FUSARIA WILT DISEASE OF STRAWBERRY CAUSED BY *Fusarium oxysporum* f. *sp. fragariae* IN IRAQ AND ITS CONTROL

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**KEYWORDS**

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*Fusarium oxysporum* f. *sp. Fragariae*

Strawberry

Disease management

Iraq

**ABSTRACT**

Study was conducted to isolate, identify and test the pathogenicity of the pathogen of Strawberry wilt disease. The efficiency of *Azotobacter chroococcum*, *Pseudomonas fluorescens*, Tchigarine and PreservePro was tested for managing the disease. Results of isolation and identification revealed the presence of *Fusarium oxysporum* f. *sp. fragariae*. The frequency of occurrence ranged from 40 - 60 %. Results of pathogenicity revealed that isolated isolates of *F. oxysporum* *f. sp. fragariae* was highly pathogenic to Strawberry plants. The percentage of disease index was recorded 75.0 – 87.5 % in all the treated samples as compared with control treatment. The disease management potential of tested control agents i.e. Tchigarine, *A. chroococcum* and *P. fluorescens*, have shown high efficiency against *F. oxysporum* f. *sp. fragariae* on culture media (PDA). These treatments shows a significant inhibition in the pathogen multiplication and it reach to 100 %. All the tested treatments are not only showing in-vitro efficacy but were equally affective under green house conditions. The disease severity on root systems was recorded highest in untreated check (86.7%) while it was 0% in all the control agent treatments. Highest fresh weighs of shoot (9.9g) and root systems (10.47g) have been reported from *A. chroococcum* and Tchigarine respectively. Similar types of trends were reported in case of dry shoot and root weight. This is the first report of the occurrence of Fusarium wilt caused by *Fusarium oxysporum* f. *sp. Fragariae* on strawberry plants in Iraq.

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1 Introduction

Strawberry (Fragariae ananassa Duch.) is a perennial economically important crop, grown under a wide range of climatic conditions. Total area under cultivation of strawberry at world level was reported 72000 hectares, among this 16160 .4 hectare was in Asia. The world production of strawberry was estimated as 3.9 million tons, of this 721566 tons produced from Asia (FAO, 2007). Wild and cultivated plants are producing small sized fruits with high nutrient value (Raab et al., 2006) and were also used as remedy for many human diseases including kidney and liver diseases. Furthermore, extracts from strawberry shoots were used to treat diarrhea (Raab et al., 2006; All-Freon & Habeeb, 2013). Strawberry cultivars are highly susceptible to several destructive and economically important soil borne pathogens such as Fusarium (Golzar et al., 2007), Rhizoctonia (Fang et al., 2012b, Fang et al., 2013; Ceja-Torres et al., 2014), Cyclindrocarpon (Manici et al., 2005), Macrophomina (Aviles et al., 2008; Hutton et al., 2013), Pythium (Mansour, 2005; Abdel-Sattar et al., 2008), Gnomonia, Phoma (Morocco, 2006; Ceja-Torres et al., 2014) and Phytophthora (Mingzhu et al., 2011). These pathogens can cause disease to strawberry either individually or in combinations. Crown disease, root rot and wilt disease of strawberry have been commonly reported from various countries like Argentina, Australia (Fang et al., 2013) China (Fu-chun et al., 2006) South Korea (Nagarajan et al., 2006) Spain, South Carolina (Williamson et al., 2012) California (Koik et al., 2009) Egypt (Abdel-Sattar et al., 2008) and Japan (Suga et al., 2013).

