silver salt to Ag NPs and confirmed stable brown color visually in mixture solution. Consequently, UV-
Vis spectral analysis to reaction mixture of cells filtrates and silver nitrate salt were showed strong
absorbance at peak 420nm which was specific for the silver NPs performance. In addition to, Fourier
transform infrared (FTIR) of dried powder samples of AgNPs from R.soleni, Sc. rolfsii, F.oxysporum
mycelium showed the presence of nine bands at cm⁻¹. Moreover, Energy Dispersive X-ray (EDX)
spectroscopy analysis for AgNPs conformation showed presence of elemental silver by sharpening
signals at optical absorption band peak exactly at (3 keV) to the three fungi. As size of AgNPs,
Scanning Electron Microscopy (SEM) showed synthesis of polydisperse spherical ranged of 47-62
nm to R. solani, ranged from 84-134 nm to Sc.rolfsii and 31-47 nm to F.oxysporum and rose
like(pentagons). Mycelium of tested fungi contained Ag NPs of 5.36, 9.82 and 10.38% of mass,
respectively. F.oxysporum was the most producer one but R. solani and Sc. rolfsii exhibited a new
source to Ag NPs. In vitro, Trichoderma hamatum have mycoparasitism ability of Sc.rolfsii and R.solani fungi mycelium. Trichoderma inoculum at 4, 2g/hill were superior to 60 and 120g/ plot as soil
drench in the revers, Trichoderma (1cm disk) / hill grown on PDA was the lowest one in controlling
of root rot disease in sugar beet. F. oxysporum followed by Sc.rolfsii were more effective than R.solani
by Trichoderma application. At all, Trichoderma led to reduce damping off by 3.22-82.71%; root rot
disease severity by 9.36-43.07%; disease index by 51.82-85.79% ; sucrose and total soluble sugar
enhanced by 2.37-36.24%.

Keywords: R.solani, Sc. rolfsii and F. oxysporum, Ag NPs, trichoderma parasitism, sugar beet.

1. Introduction

In Egypt, sugar-beet is attacked by several root-rot pathogens mainly Rhizoctonia solani Kuhn,
perfect stage (Thanatephorus cucumeris), Sclerotium rolfsii (Sacc), perfect stage (Athelia rolfsii) and
Fusarium oxysporum (Schlech), the most aggressive pathogens to cause root rot infection at both pre
and post–emergence stage (Aly and Hussein 2009).Sclerotium root-rot is a serious disease of sugar
beet in the irrigated region and disease incidence varied from traces to 50% (Abo El-Yazied, 2019).
R. solani, causes one of the most damaging sugar beet diseases more than 24% and incidence of this
disease seems to be increasing (Barholomaus et al., 2017).

Biosynthesis of nanoparticles by soil microorganisms constitute one of the vast and strong natural
factories and harness beneficial effects through biotechnology (Khan 2007) and nanotechnology
(Gurunathan et al. 2009). Synthesize silver nanoparticles (AgNPs) extracellularly by many numbers of
fungal strains are capable to among which F.oxysporum (Ahmad et al. 2003), Aspergillus fumigatus
(Bhainsa and D'Souza, 2006), A. niger, (Gade et al.2008), F. semitectum (Basavaraja et al. 2008),
Penicillum brevicompactum (Shaligram et al. 2009), Cladosporium cladosporioides (Balaji et al.2009)

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2. Material and Methods

The present work was carried out at Sakha Agric. Res. Station during 2019/2020 and 2020/2021 growing seasons.

2.1. Microbial synthesis of Ag-NPs

Three isolates of *F. oxysporum*, isolate of *R. solani* isolated from sugar beet, *cv* pelatos (kafr elShiekh location) isolate of *T. hamatum* iso2 and *T. harzianum* and one from *S. rolfsii* isolated from sugar beet, *cv* maxime (El-Ryad location); *F. semitectum*, *F. verticillioides* and *Aspergillus niger* were isolated from maize, *cv* balady and four isolates of *Alternaria alternate* were isolated from forage sorghum, *cv* Sudan grass (kafr elShiekh location) were used for the synthesis of Ag-NPs. The fungi were subjected to Ag-NPs as methods adopted by Gajbhiye et al. (2009) with some modification, which BD broth medium and cell filtrate were challenged with AgNo3 aqueous solution (0.078 g/100 ml), as El-Rafie et al. (2012) and biomass challenged with 100 ml of AgNo3/10 g biomass and incubated at room temperature until appearing visual observation stable dens color up to 3 days as follows: -: light pink (AgNo3); +: dens pink; ++: dark red color; +++: dark brown.

2.2. Ag NPs characters:

The mixture solutions from cells filtrate of all tested fungi separately and AgNo3 aqueous were subjected to optical measurements, which were carried out by using a UV-Vis spectrophotometer (Model spectronic 21) and scanning the spectra between 300 to 600 nm at the solutions, the purified solution yielded maximum absorbance peak at 420nm, Gajbhiye et al. (2009). In Fourier transform infrared (FTIR) analysis, the FTIR spectrum of dried mycelium of *R. solani*, *S. rolfsii* and *F. oxysporum* fungi was recorded on PerkinElmer 1600 instrument in the rang 400-4500 cm$^{-1}$ at a resolution of 4 cm$^{-1}$. Energy dispersive x-ray (EDX) spectroscopy analysis for confirmation of detection and *A. clavatus* (Verma et al. 2010) have been previously described. El-Rafie et al. (2012) used fungus *F. solani* for biosynthesis of silver while Raida (2013) used *T. longibrashiatum*. Moreover, Magdy et al. (2014) added that, *F. oxysporum*, *Alternaria solani* and *A. flavus* were common ones in biosynthesis of silver NPs. Noshad et al. (2020) studied the synthesize AgNPs green chemistry route using mycellial aqueous extract of *Pythium oligandrum*.

