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# The effect of addition of some chemical fortificationson soil PH and their relatedness to control *Fusarium solani* causing cowpea root rot

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***Abstract:*** *This study was initiated to evaluate the efficacy of some chemical fortifications to control the pathogenic fungus Fusarium solani infecting cowpea roots. F.solani isolation and identification were confirmed on PDA medium and microscopic examination. Calcium sulfate treatment showed the highest inhibition percentage up to 25.87%on mycelium growth at the concentration of 9%.Whereas, perlite treatments scored 14.28% growth inhibition at the same concentration. All chemical fortifications added significantly reduced F.solani infection on cowpea plants showing significant differences compared to pathogenic fungus alone treatment.The infection severities of $CaSO_4$ treatment were 60.00, 55.00 and 36.67%at concentrations 3,6 and 9%respectively.While, they were, 61.66, 51.66 and 26.67% for the perlite treatment at the same concentrations, respectively compared to fungus alone treatment which was 86.77%. In pots experiment ,roots size of cowpea plants, was the highest at the three concentrations tested which were 4.0, 4.67, 4.67 mm and 4.67, 5.0 and 4.6 mm for $CaSO_4$ and perlite, respectively compared to 2.33 mm of F.solani treatment. The numbers of branches in $CaSO_4$ and perlite treatments was 10.67, 10.00 and 9.67 and 14.67, 14.67 and 14.50branches / plant for the three concentrations, respectively. These not differ significantly from control treatment with 12.00 branch / plant, compared to 6.67 branch / plant for fungus alone treatment.Results indicated a slight change in the pH of the soil treated with chemical fortifications ranged 7.9-8.95.*

***Keywords*:** *Fusarium solani, $CaSO_4$ , perlite , infection severity*

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## I. Introduction

Soil borne pathogens are causing many diseases on a wide range of crops resulted in significant crop losses.Fusarium species are one of the most soil inhabited fungi causing rot and wilt of leguminous crops (1). Many Fusarium species are causing similar symptoms on different crops including root rot, wilt, yellowing and premature death of infected plants. In addition, they were producing toxins responsible of serious diseases on animals due to feeding on mycotoxins contained broad bean seeds(2). Soil fortified with some chemicals can decrease infection severity through rising PH (3). (4) found that the addition of lime (calcium carbonate) increased soil PH value and decreased the infectivity percent of fusarium wilt. Furthermore, gypsum ($CaSO_4$) decreased infectivity percent of *Phytophthora cinnamomi* as it reduced fungal inoculum through changing PH medium to acidic which affected unsexual spores production, spore motility and sporangia formation (5,6).(7) showed the effectiveness of CaSO4 to reduce peanut pod rot infectivity caused by *Pythium myriotylum* and *Rhizoctonia solani*. This study was aimed at the assessment of some chemical fortification to affect soil PH and instigating it relatedness to control *Fusarium solani* the causal agent of cowpea root rot.

## II. Material and Methods

2.1-Isolation and identification of *F.solani* from cowpea roots

Root samples were collected from diseased cowpea plants grown in El-Msaib-Babylon then transferred to plant pathology lab. at Plant Protection Dept., College of Agriculture/University of Baghdad. Root samples were washed and chopped into small pieces ranged about 0.5-1 cm, treated with 2% of sodium hypochlorite , washed with distilled water then placed on filter paper to dry. Root pieceswere pretreated with 2% sodium hypochlorite and washed with distilled waterplaced on filter-paper and let to dry.Four piecesper plate were plated on Petri-dishes containing Potato Dextrose Agar (PDA) medium, then incubated for 5 days at 25 °C. Fungi were identified following (8) taxonomic key.

2.2-Pathogenicity test on cowpea seeds

Pathogenicity of three isolates belong to the pathogenic fungus was tested according to (9) approach. About 5 mm discs were excised from the edge of 5 days aged fungus colonies using acork borer. Discs were

placed in the middle of PDA containing plates. Three plate replicates were used for each isolate while PDA containing plates without inoculation were used for control treatment. Plates were incubated for 3 days at 25 °C . Cowpea seeds were pre sterilized with 2% sodium hypochlorite for 2 min then 5 seeds per plate were placed about 1 cm from the edge around each colony.Plates were incubated at 25 °C until germination of cowpea seeds in the control treatment. The fungus isolate shown the highest pathogenicity was selected for the next experiments.