F. oxysporum is a major soil borne pathogen and responsible for causing wilt diseases on a variety of crop plants (Nelson et al., 1981). F. oxysporum distributed in soils throughout the worlds, and showing symptoms consisted of wilting of foliage (drying and withering of older leaves), stunting of plants, and reduced fruit production. At severe stage plants eventually collapsed and died. Internal vascular and cortical tissues of plant crowns showed a brown to orange brown discoloration. F. oxysporum is the most frequently occurring and damaging strawberry plants infected by crown and root diseases (Koike et al., 2009; Fang et al., 2011a; Fang et al., 2011b, Fang et al., 2013; Koike et al., 2013). Fusarium wilt of strawberry was caused by F. oxysporum f. sp. fragariae and responsible for causing yield losses in commercial strawberry production (Fang et al., 2012a). The causal agent, F. oxysporum f. sp. fragariae Winks & Y.N. Williams, is a haploid fungus and is difficult to control because the pathogen survives as chlamidospores in soil for many years. Early detection and diagnosis of the pathogen in plants and soils is essential for development of an effective disease control strategy. The control of Fusarium wilt disease is currently accomplished primarily through the use of fungicides. However the frequent and discriminate use of fungicides leads to atmosphere pollution and create imbalance in the microbial community, which maybe unfavorable to the activity of beneficial organisms and may lead to development of resistance strains of pathogen.(Martin & Bull, 2002) Resistance cultivars have been used against Fusarium wilt but it has been overcome by appearance of new races of the pathogen (Koike et al., 2009). In recent years biological control has become a promising safer and ecologically acceptable alternative to chemical control in the management of soil borne diseases (Shalini & Srivastar, 2007).

Among the bacteria bio-control agents, genus Pseudomonas and Azotobacter has received more attention than many other bacterial groups (O’ Sullivan & O’ Gara, 1992, Hillel, 2005). Strawberry plants newly cultivated in green houses in Iraq, and we observed symptoms of wilting manifested on Strawberry plants at different sites associated with root and stalk rot. This study was conducted to isolate and identify the causal agents of these symptoms and evaluate some chemicals and biological agents to manage the diseases.

2 Materials and Methods

2.1 Isolation and identification of pathogen

Diseases strawberry plants showing wilting symptoms (Fig.1) were collected in April 2013 from Four commercial strawberry lath houses of Diyala (90 km north of Baghdad). Twenty affected plants from each lath house were used for isolation of pathogens. Roots were washed carefully under running tap water and the crown of each plant was cut into approximately 0.5 -1 cm and surface sterilized in 1% sodium hypochlorite, after surface sterilization it rinsed with sterile water and dried in alamina flow cabinet. Four pieces of surface sterilized plant materials were separately plated in each petridish containing Potato Sucrose Ager (PSA) supplemented with 100 mg/L chloromphenicol. After 72 h of incubation at 25°C single spore isolation from each developing colony was done to have pure culture. Isolates were identified to species level according to their cultural and morphological features (Booth, 1977; Nireenberg & O’Donnell, 1998; Leslie & Summerell, 2006).

The isolation frequency of the species was calculated as follows:

\[ Frequency(\%) = \frac{\text{No of plant segments of species occurrence}}{\text{Total No of segments used}} \times 100 \]

2.2 Preparation of Inoculum

F. oxysporum f. sp. fragariae (FOF) isolates inoculums were prepared by using local Millet Panicum millaceum L. seeds. Fifty gram of clean Millet seeds were placed in each of 250 ml flasks. The seeds were autoclaved twice at 121°C and 1.5 kg/cm² for one hour for two successive days.
Each flask was inoculated with 4 disks of 0.5 cm diameter from pure fungal culture grown on PSA. Uninoculated flasks containing seeds only were used as control. All the flasks were incubated at 25± 1°C for 10 days with agitation every 3 days for homogenization; the inoculum was conserved at 4 °C until use.

2.3 Azotobacter chroococcum isolate

An isolate of A. chroococcum was obtained from biocides production division, Agriculture research center, Ministry of Science and Technology. A.chroococcum was maintained on activation medium described by Thompson & Skerman (1979). Fifty ml of the activation medium were transferred into 100 ml conical flask after sterilization. The medium was inoculated with the bacteria from culture of 24hr. The flasks were incubated at 28±2°C for 2 days and the number of CFU/ml was determined.

2.4 Pseudomonas fluorescens isolate

An isolate of P. fluorescens obtained from organic culture center plant protection office ministry of Agriculture. The isolate was grown on King’s B broth medium at 28 C° in 100 ml flasks for 2 days.