Biocontrol encompases of plant diseases were approaches, *Trichoderma* (sexual teleomorphic stages *Hypocrea spp.*, Seidl et al. 2009) biocontrol in mechanisms, via competition for space and nutrients, antibiosis and stimulation of plant defense mechanisms or direct mycoparasitism or combination of both, Lorito et al. (2010). *T. hamatum*, *T. harzianum*, *T. polysporum* and *T. viridea* used to reduce the mycelial growth of the *Macrophomina phaseolina*. *T. harzianum* was the most effective inhibition and recorded the least root rot incidence of 5% under field conditions (Ramezani, 2008). *T. viridea* suppressed disease severity of the sugar beet root rot in presence of *F. solani* and *R. solani*. (Aly and Hussein 2009). Yadav (2012) stated that *T. viridea* and *T. harzianum* completely colonized *F. oxysporum*. Nawar (2013) mentioned that the maximum reduction radial growth against *Sc. rolfsii* was observed in the *T. album* followed by *T. viridea* responsible for 44.66% and 29.08% inhibition with significant difference. *T. harzianum*. *T. hamatum* showed maximum growth inhibition of *R. solani* and *Sc. rolfsii* whereas *T. virens* was most aggressive against *R. bataticola*, Divya et al. (2015). *T. harzianum* and *T. viridea* isolates have reduced the severity of the disease in greenhouse conditions against *R. solani* that show high inhibition rate (Durak, 2016). Singh et al. (2013), Ahmed and Ahmed (2015) observed that *T. harzianum* reduced disease severity of *Sc. rolfsii* of tomato and *Allium* white rot caused by *Sc. cepivorum*, respectively. El-Tarabily (2003) recorded that, *T. asahii* was capable of colonize sugar beet roots and protecting seedlings and mature plans from *R. solani* disease. Most of the *Trichoderma* isolates showed a strong antagonistic activity, thigh lights the possibility of using *T. harzianum* in IPM programs as an effective biological agent against *R. solani*, *P. ultimum*, *A. solani*, Mazrou et al. (2020). Moreover, *T. hamatum* recorded 81.80% antagonist of *F. oxysporum*, Mao et al. (2020). Information related to biosynthesis of Ag nanoparticles by root rot fungi pathogens of *Fusarium oxysporum*, *Sclerotium rolfsii*, *Rhiococtonia solani* and other pathogens for the best of our Knowledge, lacking in the literature, therefore, investigate the aim of this study and achieve *Trichoderma* biocontrol of root rot disease management.
elemental silver was carried out. A scanning electron microscopy (JEOL JSM-IT100, Tokyo, Japan) was used to take micrograph images of synthesized AgNPs to know the shape and size.

2.3. Biological control of root rot pathogens:

2.3.1. In vitro:

Two isolates of *T. hamatum* and one of *T. harzianum* were isolated from soil surrounded of sugar beet of cv. maximeouse (El-Ryad location). Antagonism procedure between these isolates and major causal of sugar beet root rot fungi, *i.e.* *F. oxysporum*, *R. solani* and *Sc. rolfsii* was done as Ferreira et al. (1991) and examined microscopically at 10 and 40X magnification (Optika, B-193 Germany) with helping computer unit with Toup view 3.7 program after complete contact the two tested fungi (2-3 days) and take a photo. Growth of pathogenic fungi was estimated /cm after 7 days , four replicates were used for each treatment . Percentage of growth inhibition (PI) as efficiency of bio-control agent for antagonist the causal organism was determined using the equation adopted by Rewal and Thooty (1995) as follow:

\[
\text{PI} = \frac{\text{Control- Treatment} \times 100}{\text{Control}}
\]

Sclerotia and/ or like / plat for *R. solani* and *Sc. rolfsii* were counted and sporulation of *F. oxysporum* / plat were estimated microscopically at 40x magnification by helping of hymacytometer plate as Mandeel and Baker (1991).

2.3.2. In vivo (green house):

*T. hamatum* iso 2 was selected which have antagonism by parasitism in vitro, grown in autoclaved moistened wheat bran medium for two weeks at 25°C. *Trichoderma* inoculum added with planting as follows : disk/ hill(1cm) grown in PDA ; 2 , 4 g / hill (60, 120g / micro plot) and 60 , 120g / micro plot added as soil drench over row. *F. oxysporum*, *R. solani* and *Sc. rolfsii* fungi were grown in autoclaved moistened corn meal sand medium for two weeks and used at rate of 5g/hill separately, added with planting. Control treatment was every pathogenic fungus alone. Complete randomize design with three replicates was used. Sugar beet, cv. pelatos was planted in microplots (2x1.8m) in wire house in rows 60cm apart and 5 seeds / hill. All cultural practices were done at proper time. Seedlings were counted after 25 and 45 days from planting to calculate of pre- post emergence damping off percentages. Plants were uprooted and roots were checked for root-rotting. Disease severity recorded as rating 0-4 grades as follows :0 = no disease, 1=less than 25% of vascular element necrotic or localized lesions on root,2=26-50% vascular necrosis or less than10% of taproot rotted , 3= over 50% necrosis vascular elements and 10-25of taproot rotted, and 4= more than 25% taproot rotted after harvesting directly at 180 days of sowing date and disease index (DI) were calculated from root disease rating as of Harveson and Rush (1994) using equation : 