2.3-Testing the effect of chemical fortifications on mycelium growth of *F. solani* under laboratory conditions

Three concentration 3, 6 and 9% ofperlite and $CaSO_4$ were mixed with PDA medium separately and poured in petri dishes then inoculated with the pathogenic fungus.Three replicates were made for each treatment. Inhibition was estimated by measuring the radiiof fungal colonies in each treatment compared to control treatment. The inhibition percent was calculated according to following the equation(10):

$$\text{Inhibition \%}=\frac{mean\ of\ control\ \ redius-mean\ of\ treatment\ \ redius}{mean\ of\ control\ \ redius}\text{X100}$$

2.4-Efficacy assessment of some chemical fortifications to reduce *F. solani* infection on cowpea plants

One kg pots filled with autoclaved soil was prepared then about 10 g/pot of fungal inoculum grown on millet seeds was added. The mixture was wettedand covered with polyethylene bags. Two days later of inoculation,of perlite and CaSO4 were added atthree concentrations 3, 6 and 9% per pot for each chemicals. Three replicates were made for each treatments. Four seeds of cowpea per pot were sown 24 h later after chemical addition. Pots were kept in Plant Protection Department glass house. Complete Random experimental design (CRD) was applied. Data were taken 40 daysafter sowing. The following parameters were calculated: seed germination percent, infection severity, plant heights, number of leaves and branches, root system size, wet and dry weights. In addition, soil PH was measured after addition of fortifications and cowpea seed sowing. Three PH readings were made with 10 intervals between readings as follow: about 10 gm soil from each treatment was weighed and mixed thoroughly with 10 ml distilled water for 5 min. The supernatant was collected then PH was calculated using PH meter.

## III. Results and discussion:

3.1-Identification of *Fusarium solani* isolated from cowpea roots

Isolation from diseased cowpea roots, exhibiting root rot and coloration showed to be a fungal infection when a white-grey mycelium fungus was observed. The microscopic examination confirmed three type of spores, namely microconidia, macroconidia and chlamidospores with presence of a long phialide characteristic to *F.solani* (11; 8).Results in published works indicated the isolation of *F. solani* from leguminous roots which is one of biggest problems limiting leguminous crop growing (12;1).

3.2-Pathogenicity of *F. solani* on cowpea seeds under laboratory conditions

Three isolates of *F. solani* tested on cowpea seeds grown in PDA media showed to be pathogenic (Figure 1) when significantly reduced seed germination up to 30% compared to control treatment

**Figure1:** Pathogenicity test of the most virulence *F. Solani* isolate on cowpea seeds

Which was 100%. *F. solani* pathogenicity is related to the virulence variation which is based on isolate ability to produce toxins. *F. solani* isolates produce number of toxins including Marticin, Isomarticin and Fusarubin which are responsible for symptoms expression (13).

3.3-The effect of chemical fortifications on mycelium growth of *F.solani* under laboratory conditions

The addition of perlite and $CaSO_4$ to PDA medium successfully inhabited mycelium growth of *F. solani* (Table 1).The 9%$CaSO_4$was the highest among other treatments when inhibit fungus growth by 25.87% followed by 14.28% for 3% and 6%$CaSO_4$and 9% perlite treatments compared to 0% growth inhibition for control treatment. It was observed that when $CaSO_4$ and perlite concentration sincrease the inhibition percent increase as $CaSO_4$ addition makes unsuitable environment to pathogenic fungi for growth and virulence.

**Table 1:** The effect of chemical fortifications om mycelium growth of *F. solani* (F.s.) on PDA medium

| Treatment | Inhibition% |
|---|---|
| F.s.+ 3% $CaSO_4$ | 14.28 |
| F.s.+ 6%$CaSO_4$ | 14.28 |
| F.s.+ 9% $CaSO_4$ | 25.87 |
| F.s.+ 3% perlite | 2.63 |
| F.s. 6% perlite | 11.60 |
| F.s.+ 9%perlite | 14.28 |
| F.s. | 0.00 |
| LSD prob. value0.05 | 5.16 |

3.4-The evaluation of chemical fortifications on *F.solani* infectivity on cowpea plants