2.5 Bacterial cells count

The colony forming unit (CFU) was calculated by plate count method. A series of dilution up to 10^8 in test tubes containing sterile distilled water were done. One ml of each of 10^3 to 10^8 dilutions was transferred into each of Petri dishes containing nutrient agar and homogenized. The plates were incubated at 27±2°C for two days, and the CFU / ml were calculated as following:

The number of CFUs per ml of sample = The number of colonies (30-300 plate) × The dilution factor of the plate counted ( Harrigan, 1976 )

2.6 Activity of biocontrol and Tchigarine against F. oxysporum on PDA

One ml of P. fluorescens at 10^5 CFU /ml , A. chroococcum at 10^5 CFU /ml and 1 ml/L Tchigarine added separately to PDA before solidification and poured in petridish of 9 cm diameter. The center of each plate was inoculated with 5 days old 0.5 cm disc of FOF. The plates were incubated at 25 ±1°C until the fungal growth reach to the plate edge in control (4 plates for each concentration).The fungal growth diameters were calculated and the inhibition percentages were determined by the following equations :

% inhibition = (Fungal growth diameter in control –fungal growth diameter in treatment /fungal growth diameter in control) ×100.
2.7 Pathogenicity of *Fusarium oxysporum* f. sp. *fragariae* isolates

Pathogenicity of *Fusarium* isolates was tested on Strawberry cultivar Ruby in a glass house experiment. Sterilized soil (1 kg / pot) was distributed in 14 cm in diameter experimental pots. Four FOF isolates ( KFO1 - KFO4) grown on Millet seeds were added into potting soil at 1% W: W and planting with healthy Strawberry plants at five leaf stage , one plant / pot . Plants were watered with Hoagland solution once a week. Control treatments were inoculated with sterilized millet seeds and the pots were distributed in the glasshouse at complete randomized design with 4 replicates. 40 days after inoculation disease severity was assessed for each plant on a zero - 4 rating scale according to percentage of shoot, roots and crowns affected by , acropetal chlorosis , necrosis , wilt, dark brown colors( Bejarano et al., 1995; Zhang et al., 2012 ) ; 0=healthy plants , 1= 1 to 33% , 2 = 34 to 66 % , 3 = 67 to 97 % , 4 = dead plants. Disease severity was calculated as shown in the formula below :

\[
\text{Disease severity} = \frac{\sum (\text{No. infected plants} \times \text{their infected degree})}{(\text{total examined tested plants} \times \text{upper infected degree})} \times 100.
\]

2.8 Evaluation of biocontrol agents activity in reduction of Strawberry wilting severity under glasshouse conditions.

Pot experiments were conducted at plant protection department green house, college of Agriculture, University of Baghdad. The inoculum of pathogenic fungi was prepared and maintained on the sterile local millet seeds. This inoculum was mixed with the autoclaved soil and distributed in pots of @ 1 kg/pot. The pots have been assigned to 4 groups with four replicates. The pots of the first group were amended with *A. chroococcum* (Ac ) inoculum grown on sterile peat peat moss @10^9 CFU/g (2g/kg). Pots of group second were treated by *P. fluorescens* (Pf ) @ 2g/kg soil containing 10^9 CFU /g. The soil in the pots of the third group were watering with preserve pro (pp)(Preserv pro product was provided by Arysta life science 2% Ascorbic acid) at 1ml /L, while the soil of the 4 th group was watering with Tchigarine solution at1ml/L .The 4 groups pots were contaminated with FOF grown on millet seeds at 5g/kg soil, three days after the addition of the bioagents and fungicide. Four pots contaminated with FOF only as above used as control. Other pots related with biocontrol agent non – contaminated with pathogenic fungi were individually used as control. The pots were planting with strawberry plantlets, Ruby variety, one plant/ pot and distributed in the glass house at complete randomized design. Root and shoot disease was determined after 2 month of planting assessed on a 0–5 disease severity scale as used previously (Bejarano et al., 1995; Zhang et al., 2012 ). The disease severity was calculated according to Mckinney formula (McKinney 1923), with this fresh and dry weight of plants was determined for determining the effect of fungal infraction on biomass.