\[
\text{DI} = \frac{\text{DR1x1} + \text{DR2x2} + \text{DR3x3} + \text{DR4x4}}{\text{EDR0-4}},
\]

where 

\[
\text{DR0} = \text{number of roots rated 0, DR1 = number of roots rated 1,et.}
\]

Sucrose and total soluble solids (TSS) were determined in fresh roots using Sacarometer and Refrectometer according to AOAC (2005) and McGinnis (1982).

2.4. Statistical analysis

Data were analyzed statistically using the analysis of variance and the means were further tested using the least significant difference test (LSD) and the means were further tested using DMART test as outlined by Steel and Torrie (1980).

3. Results and Discussion

3.1. Microbial biosynthesis of AgNPs:

After addition of aqueous AgNO3( 0.078 g / 100 ml) to fungal cells filtrate, biomass and PD broth medium according to protocol listed above by Gajbhiye et al. (2009), Ag NPs was synthesized by all tested fungi in Table (1). *i.e.* *F. oxysporum* (3 isolates) as reported by (Ahmed et al. (2003) and (Magdy et al. 2014); *Sc. rolfsii*, *R. solani*;*T. harzianum*; *T. hamatum* iso 2 as found with *T. longibrashiatum* by (Raida 2013); *F. semitectum* and *F. verticillioides*, similarly, extracellular biosynthesis of silver NPs was done by *F. semitectum*, (Basavarja et al. 2008) and , *F. solani* (El-Rafie et al. 2012); *A. niger* as which produced by *A. niger* (Gade et al. 2008) and *A. fumigates* (Kuber et al. 2006); and *A. alternare* (4 isolate), as biosynthesis by *A. alternare* (Gajbhiye et al. 2009), *A. clavatus* (Verma et al. 2010) and
(F. oxysporum, Alternaria solani and A. flavus, Magdy et al. 2014), color changes appeared within 1-3 days in the reaction mixture at lab. temperature indicating the completing of the reaction. The intensity of colors steadily increased along the incubation period. In contrast, silver nitrate solution without fungi filtrate and/or biomass showed negative reaction (no color changes, light pink). AgNPs solutions exhibited dark red to brown color as Fig (1), this due to reduction of aqueous solution by silver ions and formation of AgNPs, Gajbhiye et al. (2009). Silver ions required the NADH – dependent nitrate reductase enzyme for their reduction, Roh et al. (2001) or electron shuttle for F. oxysporum, Jain et al (2011) or both and secreted by the tested fungi in its extracellular. In the most cases, filtrates of fungi recorded dens color more than biomasses while PD broth was the least color ones, this mean extracellular biosynthesis of AgNPs than intracellular one. F. oxysporum iso3 was the most one in this respect followed by F. semitectum. The vast and strong natural factories to biosynthesis of nanoparticles were constituted by soil microorganisms (Khan 2007) like Fusarium spp. and Aspergillus spp. (Rai and Kratosova 2015), Penicillium brevicompactum (Shaligram et al. 2009), Cladosporium cladosporioides (Balaji et al. 2009). Noshad et al. (2020) synthesized AgNPs using mycellial aqueous extract of Pythium oligandrum, suggesting a strong candidate for industrial scale production of AgNPs.

Table 1: The listed fungi were used to biosynthesis of Ag nanoparticles as nature source.

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Source</th>
<th>Reaction</th>
<th>Filtrate</th>
<th>Biomass</th>
<th>PD broth</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. oxysporum iso1</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>F. oxysporum iso2</td>
<td></td>
<td></td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>F. oxysporum iso3</td>
<td>Sugar beet root</td>
<td></td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Sc. rolfsii</td>
<td></td>
<td></td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>R. solani</td>
<td></td>
<td></td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>T. harzianum</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>T. hamatum iso2</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>F. semitectum</td>
<td>Maize grains</td>
<td></td>
<td>+ +</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>F. verticillioides</td>
<td></td>
<td></td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A. niger</td>
<td></td>
<td></td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A. alternate iso1</td>
<td>Sorghum grains</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A. alternate iso2</td>
<td></td>
<td></td>
<td>++</td>
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<tr>
<td>A. alternate iso3</td>
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<td>+</td>
</tr>
<tr>
<td>A. alternate iso4</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Light pink (AgNO₃); +: dens pink ; ++ : dark red color ; +++: dark brown color.

Fig. 1: Dark stable brown color with reaction mixture of F, Fo (F. oxysporum), Rs (R. solani) and Sc (Sc. rolfsii) filtrates and biomass means confirmed of Ag NPS and AgNO₃ in the left.

