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Efficacy of Lemongrass Oil in Managing Fusarium Root Rot in Okra (Fusarium Solani)

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ABSTRACT
This study was aimed at assessing the intensity of okra root rot in district Charsadda and evaluating the effectiveness of lemon grass oil against the obligate parasitic fungus associated with the root rot of okra (Fusarium solani). Surveys were conducted in the district of Charsadda during April and May 2021 when the okra was at seedling stage. The survey showed that the seedlings were planted in 15 different localities in which okra was grown. The incidence of Fusarium solani was recorded in soil samples. The minimum incidence was reported from Rahim Abad (9.00% seedling mortality) and the maximum seedling mortality (20.14%) was reported from Bad Shah Kali. The in- vitro study of lemon grass oil revealed that the oil adversely affected the mycelia growth, biomass and spore count/ml of the pathogen (F. solani). The treatments showed that lemon grass oil concentrations applied at max. (3%) was maximum for min. of colony diameter, fresh biomass and spore count/ml of Fusarium solani. While it was min. where lemon grass oil concentrations applied @ 0.5% and @1%. The lemon grass oil @ 3% had significant effect in limiting the colony diameter, fresh biomass and spore count/ml. In in-vivo study, lemon grass oil stimulated okra seed germinations. A significant effect was shown for germination and mortality percent. The study also showed that lemon grass oil had a significant effect on controlling okra root rot condition in-vitro and in-vivo. The maximum concentration 3% had a significant effect on controlling Fusarium solani in both in-vitro and in-vivo conditions. It is possible to state that lemon grass oil can be used against F. solani as a disease management method.
Keywords: Okra root rot, Fusarium solani, lemon grass oil, biocontrol, seedling mortality

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INTRODUCTION
Okra is sometimes also referred to as ladies' fingers or ochro. The sweet green pods produced are a highly edible good. Geographically potential origins of okra (Abelmoschus esculentus L.) include West Africa, Ethiopia and South Asia. Okra was grown commonly in all warm temperate, tropical and subtropical regions (Singha et al., 2014).Okra is primarily composed of water (90%) and has low nutritional values with (2%) protein, (7%) carbohydrates, and small amounts of fats. Okra in the fresh state was found to have more vitamins and minerals than fruits and vegetables such as (K, C), fiber and only moderate levels of magnesium, folate and thiamin. The mucilage of the okra plant has flocculent properties which may be used to reduce the turbidity of waste water (Agarwal et al., 2001). In Pakistan, the okra crop was grown on 15,923 hectares with a production of 12, 2157 tonnes of fresh okra pods. The Gross production of okra in KPK was on 2138 hectares, with a production of 17,274 tonnes (MNFSR, 2019).
Okra is in the Malvaceae family and is grown specifically for its immature, edible, fibrous free green

fruits found in the tropical and subtropical areas in the world. The crop will also suffer tremendously from a variety of nematodes, viruses and fungi, which differ in the severity of the invasion.The most serious crop diseases include: Cercospora's blight, wilt, root rotting, powdery mildew, damping off and yellow vein mosaic virus disease. (Anonymous. 2011). Also, a number of fungal attacks on okra crops (Mithal, 2006). Okra plants infected with F. solani leaf wilting and shrinking of leaves, as well as the death of seedling roots. Infected plants have obvious root rots and symptoms of extensive root rotting. In screened houses, F. solani was the primary pathogen in reduced yield and leaf mass of okra. (Godoy et al., 1990).

Okra's yield and quality are influenced by Fusarium solani, which has an effect on other vegetables (Ghaffar, 1995) likely beans (Mousa, 1994). Okra root rot disease is caused by fungus Fusarium solani (Rahim et al., 1992). It appears to have an estimated prevalence of approximately (10-80 %) maximum (55-88 %) for kitchen/home gardening as the least occurrence of (10-45 %) of all commercial fields; it appears that it can survive on infected plants as well from the soil; for naturally infected okra seeds were described as hazel to black appearance (Mithal, 2006).

Plant disease management employs many management practices including cultural, biological, chemical and crop rotation control methods.