3 Results and Discussion

3.1 Isolation and Identification of pathogen

Results of isolation and identification from infected Strawberry plants revealed the presence of *Fusarium oxysporum* in all the samples in percentage of frequency ranged 40-60 % (Table 1). Hyaline branching mycelium was formed from the infected pieces of strawberry plants on PSA, the colonies of the isolates varies in color , most of them turned to white grey in age , others showed pale violet color .The microscopic observation showed straight to slightly curved, relatively slender and thin walled Macroconidia with curved Apical cell and Foot shaped Basal cell varied in size 30-60 μm X3-5 μm with three septa. Abundant oval ellipsoidial or kidney shaped and usually 0- sepete Microconidia borne on short monophilides in the aerial mycelium clustered into so called false heads were observed .Chlamydospires formed abundantly after 2-3 weeks by all the isolates .Usually formed singly or in pairs but also formed in clusters or in short chains, either terminal or intercalary in aerial mycelium, these cultural and morphological characteristics on culture media similar to those described by Booth(1977), Nirenberg& O onnell (1998) and Leslie & Summerell (2006). *F. oxysporum f. sp.fragarae* was identified as the predominant pathogen in many countries in the world (Abdel-Sattar et al.,2006;Zhaao et al.,2009; Suga et al., 2013),the first recording of this in western Australia (Philips & Golzar ,2008).Williamson et al.(2012) reported that the main causal agents of strawberry wilt was *F.oxysporum* that isolated from crown and root in south carelinea. Several other studies indicated that *F. oxysporum* was among major causal agent of strawberry wilting (Nagarajan et al ., 2006 ; Koik et al ., 2009; Ebihara & Uematsu, 2014).This is thought to be the first report of *Fusarium* wilt of strawberry in Iraq.

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Location</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bagobia-Kennan</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>Bagobia-Benysaad</td>
<td>45</td>
</tr>
<tr>
<td>3</td>
<td>Khalis-Al-Manchuria</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td>Khalis-Hbhb</td>
<td>60</td>
</tr>
</tbody>
</table>

Table 1. Frequency of *F. oxysporum* f. sp. *fragariae* in strawberry samples.
Table 2 Effect of *Fusarium oxysporum* f. sp. *fragariae* isolate on strawberry plants (Cv. Ruby).

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Disease index ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>KFO1</em></td>
<td>75.0 ± 2.37 c**</td>
</tr>
<tr>
<td>KFO2</td>
<td>81.5 ± 2.89 b</td>
</tr>
<tr>
<td>KFO3</td>
<td>75.0 ± 2.41 c</td>
</tr>
<tr>
<td>KFO4</td>
<td>87.5 ± 3.04 a</td>
</tr>
<tr>
<td>Control</td>
<td>0.0 ± 0.00 d</td>
</tr>
<tr>
<td>LSD Value (P≤0.05)</td>
<td>5.585</td>
</tr>
</tbody>
</table>

*KFO1= Kenman isolate, KFO2= Benysaad isolate, KFO3= Almansuria isolate, KFO4= Hbhb isolate. Values in the table are an average of four replicates. **Results followed by different letters are significantly different (P<0.05) as determined by LSD test.

Table 3 Growth inhibition of *Fusarium oxysporum* f. sp. *fragariae* by bioagents and Tchigarine on PDA.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Inhibition rate ± SEM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>F. oxysporum</em> f. sp. <em>Fragariae</em> (FOF)+ Tchigarine</td>
<td>93.80 ± 4.93 b*</td>
</tr>
<tr>
<td>FOF + <em>P. fluorescens</em></td>
<td>75.12 ± 2.46 c</td>
</tr>
<tr>
<td>FOF + <em>A. chroococcum</em></td>
<td>100.00 ± 1.20 a</td>
</tr>
<tr>
<td>Control</td>
<td>0.00 ± 0.00 d</td>
</tr>
<tr>
<td>LSD Value (P&lt;0.05).</td>
<td>1.806</td>
</tr>
</tbody>
</table>

*Results followed by different letters are significantly different (P<0.05) as determined by LSD test. Values in the table are an average of four replicates.