The UV-Vis spectral analysis of reaction mixtures confirmed the synthesis of Ag NPs from filtrates aqueous solutions by the using of wave length scan at 240nm (absorbance peak, Gajbhiye et al., 2009) and showed strong absorbance (Fig. 2 and 3). F. oxysporum iso1 was the most one followed by F. semitectum. Dark red color and/or brown were appeared as shown in Fig (1) in chemical reaction due to reduction of aqueous solutions by Ag NO3 (silver ions) and formation of Ag NPs from fungi filtrates aqueous solutions. The colloidal suspension of Ag NPs are stable for many months, interestingly possible used in many application fields. So, the isolated fungi from rotted infection of sugar beet roots and others exhibited fungi Ag NPs producer and new sources. Presence of NADH –
dependent nitrate reductase enzyme in extracellular cell filtrate of fungi used for the synthesis of Ag NPs had been confirmed, Anilkumar et al. (2007). Ingle et al. (2008) and Magdy et al. (2014) demonstrated that, fungal cell filtrates challenged by silver nitrate solution showed peak around 420 nm with high absorbance indicating synthesis of Ag NPs.

Fig. 2: UV-visible spectral of filterates containing AgNPs of fungi isolated from sugar beet roted root.

Fig. 3: UV-visible spectral of fungi filterates of *F. semitectum*, *F. verticilliodes* and *A. niger* isolated from maize grains and four isolates of *A. alternata* isolated from surgum seeds containing Ag NPs.

FTIR measurements of dried powder mycelium of AgNPs of *R.solani*, showed the presence of nine bands at cm$^{-1}$ as figure (4a), band at 3793,3567,2866,1651,1556,1419,1277,1067,631. FTIR measurements of dried powder mycelium of AgNPs of *Sc. rolfsii*, showed the presence of nine bands at cm$^{-1}$ as figure (4b), band at 3745,3436,2897,1687,1524,1439,1315,1038,745. Finally, FTIR measurements of dried powder mycelium of AgNPs of *F. oxysporum* iso1, showed the presence of mean nine bands at cm$^{-1}$ as figure (4c), band at 3479, 2923, 2235, 1759, 1405, 1345, 1235, 1058, 743. Present results supported by the finding of Gajbhiye et al. (2009), who found presence of nine bands with Ag NPs of *A. alternata* and Gole et al.(2001) found that, proteins can band to nanoparticles through free amine or the electrostatic attraction of negatively charged carboxylate groups in enzymes present in cell wall of mycelium and stabilization of AgNPs by protein occurs and secreted by *F. oxysporum* (Ahmed et al.2003).
Fig. 4: FTIR spectra of powder mycelium of AgNPs synthesized by *R. solani* (a), *Sc. rolfsii* (b) and *F. oxysporum* (c) fungi.

EDX spectroscopy analysis for conformation of AgNPs was performed and confirmed the presence of elemental silver by sharpening signals as showed in figures (5a, b, c).
Energy KeV

Fig. 5: Spectrum of Ag NPs by EDX spectroscopy to *R. solani* (a), *Sc. Rolfsii* (b) 3and *F. oxysporum*(c) fungi.

Cu: copper; Zn: Zinc; PS: potassium; N: nitogen; O: oxygen; C: carbon; P: phosphorus; S: sulphur; Si: silicon; Cl: color.

The optical absorption band peak in the range of 2-4keV exactly at (3 keV) to *R. solani* (a), at 3 keV to *Sc. Rolfsii* (b) and 2-4keV exactly at (3 keV) to *F. oxysporum*(c) as was typical for the absorption of metallic AgNPs crystals as reported by Magudapathy et al.(2001) . Mycelium of the tested fungi contained of 5.36, 9.82 and 10.38% mass of Ag NPs, respectively, so *F. oxysporum* was the most producer one to Ag NPs but *R. solani* and *Sc. Rolfsii* were a new producer to it.
Topology and size of AgNPs was carried out by scanning electron microscopy and showed the synthesis of polydisperse spherical AgNPs in the range of 47-62 nm with average size of 54.5nm as showed in figure (6 a) to *R. solani*, polydisperse spherical, rose like(pentagons) in the range of 84-134 nm with average size of 109 nm to *Sc. rolfsii*, figure (6b) and polydisperse spherical AgNPs in the range of 31-47 nm with average size of 39nm to *F. oxysporum*, figure (6c) fungi. Many scholars found that, mostly spherical AgNPs in the range of 5-15nm exposed by *F. oxysporum* (Ahmed et al. 2003), 10-60 nm exposed by *F. semitectum* (Basavaraja et al. 2008) and 5-25nm by *A. fumigates* (Kuber et al. 2006),10-100nm by *F. oxysporum* with shape hexagons, pentagons, circular, squares and rectangular in both intra and extra production (Mohammadian and Rezaee 2007).

Fig. 6: Scanning electron microscopy of aggregation and spherical of Ag NPs (47-62nm) to *R. solani* (a), (84-134nm) to *Sc. rolfsii*(b) and (31-47nm) to *F. oxysporum*(c) fungi.
3.2. Biological control of sugar beet root rot pathogens:

3.2.1. In vitro:

Data stated in table (2) showed that, three Trichoderma isolates were subjected to antagonist test on PDA medium with main causal pathogens of sugar beet root rot, results showed that *T. harzianum* retarded the growth of *F. oxysporum* fungus and exhibited the most Trichoderma effective one, ie. 1.93 cm compared to 8.11 cm of control, causing growth inhibition by 76.20% followed by *T. hamatum iso2* and 1 which recorded growth inhibition by 73.36 and 69.66%, respectively. These Trichoderma isolates led to reduce of sporulation of this fungus wherever *T. harzianum, T. hamatum iso1* were superior followed by iso2, recording inhibition of spores production by 71.52,71.32 and 43.04%, respectively.

