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Effect of Biofumigation with Radish (Raphanus sativus) Leaves Fresh and Seed Meals to Control Root Knot Nematode and Fusarium wilt Disease Complex Infecting Eggplant

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Abstract:
The study was conducted to evaluate the biofumigation of crushed radish leaves and seed meals to manage the disease complex caused by *Fusarium oxysporum* and *Meloidogyne* spp in eggplants under greenhouse conditions. Pathogenic isolates of *F. oxysporum* were isolated from infected eggplants on PDA and the Meloidogyne was isolated from cucumber plants. Results showed that the concentration, 10, 5, 2.5 g/pot of crushed leaves and seed meals were effective and cause significant reduction in fungal growth on PDA. The addition of radish crushed leaves into the soil at 10,5,2.5g/pot reduced the disease incidence to 5,10,15 % compared with 77.5% in control , and the disease severities to 5,10,10% compared with 75%in control.The amendment of the soil with seed meals at 10,5,2.5 g/pot has reduced the disease incidences to 15,20,35% , and the disease severities to 12.5,18.8,28.8% compared with 77.5%and 75% in control respectively . Plant heights , leaf area , fresh and dry weights were found to be34.75,32.00,28.00cm,7.40,7.325,6.750 cm , 8.075,7.075,4.400 g/plant , and 1.420,0.900,0.675 g/plant respectively in plants grown in the soil containing 10,5,2.5 g/pot crushed leaves ,26.50,24.75,21.00 cm , 8.475,6.975,6.350 cm ,8.00,5.560,4.400 g/plant, 0.950,0.750,0.622 g/plant respectively ,in plants grown in soil containing 10,5,2.5 g/pot of seed meals compared with 13.75 cm , 3.088, 1.805 g/plant and 0.235 g/plant in control respectively .
Keywords: *Fusarium oxysporum*, *Meloidogyne spp* , Biofumigation , eggplants .

1 .Introduction

Soil borne pathogens are considered as the most important factors affecting plant production worldwide. Of these pathogens, *Fusarium oxysporum* and root knot nematodes,*Meloidogyne* spp are the more prevailing and causes heavy losses in vegetable yields reaching to 80% (Nchore *et al.*, 2011; Cetintas & Yarba ,2010).

Eggplants (*Solanum melongena* L.), Family solanacea is one of the most important vegetables in Iraq and intensively cultivated in most Iraq governorates. It has been reported that eggplants were highly infected with *F. oxysporum* that causing root rot and wilt, and *Meloidogyne* spp causing root knot wherever grown. These disease infected roots limiting nutrients uptake resulting in deterioration and death of plants (Cetintas & Yarba , 2010 ; Nchore *et al.*,2010 &2011).

Soil borne pathogens were effectively controlled by using fungicides and nematicides , but the misuse of these compounds has led to enormous problems to environment and human health as well as resistant strains of pathogen were developed making the use of these chemicals ineffective .These problems have stimulated the research of alternative measures for disease control including biofumigation that may offer a strategy ecofriendly to mange soil borne fungal pathogens (Ramirez-Villapudua & Munnecke ,1988;Subbarao *et al.* ,1999),and nematodes (Mojtabe *et al.* ,1991; Johnson *et al.* ,1992). Biofumigation is the use of volatile compounds released during Brassica plant decomposition to mange plant diseases caused by soil borne pathogens (Gruver *et al.*, 2010). Oloo *et al*. (2011) found that *Brassica juncae* was extremely effective for killing *F. oxysporum* f.sp. *rosae* spores . Biofumigation of radish, cabbage, cauliflower and Chinese cabbage leaves caused high reduction in *Meloidogyne hapla* attained to 60.6% with radish , the more effective (Anita, 2012). The aim of this study is to evaluate the efficacy of biofumigation with radish fresh leaves and seed meals at three rates for managing root knot nematode *Meloidogyne* spp and Fusarium wilt infecting eggplants under greenhouse conditions.

2 .Materials and Methods

2.1 .Fungus isolation:

Fusarium oxysporum was isolated from infected eggplants collected from different locations at Abughraib, Baghdad, Iraq, on potato dextrose agar (PDA) medium. The fungus isolates were purified by single spore culture technique as described by (Juber *et al*., 2014).*F. oxysporum* was identified based on cultural and microscopic characters according to, Booth (1977) .The pathogenicity of the isolates was carried out on susceptible eggplants.