3.2 Pathogenicity of *F. oxysporum* f. sp. *Fragariae* isolates

Results of Pathogenicity test showed that *F. oxysporum* f. sp. *fragariae* isolates were highly pathogenic to strawberry plants (Table 2). The percentage of disease index ranged between 75.0 – 87.5 % compared with zero in control. These results were in agreement with Fang et al. (2011a; 2011b). That *F. oxysporum*, was reported as most virulent pathogen responsible for causing crown and root diseases of strawberry in Western Australia. Koike et al. (2009) and Williamson et al. (2012) first time reported of the occurrence of *Fusarium* wilt caused by *F. oxysporum* on strawberry plants in California and South Carolina. Phillips & Golzar (2008) reported high numbers of plant deaths in strawberry crops from Western Australia in a prompted survey to identify the causal agent. Previous research work showed that *Fusarium oxysporum* was most frequently isolated from diseased crown and root tissues of strawberry (Nagarajan et al., 2006; Suga et al., 2013, Ebihara & Uematsu, 2014).

Figure 2 Activity of bioagents, Tchigarine against *F. oxysporum*

A= FOF, B= FOF + T; C= FOF + Pf; D = FOF + PP; E = FOF + Ac
Table 4 Effect of biocides and Fungicide on Strawberry wilting under greenhouse conditions.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Disease severity(%)</th>
<th>Fresh weight (gm / plant)</th>
<th>Dry weight (gm /plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root Shoot</td>
<td>Root Shoot</td>
<td>Root Shoot</td>
<td>Root Shoot</td>
</tr>
<tr>
<td><em>F. oxysporum</em> f.sp. Fragariae (FOF)</td>
<td>86.7±2.73 a</td>
<td>2.43±0.06 c</td>
<td>0.33±0.02 c</td>
</tr>
<tr>
<td><strong>FOF+ Tchigarine</strong> (T)</td>
<td>0.0±0.00 b</td>
<td>7.27±0.67 b</td>
<td>2.17±0.05 b</td>
</tr>
<tr>
<td><strong>FOF+ A.chroococcum</strong> (Ac)</td>
<td>0.0±0.00 b</td>
<td>14.03±0.92 a</td>
<td>3.87±0.06 a</td>
</tr>
<tr>
<td><strong>FOF + Preserve Pro(PP)</strong></td>
<td>0.0±0.00 b</td>
<td>11.70±0.82 ab</td>
<td>2.90±0.02 a</td>
</tr>
<tr>
<td><strong>Preserve Pro(PP)</strong></td>
<td>0.0±0.00 b</td>
<td>13.27±0.65 a</td>
<td>3.57±0.07 a</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td>0.0±0.00 b</td>
<td>12.70±0.77 ab</td>
<td>3.27±0.03 a</td>
</tr>
<tr>
<td>LSD Value <em>(P≤0.05).</em></td>
<td>9.34</td>
<td>4.1961</td>
<td>1.328</td>
</tr>
</tbody>
</table>

*Results followed by different letters on each column are significantly different *(P<0.05)* as determined by LSD test; Values are an average of four replicates.