Trichoderma isolates were reduced *R. solani* fungus growth with no significant between them compared to control and *T. hamatum iso2* was recorded the most growth inhibition followed by *T. harzianum* and *T. hamatum iso1*, ie. 58.77,57.11 and 55.33%, respectively. Sclerotia and/or like formation was completely stopped as antagonistic effect of this isolates. Mycelial growth of *Sc. rolfsii* was reduced as in *R. solani* and *T. hamatum iso1* was recorded the most growth inhibition followed by *T. harzianum* and *T. hamatum iso2*, ranged from 72.54-77.01%. Sclerotia and/or like formation was significantly reduced especially with *T. harzianum* and *T. hamatum iso1*, while *T. hamatum iso2* was the least one in this target, ranged from 60.04-96.22%. Many scholars supported this investigation, ie. Ramezani (2008) reported that, *T. hatman, T. harzianum, T. polysporum* and *T. viride* reduced mycelial growth of *M. phaseolina*, *T. viride* and *T. harzianum* completely colonized *F. oxysporum* as stated by Yadav (2012). "Nawar (2013) mentioned that, max. radial growth reduction of *Sc. rolfsii* was observed in the *T. albium* (44.66%), while *T. viride* responsible for 29.08% inhibition with significant difference with *T. harzianum*. In vitro, efficacy of three bioagents, *T. harzianum* was the most effective followed by *T. viride* and *T. viride* proved least effective in inhibiting the mycelial growth of *A. solani*, Ganie et al. (2013).

Additionally, Divya et al. (2015) found that, *T. harzianum* showed max growth inhibition of *M. phaseolina*, ie. 81.11%, *R. solani* (82.59%) and *Sc. rolfsii* (76.67%) whereas *T. viride* was most one against *R. bataticola* (68.15%). *T. harzianum* has the highest activity in reducing the radial growth of *Sc. rolfsii* on PDA media and also for glucanase enzyme production. Mostly, the strong antagonistic activity against *R. solani, P. ultimum, A. solani* was done with Trichoderma isolates showed, possibility of using *T. harzianum* in IPM programs, Mazrou et al. (2020). Moreover, *T. hamatum* recorded inhibitory rate by 81.80% of *F. oxysporum*, Mao et al. (2020).

Table 2: Effect of *Trichoderma* isolates of growth and sporulation of *F. oxysporum*, growth and sclerotia production of *R. solani* and *Sc. rolfsii* fungi in vitro.

<table>
<thead>
<tr>
<th>Trichoderma isolates</th>
<th><em>F. oxysporum</em></th>
<th><em>R. solani</em></th>
<th><em>Sc. rolfsii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Growth (cm)</td>
<td>Growth Inhibition %</td>
<td>Growth (cm)</td>
</tr>
<tr>
<td><em>T. harzianum</em></td>
<td>1.93c</td>
<td>76.20a</td>
<td>3.86b</td>
</tr>
<tr>
<td><em>T. hamatum iso1</em></td>
<td>2.46b</td>
<td>69.66c</td>
<td>4.02b</td>
</tr>
<tr>
<td><em>T. hamatum iso2</em></td>
<td>2.16bc</td>
<td>73.36b</td>
<td>3.71b</td>
</tr>
<tr>
<td>Control</td>
<td>8.11a</td>
<td>-</td>
<td>9.00a</td>
</tr>
<tr>
<td>Trichoderma isolates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spores</td>
<td>Spores</td>
<td>Sclerotia</td>
</tr>
<tr>
<td></td>
<td>x10^6 Inhibition %</td>
<td>No. Inhibition %</td>
<td>No. Inhibition %</td>
</tr>
<tr>
<td><em>T. harzianum</em></td>
<td>1.33c</td>
<td>71.52a</td>
<td>0.00b</td>
</tr>
<tr>
<td><em>T. hamatum iso1</em></td>
<td>1.34c</td>
<td>71.32a</td>
<td>0.00b</td>
</tr>
<tr>
<td><em>T. hamatum iso2</em></td>
<td>2.66b</td>
<td>43.04b</td>
<td>0.00b</td>
</tr>
<tr>
<td>Control</td>
<td>4.67a</td>
<td>-</td>
<td>9.00a</td>
</tr>
</tbody>
</table>

In the same column, means followed by the same letter are not significantly different according to DMRT at 5% level of significance.