Three concentrations (3, 6 and 9%) of each chemical fortifications (perlite and $CaSO_4$ )were assessed through addition to *F. solani* contaminated soil planted with cowpea. Results revealed all treatment significantly decreased the infection severity of *F. solani* compared to *F. solani* alone treatment (Table 2). Infection severity were 60.00, 55.00 and 36.67% and 61.66،51.66،26.67% for the three concentrations (3, 6 and 9%) of each $CaSO_4$ and perlite, respectively, compared to 86.77% infection severity for the *F. solani* alone treatment. Chemical fortifications affected growth parameters when root system sizes were 4.0, 4.67and 4.67 and 4.67, 5.0 and 4.6 mm for the three concentrations (3, 6 and 9%) of each CaSO4 and perlite, respectively compared to 2.33 and 6 mm for the *F. solani* alone and healthy control treatments, respectively. Whereas, number of branches for the three concentrations of $CaSO_4$ and perlite were 10.67, 10.00and 9.67 and 14.67, 14.67and 14.50 branch/plant, respectively compared to 6.67 and 12 branch/plantfor the *F. solani* alone and healthy control treatments, respectively. No significant differences for other growth parameters were shown between treatments and *F. solani* alone and healthy control treatments. The reason behind pathogenicity decrease is attributed to the mechanism of calcium absorption by plant may decrease the pathogenicity through inhibition of mycotoxins activity, in addition to the plant use of Ca to support cell walls which increase plantresistance against pathogens and attenuate the fungus ability to penetrate host cells.(4; 7).

**Table2**: The evaluation of chemical fortifications efficacy to decrease *F.solani* infectivity on cowpea plants

| Treatment | Infection severity | plant height (cm) | No. of leaves | No. of branches Branch/plant | Wet weight(gm) | Dry weight (gm) | Root size (mm) |
|---|---|---|---|---|---|---|---|
| F.s.+ 3% CaSO4 | 60.00 | 31.66 | 9.00 | 10.67 | 14.00 | 3.73 | 4.15 |
| F.s. + 6% CaSO4 | 55.00 | 36.33 | 10.33 | 10.00 | 12.33 | 3.20 | 4.67 |
| F.s.+ 9% CaSO4 | 36.67 | 35.33 | 10.67 | 9.67 | 12.33 | 3.16 | 4.67 |
| F.s.+ 3% perlite | 61.66 | 29.83 | 11.33 | 14.67 | 15.67 | 3.20 | 4.67 |
| F.s. 6% perlite | 51.66 | 32.00 | 10.33 | 14.67 | 15.67 | 5.00 | 5.00 |
| F.s.+ 9% perlite | 26.67 | 34.00 | 10.67 | 14.50 | 15.00 | 5.13 | 4.25 |
| F.s. | 86.77 | 34.66 | 10.00 | 6.67 | 12.66 | 2.33 | 2.33 |
| control | 0.0 | 36.00 | 9.67 | 12.00 | 13.33 | 3.23 | 6.00 |
| LSD prob. value 0.05 | 1.501 | 5.851 | 2.596 | 2.935 | 3.568 | 1.624 | 1.951 |

Perlite consists of 70-75% silicon dioxide ($SiO_2$)which has been found silicon has a toxic effect on *Fusarium* at high concentrations (> 7640 mg). It inhibits fungal mycelium growth and spore germination. At low concentrations, silicon attenuates fungal mycelium growth. It has been found that addition of silicon to banana trees and legumes was resulted in decrease the infection severity of Fusarium wilt (14).

PH estimation results revealed soil PH values for the three concentrations ranged 7.10-7.9 and 8.15-8.95 for $SaCO_4$ and perlite, respectively (Table 3). These results indicate a slight change occurred in PH due to the use of chemical fortifications in three concentrations previously mentioned.Results in published works indicated *F. solani* can survive under PH values ranged7-8 (15), which may explain why chemical fortifications did not affect *F. solani* infection severity using the three concentrations.

**Table 3:** PH estimation of the soil after chemical fortification addition

| Treatment | PHvalue | | |
|---|---|---|---|
| | After 10 day | After 20 days | After 30 days |
| F.s.+ 3% CaSO4 | 7.63 | 7.10 | 7.62 |
| F.s. + 6% CaSO4 | 7.83 | 7.75 | 7.30 |
| F.s.+ 9% CaSO4 | 7.70 | 7.47 | 7.90 |
| F.s.+ 3% perlite | 8.82 | 8.95 | 8.23 |
| F.s. 6% perlite | 8.16 | 8.57 | 8.90 |
| F.s.+ 9% perlite | 8.62 | 8.15 | 8.42 |
| F.s. | 7.58 | 7.57 | 7.30 |
| Control | 7.64 | 7.62 | 7.63 |
| LSD prob. value 0.05 | 0.022 | 0.011 | 0.705 |

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