F. oxysporum isolates were grown on sorghum seeds medium prepared by adding 100g of sorghum seeds to enough water in bottle of one liter size and autoclaved at 121 C˚and 1.5 kg/cm^2 for one hour. The medium was inoculated with *F. oxysporum* incubated at 25±2 C˚ for 15 days and used as fungus inoculums.

2.2. Meloidogyne isolation

Meloidogyne spp were isolated from cucumber plants and maintained on eggplant 'black beauty' grown is polarized sandy loam soil in the greenhouse. The mean of initial population level of *Meloidogyne* spp in soil samples was estimated before treatment, and the active juveniles / ml were extracted by sieving and decanting method and counted by compound microscope (Barker, 1985).

2.3. Effect of radish material on survival of *F. oxysporum i*n vitro

The experiment was conducted at plant protection department /College of Agriculture/University of Baghdad,Iraq .A plug ,5 mm diameter taken from the margin of *F. oxysporum* colony on PDA,7 days old, was placed in the centre of PDA plate amended with chloramphenicol at 100µg ml^{-1}. Crushed fresh radish leaves or seed meals were placed in 5cm glass plate onto the upturned lid of the plates with the inverted bottom containing the fungal plug. Sterile distilled water at a rate 1:2 (w /v) was added to the radish material to induce releasing the isothiocyanates (ITCs) and the plate was immediately sealed with parafilm. Five concentrations 0,5,10.15 and 20 mg plate $^{-1}$ with four replicates were applied .The plates were incubated at 25±2 C° for 8 days .The colonies typical for *F. oxysporum* were counted the growth inhibition percentage was calculated by the formula I= C-T / C ×100 ,where I= percent of growth inhibition , C= radial growth in control , and T= radial growth in treatment .

2.4. Effect of radish material on survival of *F. oxysporum* and *Meloidogyne* spp in vivo:

Sandy loam soil was autoclaved twice at 121C° and 1.5 kg/cm^2 for one hour for two successive days and left stand for a month to avoid toxic gas produced by the degradation of the organic matter (Escand, 2007). The soil was distributed in pots of 15 cm diameter and inoculated with Fusarium inoculums at 3% (w/w). The inoculated pots were watered and mixed thoroughly for one week to ensure inoculums distribution .After distribution of the Soil in the pots , crushed radish leaves or seed meal were mixed with the soil at different rates .The pots were then covered with plastic sheets to hold back the fumes resulting from leaves or seed decomposition in the soil .The sheets were removed after ten days and the pots were cultivated with 30 days old of eggplant seedling , 5 seedling/pot and 4 pots for each treatment. After 4 days of seedlings transplanting, 1000 freshly hatched juveniles of Meloidogyne were added to each pot. The pots were distributed in the greenhouse at complete randomized designed (CRD) as following:

1- Non inoculated soil.
2- Inoculated soil.
3- Inoculated soil+ crushed radish leave at (2.5 g/pot).
4- Inoculated soil + crushed radish leave at (5 g/pot).
5- Inoculated soil +crushed radish leave at (10 g/pot).
6- Inoculated soil + seed meal at (2.5g/pot).
7- Inoculated soil + seed meal at (5g/pot).
8- Inoculated soil + seed meal at (10g/pot).
9- Non inoculated soil + crushed radish leave at (10 g/pot).
10- Non inoculated soil + seed meal at (10 g/pot).

The percent of disease incidence of *F. oxysporum* was recorded 30 days after germination and the disease severity was determined after 60 days according to a scale of five degree proposed by Ishikawa *et al.*(2005) with slight modification based on amount of browning on root and stem (0= healthy , 1= 1-25% browning , 2= 26-50% browning , 3= 51-75% browning , 4= 76-100% browning).

Plant growth parameters including, plant heights, leaf area, fresh and dry weight of plants were taken. The plants were uprooted after 60 days of inoculation and the nematodes in the soil were extracted by sieving and decanting method (Barker ,1985).Root –gall index was determined in each pot using a scale of 5 degree according to Dube & Smart (1978).

3. Results

3.1. Pathogencity test *of Fusarium oxysporum*

Based on cultural and microscopic characters, the fungus was identified as *F. oxysporum* (Booth, 1977).Results obtained indicated that all *F. oxysporum* were found pathogenic and caused wilting of eggplants after 10 days of inoculation.