3.2.2. Mycoparasite studies

Microscopical examination to antagonism between *T. harzianum* and *F. oxysporum*, *R. solani* and *Sc. rolfsii* fungi showed no parasitism phenomena was happened, so it was inhibited the pathogenic fungi by competition on space and may be competition on nutrients. *T. hamatum iso1* and 2 recorded parasitism on hyphae of *R. solani* and *Sc. rolfsii* only and the reverse was true in case of *F. oxysporum* as showed in figures 7,8,9 with *T. hamatum iso2*. As figure (7): the scanning micrograph of interaction...
between trichoderma and *Sc. rolfsii* showed that, condensed coiling of trichoderma hyphae around a hypha of *Sc. rolfsii* (a,b,c), partial degradation of *Sc. rolfsii* mycelium (d), hock like and penetration by appressorium like of trichoderma hyphae to *Sc. rolfsii* mycelium (e) meaning of parasitism presses by many modes of actions in comparison normal hyphae of *Sc. rolfsii* (f).

![Fig. 7: a-e, Scanning micrograph of interacting of *T. hamatum*. Iso 2 hypha with *Sc. rolfsii*. a, b, c: Condensed coiling of *T. hamatum* around a hypha of *Sc. rolfsii* (10x and 40x, 100), d: Partial degradation of host mycelium (40x, 100), e: Hock, penetration by appressorium like and coiling of *T. hamatum* to hypha of *Sc. rolfsii* (40x, 100) means parasitism. f: Normal hyphae of *Sc. rolfsii* fungus (40x, 100).](image)

Scanning micrograph of interaction between trichoderma and *R. solani* showed that, condensed coiling of trichoderma hyphae around a hypha of *R. solani* as in fig 8 (a-d) hock like and penetration by appressorium like of trichoderma hyphae to mycelium (d) meaning of parasitism process by many modes of actions in comparison normal hyphae (d).

![Fig. 8: a-d, Scanning micrograph of interacting of *T. hamatum* iso 2 hypha with *R. solani*. a, b, c: Condensed coiling of *T. hamatum* around a hypha of *R. solani* (10x and 40x, 100), d: Hock, penetration by appressorium like and coiling of *T. hamatum* to hypha of *R. solani* (40x, 100) means parasitism and normal hyphae of *R. solani*.](image)
Scanning micrograph of interacting between both of trichoderma and fusaium as showed in figure (9) recorded normal mycelium and spores of fusarium fungus(a-d) with spores clusters of trichoderma, this means no parasitism action was happened and the effect was done by competition in space and/or nutrients. Mycoparasitism play an important role in biocontrol and divided into two types: necrotrophic that kill the host cells before or just after invasion and use the released nutrients due to the production of antibiotics, toxins, or hydrolytic enzymes (Manocha and Sahai 1993) and biotrophic parasitism, the development of the parasite is favoured by a living rather than a dead host structure, direct attack on a fungal thallus leading to its lysis and inhibition of phytopathogens, Chet et al (1981).

Fig. 9: a-d, Scanning micrograph of interacting of T.hamatum iso. 2 hyphea with condensed normal hyphea and spores of F. oxysporum means competition not parasitism.

3.2.3. In vivo (wire house):

The in vitro screening of the antagonistic potential used in this work allowed a systematic investigation of several trichoderma isolates including specific ecological factors and a selection of one effective isolate, so, T.hamatum iso2 was used which able to parasite of, R.solani and Sc. rolfsii mycelium and inhibited of F. oxysporum growth. Data in (3) stated the using of T.hamatum iso2 as biocontrol to F. oxysporum and results recorded that, damping off was affected by tichoderma wheat bran inoculum used at 4 g/hill(120 g/plot) which recorded the lowest pre-post emergence damping off, i.e. 6.25 and 4.25% compared to 30.91 and 25.21% with control and the most effective one in retarding (efficiency), i.e. 79.78 and 83.14%. Soil drench over rows by 120g/plot and 2g/hill of trichoderma inoculum were the most followed in this respect while treatment by disk (1cm) of PDA growth of trichoderma/hill was the lowest one, reduction of damping off (efficiency) ranged from 18.60-82.71%. Rot root disease severity rate (DS) and index (DI) were significantly retarded with trichoderma methods application especially treatment of 4g/hill recording the lowest ones and the highest efficiency against the disease, this led to enhance of TSS and sucrose contents in sugar beet roots in comparing of control which recorded the highest disease parameters and the lowest TSS and sugar contents. Other treatments showed satisfactory effect against the disease recording DS and DI ranged from 2.86-3.10 and 4.71-10.69% compared 3.41 and 22.19% of control, respectively, the efficiency ranged 9.09-26.68% (DS), 51.82-80.48% (DI). At all, TSS ranged from 14.03-17.60 compared to 13.66% of control showing enhancement (efficiency) ranged from 2.71-28.84%, sucrose ranged from 11.20-14.08 compared 10.93 of control recording enhancement 2.17 to 28.82%.
In the same column, means followed by the same letter are not significantly different according to DMRT at 5% level of significance. DS*: disease severity, DI*: disease index, TSS*: total soluble solids.

**Table 3:** Effect of *T.hamatum* of pre and post emergence damping off, root rot disease severity and index, total soluble solids and sucrose of sugar beet *cv.* pelatios in infested soil by *F. oxysporum* fungus in wire house.