3.3 Activity of bioagents and Tchigarine against *F. oxysporum f. sp. fragariae* on FDA

Significant reduction was reported in mycelial growth of *F. oxysporum f. sp. fragariae* (FOF) on FDA exerted by the bacteria *A. chroococcum* and *P. flourescens* (Table 3). The inhibition percentage of FOF was attained up to 100% with *A. chroococcum* as compared to 75.12 % with *P. flourescens*. Also it was observed that *A. chroococcum* was more effective than Tchigarine. The inhibition percentage of FOF was reported 93.80 % in the treatment containing Tchigarine. The reduction activity of *A. chroococcum* and *P. flourescens* may be due to the production of secondary metabolites by these two bacteria (organic compounds, Lytic enzymes and antibiotic), as well as competition with the fungi for nutrients. According to Hillel (2005) production of organic compounds, indol acetic acid, enzymes, antibiotic and hydrogen cyanide by *A. chroococcum* on cultural media was reported and in this context findings of present study are in accordance with several previous studies (Hillel, 2005; Fatima et al., 2009; Mali & Budhankar, 2009; Matloob & Juber, 2013 ). The activity of *P. flourescens* was also explained by its ability to produced several antibiotics such as Oomycine A, pyrolinriotin, competition for nutrients and direct parasitism on fungal mycelium (Walsh et al., 2001; Haas & Keel, 2003; Hillel, 2005; Abdul-hussein, 2013 )

3.4 Activity of bioagents and Tchigarine in reducing Strawberry wilting severity under greenhouse conditions

Significant reduction in disease severity was manifested on plants treated with *A. chroococcum*(Ac), *P. flourescens* (Pf) , Preservepro ( PP) and fungicide Tchigarine (T) compared with control treatment ( Table 4, Fig. 2). The disease severity on shoot systems were recorded 0.0% and in Tchigarine, *A.chroococcum*, *P. flourescens* and preservepro treated plants Compared to 86.7% in untreated check While it was in the shoot systems 20%, 20%, 26.6% and 26.6% in the treatments of the above control agents respectively compared to 100% in the untreated check. The reduction in disease severity was found associated with promotion of plant growth parameters as proved by increasing the fresh and dry weights of treated plants. The root fresh weights were 8.33, 9.90, 7.27 and, 7.57 g/plant, while the foliage fresh weights were 10.47, 9.70, 7.93 and 7.50 g / plant in treatments containing T+FOF, Ac+FOF, FO+ Pf and PP+ FOF treatments respectively compared with 2.43 g / plant root and 3.50 g / plant shoot weight of control treatment.

The dry weights of plant root and shoot were found 2.77, 2.97, 2.17, 2.23g/plant and 2.267, 2.100,1.467,1.600 g / plant in T+FOF, Ac+FOF, FO+ Pf and PP+ FOF treatments respectively as compared to 0.33 g / plant dry root and 0.267 g / plant dry shoot in control treatment. It has been observed that the control agents used in this study have stimulatory effect on plant growth parameters (Table 4). The reduction in disease severity by the application of bioagents can be the result of competition with FOF for nutrients. Several studies have been reported to the activity of various plant growth promoting bacteria like *A. chroococcum* and *P. flourescens* against soil borne pathogen (Van Loon et al.,1998 ; Verma et al., 2009, Verma et al., 2010 ; Bolwerk et al., 2003; Al-Azawy, 2010; Abdul-hussein, 2013; Bhosale et al.,2013 ; and Matloob & Juber, 2013 ).

The promotion of plant growth parameters may be result of restriction the growth of FOF or by producing compounds necessary for plant growth such as increasing the amount of available nitrogen, plants regulators as well as make other compounds more available for plants roots by PGPB (Ramamoorthy et al.,2001 ; Haas & Keel,2003 ; Hillel,2005 ; Bhattacharyya & Jha., 2012 Doornbos et al.,2012 ). The growth stimulatory effect of rhizosphere bacteria was also reported by various researchers ( Tuzun & Kloeper ,1995;
We et al. (1996). Furthermore, Mali & Bodhankar (2009) reported that 25 isolates of A. chroococcum from groundnut showed antagonistic activity against several species of fungi including F. oxysporum by producing plant hormones such as IAA and Gibberellins. PreservePro exhibited higher activity against FOF and reduced disease severity, this may be result of its content of ascorbic acid which act as antioxidant for the secondary metabolites of FOF led to lowering its effects on the plant (Abdel-Kader et al., 2012). Similar results were reported about the effect of ascorbic acid against pathogens and enhancing plant growth (Dias et al., 2011).

References


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