3.2. Effect of biofumigation on *F. oxysporum* in vitro

Results showed that all the concentration of radish crushed leaves and seed meal were effective and caused significant reduction in *F.oxysporum* growth on PDA,(Fig.1). The reduction in fungal growth was found to be correlated with the concentrations. The percentage of mycelium growth inhibition was attained to 100% at 6

mg/plate of crushed leaves, while 10 mg / plate of seed meal were needed to obtain the same percentage of inhibition .

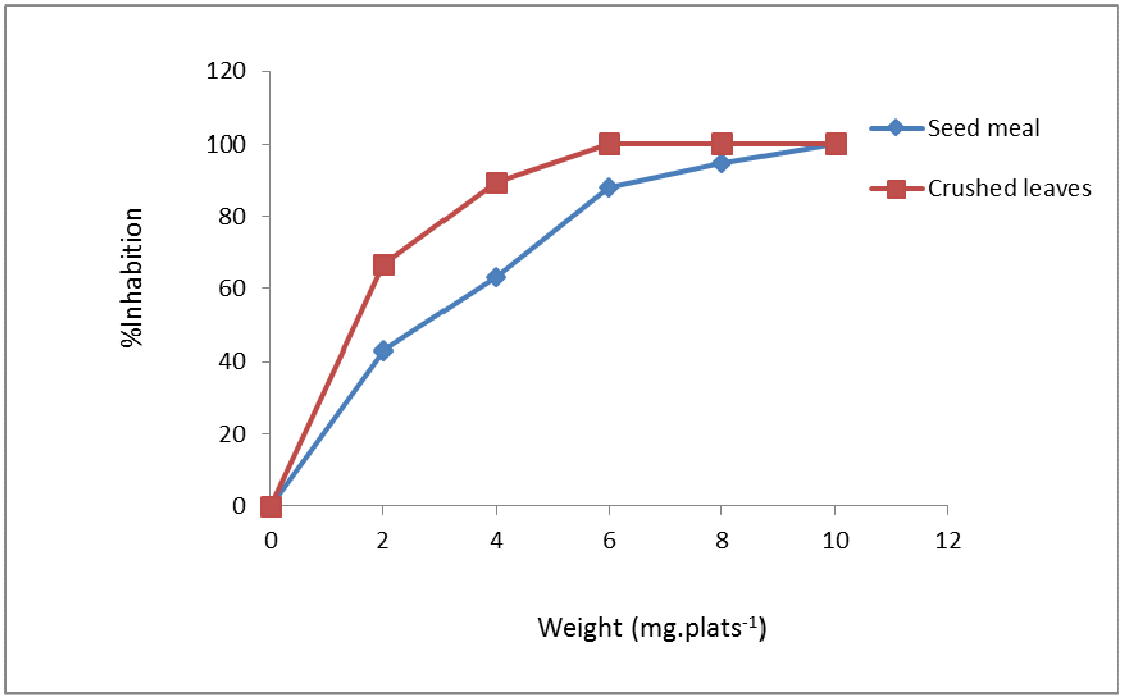

Figure 1. Effect of radish material on survival of *F. oxysporum* in vitro
LSD (P=0.05) for seed meal = 8.23, LSD (P=0.05) for crushed leaves =4.93. Four replicates for each treatment.

3.3. Effect of radish materials on disease complex on eggplants

It was found that the addition of 2.5,5,and 10 g/pot of radish materials ,10 days before cultivation of eggplants, Black beauty, induced significant reduction in disease complex incidence. The percentage of disease incidences were found to be 5.0, 10.0 , 15.0% of plants grown in soil treated with 10,5,2.5 g/pot of crushed leaves respectively , attained to 15.0, 20.0 , 35.0% on plants grown in pots containing 10,5,2.5 g/pot of seed meal respectively compared with 77.5% on plant in soil treated with the pathogens only (control).

The disease severities have attained to 5.0, 10.0, 10.0% on eggplants grown in soil with 10,5,2.5 g/pot of crushed leaves respectively , 12.5, 18.8 and 28.8% on eggplants grown in soil with 10,5,2.5 g/pot of seed meal respectively compared with 75% in control (Fig.2).