<table>
<thead>
<tr>
<th>Trichoderma</th>
<th>Pre*</th>
<th>Post*</th>
<th>DS* rate</th>
<th>DI*%</th>
<th>TSS*%</th>
<th>Sucrose%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disk (1 cm)/hill</td>
<td>25.16b</td>
<td>20.33b</td>
<td>3.10b</td>
<td>10.69b</td>
<td>14.03c</td>
<td>11.20d</td>
</tr>
<tr>
<td>2g/ hill (60g/plot)</td>
<td>8.30d</td>
<td>8.88d</td>
<td>2.86d</td>
<td>4.71e</td>
<td>16.40b</td>
<td>13.12c</td>
</tr>
<tr>
<td>4g/ hill (120 g/plot)</td>
<td>6.25e</td>
<td>4.25e</td>
<td>2.50e</td>
<td>4.33f</td>
<td>17.60a</td>
<td>14.08a</td>
</tr>
<tr>
<td>Soil drench 60 g/plot</td>
<td>14.58c</td>
<td>12.17c</td>
<td>3.00c</td>
<td>9.25c</td>
<td>16.47b</td>
<td>13.18c</td>
</tr>
<tr>
<td>120 g/plot</td>
<td>8.82d</td>
<td>4.36e</td>
<td>3.00c</td>
<td>5.36d</td>
<td>16.80b</td>
<td>13.44b</td>
</tr>
<tr>
<td>Control</td>
<td>30.91a</td>
<td>25.21a</td>
<td>3.41a</td>
<td>22.19a</td>
<td>13.66c</td>
<td>10.93e</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Efficiency percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>disk (1 cm)/hill</td>
</tr>
<tr>
<td>2g/ hill (60g/plot)</td>
</tr>
<tr>
<td>4g/ hill (120 g/plot)</td>
</tr>
<tr>
<td>Soil drench 60 g/plot</td>
</tr>
<tr>
<td>120 g/plot</td>
</tr>
</tbody>
</table>

The lowest damping off was recorded with 4,2 g/hill followed by 120 and 60 g/plot as soil drench recording damping off reduction (efficiency) from 22.50 to 55.66 %. At all, DS rate was reduced with last group of treatments and the lowest one was treatment of disk/ hill , ie. 3.50 compared to 3.69% of control. DI ranged from 5.38-13.33% compared to 29.89 % of control and DI efficiency against *R. solani* resulted treatment by trichoderma ranged from 55.40-82.00 %. As to TSS and sucrose, high disease severity led to decrease of its with control treatment, ie. 13.16 and 10.53, since trichoderma treatments showed enhancement in contents recoding rang 15.13-17.93 and 12.10-14.34 %, respectively, showing enhancement rang 14.91-36.24%. At all, 4 g/hill was the best treatment.

**Table 4:** Effect of *T.hamatum* of pre and post emergence damping off, root rot disease severity and index, total soluble solids and sucrose of sugar beet *cv.* pelatios in infested soil by *R. solani* fungus in wire house.

<table>
<thead>
<tr>
<th>Trichoderma</th>
<th>Pre*</th>
<th>Post*</th>
<th>DS* rate</th>
<th>DI*%</th>
<th>TSS*%</th>
<th>Sucrose%</th>
</tr>
</thead>
<tbody>
<tr>
<td>disk (1 cm)/hill</td>
<td>62.50a</td>
<td>46.55a</td>
<td>3.50b</td>
<td>13.33b</td>
<td>15.13c</td>
<td>12.10c</td>
</tr>
<tr>
<td>2g/ hill (60g/plot)</td>
<td>37.5d</td>
<td>26.66c</td>
<td>2.50d</td>
<td>6.83d</td>
<td>17.73a</td>
<td>13.98ab</td>
</tr>
<tr>
<td>4g/ hill (120 g/plot)</td>
<td>35.41d</td>
<td>22.58d</td>
<td>2.11f</td>
<td>5.38f</td>
<td>17.93a</td>
<td>14.34a</td>
</tr>
<tr>
<td>Soil drench 60 g/plot</td>
<td>50.00b</td>
<td>39.13b</td>
<td>2.88c</td>
<td>11.01c</td>
<td>15.26c</td>
<td>12.21b</td>
</tr>
<tr>
<td>120 g/plot</td>
<td>43.75c</td>
<td>31.51c</td>
<td>2.17e</td>
<td>5.71e</td>
<td>16.66b</td>
<td>13.33b</td>
</tr>
<tr>
<td>Control</td>
<td>64.58a</td>
<td>50.92a</td>
<td>3.69a</td>
<td>29.89a</td>
<td>13.16d</td>
<td>10.53d</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Efficiency percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>disk (1 cm)/hill</td>
</tr>
<tr>
<td>2g/ hill (60g/plot)</td>
</tr>
<tr>
<td>4g/ hill (120 g/plot)</td>
</tr>
<tr>
<td>Soil drench 60 g/plot</td>
</tr>
<tr>
<td>120 g/plot</td>
</tr>
</tbody>
</table>

In the same column, means followed by the same letter are not significantly different according to DMRT at 5% level of significance. DS*: disease severity, DI*: disease index, TSS*: total soluble solids.

As to effect of trichoderma biocontrol against *S. rolfsii*, data in table (5) showed that ability of trihodermia in suppressing of root rot disease of sugar beet plants was obvious wherever caused reduction in damping off (efficiency) in rang 21.67-80.71, treatments of 4, 2 g/hill followed by 120 and 60g/plot were superior to treatment with disk (1cm) / hill of trichoderma grown in PDA, damping...
off ranged from 6.23-30.50 compared to 32.29-39.17% of control. Superior effect longitude to DS and DI showing in range 3.62-26.74 and 55.47-85.79% , respectively ,in comparing of 3.59 and 26.95% of control. A reduction of DS and DI led to enhancement of TSS and sugar contents from 2.37-18.25%. TSS recorded 14.03-16.20 and sucrose 11.22-12.96 compared to 13.70 and 10.96% of control, respectively.

**Table 5: Effect of T.hamatum of pre and post emergence damping off, root rot disease severity and index, total soluble solids and sucrose of sugar beet cv. pelatos in infested soil by Sc. rolfsii fungus in wire house .****

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Pre*</th>
<th>Post*</th>
<th>DS*rate</th>
<th>DI*%</th>
<th>TSS*%</th>
<th>Sucrose%</th>
</tr>
</thead>
<tbody>
<tr>
<td>disk /hill(1 cm)</td>
<td>30.50b</td>
<td>25.29b</td>
<td>3.46b</td>
<td>12.00b</td>
<td>14.03c</td>
<td>11.22d</td>
</tr>
<tr>
<td>2g / hill (60g / plot)</td>
<td>10.41e</td>
<td>8.33e</td>
<td>3.00e</td>
<td>4.06e</td>
<td>16.03a</td>
<td>12.82b</td>
</tr>
<tr>
<td>4g / hill (120 g / plot)</td>
<td>8.33f</td>
<td>6.23f</td>
<td>2.63f</td>
<td>3.83f</td>
<td>16.20a</td>
<td>12.96a</td>
</tr>
<tr>
<td>Soil drench  60 g / plot</td>
<td>19.50c</td>
<td>15.22c</td>
<td>3.17c</td>
<td>4.50c</td>
<td>15.07b</td>
<td>12.06c</td>
</tr>
<tr>
<td>120 g / plot</td>
<td>12.99d</td>
<td>13.29d</td>
<td>3.11d</td>
<td>4.25d</td>
<td>16.01a</td>
<td>12.80b</td>
</tr>
<tr>
<td>Control</td>
<td>39.17a</td>
<td>32.29a</td>
<td>3.59a</td>
<td>26.95a</td>
<td>13.70d</td>
<td>10.96e</td>
</tr>
</tbody>
</table>

In the same column, means followed by the same letter are not significantly different according to DMRT at 5% level of significance. DS*: disease severity , DI*: disease index, TSS*: total soluble solids.

Data in tables (3,4,5) concluded that, *T. hamatum* iso2 as wheat bran inoculum 4.2 g / hill (120,60 g/plot) were superior to 120, 60 g / plot as soil drench over rows, while disk(1cm) of PDA growth of trichoderma was the lowest one in controlling of sugar beet root rot disease and *F. oxysporum* was the most effective followed by *Sc. rolfsii* and *R. solani* was the lowest one resulting trichoderma treatments. This results were supported by many scholars and their results, i.e. Studholme et al.(2013) stated to *T.hamatum* strain GD12 biocontrol against pre-and post-emergence of *Sclerotiorum* soil pathogens. *T. harzianum* was the most effective inhibition and recorded the least root-rot infection of 5% under field conditions (Ramezani, 2008). *T. viride* suppressed disease severity of the sugar beet root rot in presence of *F. solani* and *R. solani* (Aly and Hussein 2009). *T. harzianum* and *T. virens* isolates reduced the severity of the disease in greenhouse conditions against *R. solani* that show high inhibition rate (Durak, 2016). Singh et al. (2013) , Ahmed and Ahmed (2015) observed that, *T. harzianum* reduced disease severity of *S. rolfsii* of tomato and Allium white rot caused by *S. cepivorum*, respectively. Consequently in the greenhouse experiments, Chet et al. (1997) applied *T. harzianum* in the form of wheat bran to *R. solani* infected soil, effectively controlled damping-off of bean, tomato and eggplant seedlings enhancing the emergence and inhibits plant infection with soil pathogens. A significant reduction in the incidence of root rot caused by *R. solani* and trichoderma treatment was reported by Jeyaraj and Ramabadran (1999). Singh et al. (1999) reported that, *Trichoderma spp.* fungi as microbiological preparation components used for soil amendment is reported in numerous papers which cover protecting germinating seeds and then plant roots from infection with phytopathogens. Moreover, sugar beet root rot infection with *S. rolfsii* was significantly reduced due to using of *T. harzianum*, Abo El-Yazied(2019). Additionally, Mao et al.(2020)supported the present results and interpreted that, *T.hamatum* inhibited pepper fusarium wilt with control effect 60.61% efficiency level, and yield increase in the field.

### 4. Conclusion

Given these observation, it was hypothesized extra and intra-cellular biosynthesis of silver NPs was done by *R.solani, Sc.rolfsii* as anew sources addition to *F.oxysporum*; causal pathogens of sugar beet root rot disease by measurements of FTIR, EDX, SEM and UV-Vis analysis. Additionally, results showed that safety control by *T.hamatum,* which could be parasite on *Sc.rolfsii* and *R.solani* mycelium

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and reduced damping off, root rot index and severity and do enhancement of total soluble solids and sucrose. Also, adaption of these practice, T. hamatum was proposed as a potential biocontrol fungus.

5. Acknowledgement:
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References