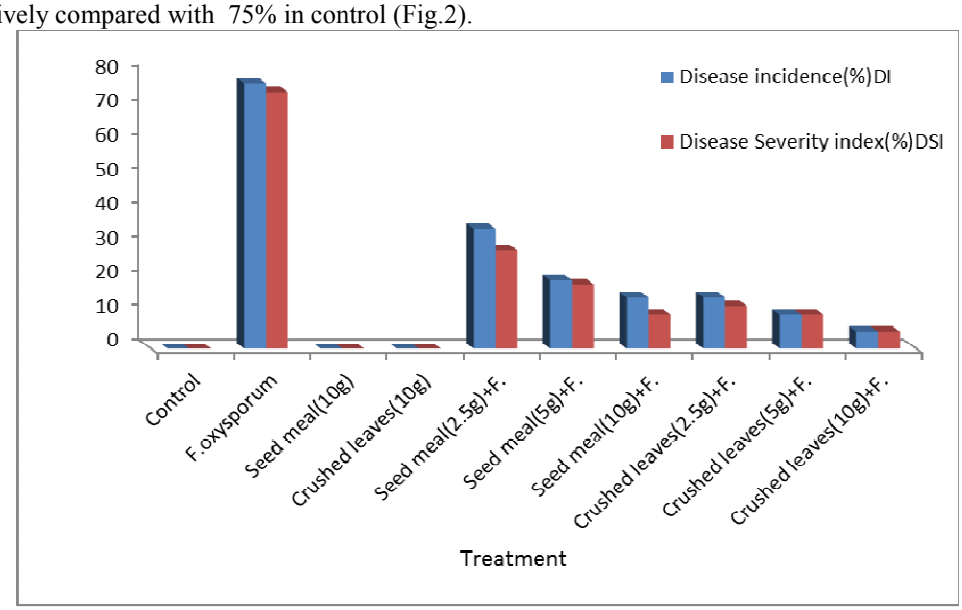

Figure 2. Effect of radish material on complex disease Incidence and severity on eggplant cv.black beauty.*Four replicates for each treatment .LSD (P=0.05) = 16.83 of disease incidence (%) and LSD (p=0.05) =16.28 of disease severity.

3.4. Effect of radish materials on plant growth parameters:

The reduction in disease incidence and severity was found associated with enhancement of plant growth parameters. The plant heights ,leaf area ,fresh and dry weights were attained to 34.75,32.00,28.00cm ,7.40,7.325,6.750 cm , 8.075,7.075,4.400g/plant, and 1.420, 0.900,0.675g/plant respectively for plants grown in the soil containing ,10,5,2.5 g/pot of crushed leaves ,26.50,24.75,21.00cm,8.476,6.975,6.350cm,8.00,5.560,4.400 g/plant and 0.950,0.750,0.622 g/plant respectively for the plants grown in the soil containing 10,5,2.5 g/pot of seed meal compared with 13.75cm,3.088cm, 1.805g/plant and 0.225 g/plant in control respectively (Fig. 3).

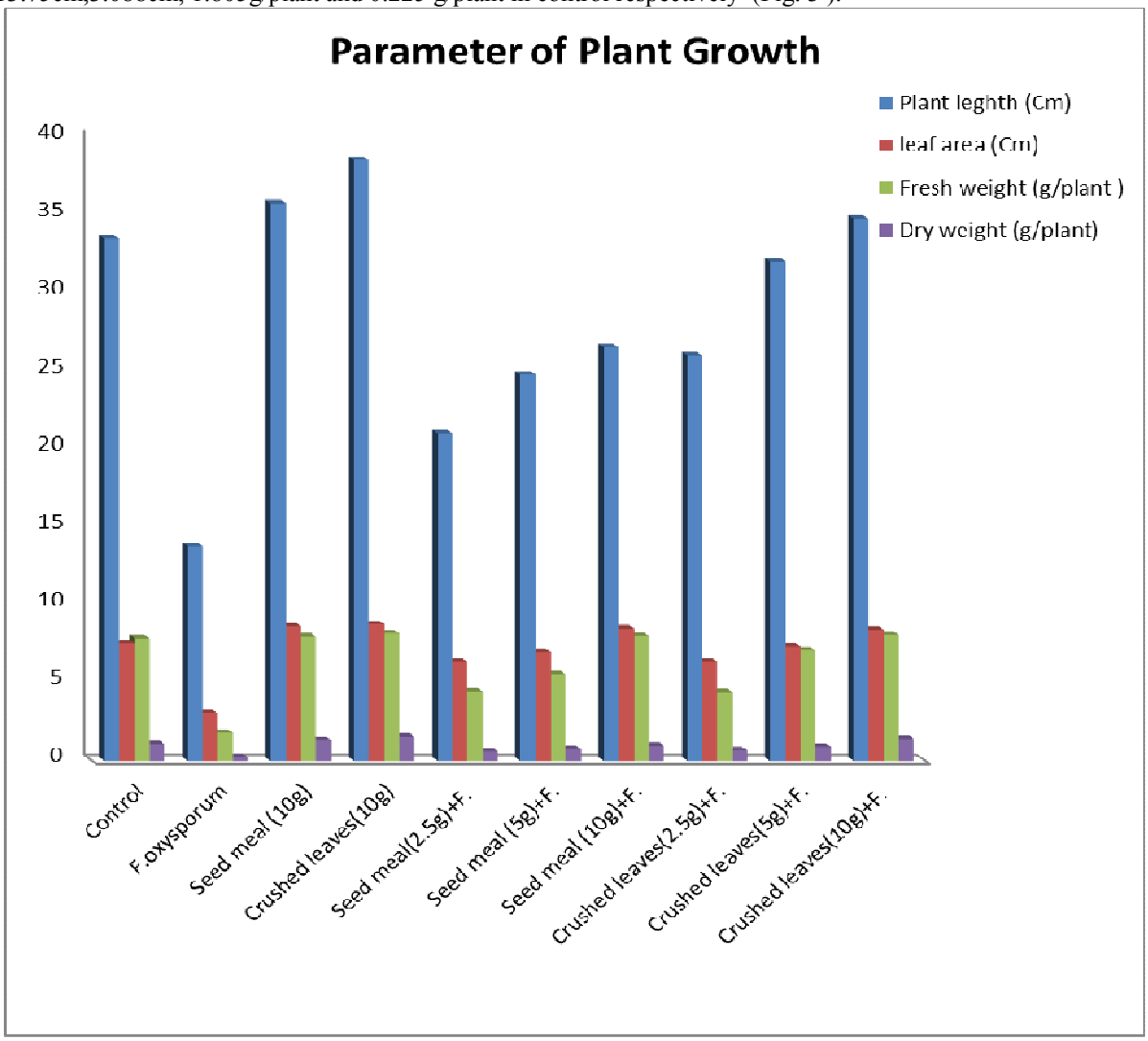

Figure 3.Effect of radish material as a biofumigant at different rates on growth parameters of eggplant infected with complex disease .LSD=0.05 0f plant length =2.912, and LSD=0.05 of leaf area = 0.678 and LSD=0.05 0f Fresh weight =0.633 and LSD =0.05 of dry weight = 0.226. * Four replicates for each treatment.

Discussion

The results of this study demonstrated that fumigation with radish crushed leaves or seed meal induced high inhibition activity against *F. oxysporum* and *Meloidogyne* spp as proved by the high significant reduction of the disease complex incidence and severity that cause, compared with the control. The antagonistic effect of radish material may come from the compounds that released in the soil during covering with the plastic sheets. It was reported that covering soil, containing radish residues, with plastic sheets, induce releasing volatile compounds including isothiocyates and induce the hydrolysis of glucosinates producing other compounds that exert toxic effects on the pathogens and decrease the incidence of various diseases (Gamliel & Staplelon ,1993;Block *et al.*,2000).Gruver *et al.* (2010) reported that decomposition of Brassica plants in the soil has released volatile compounds that posses antagonistic activity against soil borne pathogens. Lou *et al*. (1992) found that *Brassica juncae* was effective in killing the spores of *F. oxysporum* f .sp.*rosae* .The fumigation of radish ,cabbage ,cauliflower , chinese cabbage leaves caused high reduction in *Meloidogyne hapla*

(Anita ,2012).The promotion of plant growth parameters may came from increasing the availability of nutrients in the rhizosphere and inducing root growth that led to foliate the nutrient uptake .The promotion may also due to the suppression of pathogens in the soil by some compounds released during decomposition of radish residues. It was reported that the plants of solanaceae produce many active biological alkaloids having inhibitory effects against different pathogens (Iranbakhsh *et al*., 2010).The results of this study revealed that fumigation of plant material may be promising as a factor in plant diseases management as alternative measures of using fungicides and nematocides.

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