Agrochemicals, including pesticides/fungicides and fertilizers, can damage the environment and human health. Additionally, chemical control methods are the easiest and most dangerous option for beneficial organisms in the soil. Chemicals pollute the area (soils and water) and are a great risk for future generations. Alternative sources need to be explored for agrochemicals, such as plant extracts (lemongrass oil and essential oils). Plant extracts have no environmental effects, and mark a more manageable option for plant diseases. To that end, this research study was undertaken with the following objectives.

1. To establish the severity of okra root rot infection in the district of Charsadda.
2. To determine the efficacy of lemon grass oil both in vitro and in vivo against Fusarium solani.

MATERIALS AND METHODS

Distribution of Okra Root Rot in the District Charsadda

A survey was conducted throughout the district of Charsadda, in fifteen okra growing areas. In each location, five fields were each assessed to track seedling mortality. Each field collected data from five separate locations for each individual field. The total percentage of the seedlings beyond trace mortality was calculated using the following formula:

$$\text{Seedling mortality (\%)} = \frac{\text{Total number of rotted seedlings/m2}}{\text{Total number of seedlings/m2}} \times 100$$

Isolation of Fusarium solani

Diseased okra seedlings were collected from several locations in district Charsadda, cut into small pieces and soaked in a 0.1 percent ($HgCl_2$) solution for fifteen to thirty seconds before rinsing three times with sterile distilled water, then transferred to a Petri dish with PDA medium and incubated at 25o C. Petri dishes were evaluated regularly for fungal growth. The pathogen was identified with the key of Tousson and Nelson (1979).

In vitro study

Different concentrations of lemon grass oil (0.5, 1, 1.5, 2, 2.5 and 3) % were tested in an in-vitro experiment to control Fusarium solani using the food poisoning technique (Meraj Ali, 2019). Fusarium solani was inoculated on PDA poured in Petri dishes which were then taped shut and incubated at 25oC. Mancozeb was used in the positive controls and Fusarium solani was used in the negative control on PDA without adding any chemical. The experiment used a Completely Randomized Design (CRD) with five replications.

The treatments were;

T_1	= *Fusarium solani* only (negative control)
T_2	= *Fusarium solani* + Mancozeb @ 500 ppm (positive control)
T_3	= *Fusarium solani* + lemon grass oil @ 0.5%
T_4	= *Fusarium solani* + lemon grass oil @ 1%

T_5 = *Fusarium solani* + lemon grass oil @ 1.5%	
T_6 = *Fusarium solani* + lemon grass oil @ 2%	
T_7 = *Fusarium solani* + lemon grass oil @ 2.5%	
T_8 = *Fusarium solani* + lemon grass oil @ 3%	

The spore concentration/ml data, the colony diameter data, and the fresh biomass data were collected at 4, 8, and 12 days' intervals, and the spore concentration/ml was only added at the 12 days' interval. The recorded data were subjected to statistical analysis using the Analysis of Variance (ANOVA). Separation of means was done using the least significant difference test (Dana, 2001).

Screen house study

Arrangement of pots

The pots were filled with a 1:1:1 of clay, farm yard manure (FYM), and silt. The pot diameter was 6.5 cm and the depth was 8 cm. Fort (40) pots were filled and arranged under a Completely Randomized Design (CRD) with five replications. Ten seeds were sown in each pot. Lemat grass oil was applied to these okra seeds (Shaheen) using different concentrations with two controls (negative and positive). The pots were injected with Fusarium solani @ 2.0×105 spore/ml at the root zone (Jain and sharma, 2001). In order to record data on germination percentage and seedling mortality after twenty two days of germination the following formulas were used:

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated/pot}}{\text{Total number of seeds/ pot}} \times 100$$

$$\text{Mortality (\%)} = \frac{\text{Number of rotted seedlings/pot}}{\text{Total number of seeds/pot}} \times 100$$

Experimental Layout

The experiment was performed using a five times replicated Completely Randomized Design (CRD) so we can examine the in-vivo effect of Lemon grass oil. The pots were arranged as shown in Table 3.1.

Data Recording and Statistical Analysis

All recorded data was analyzed using the analysis of variance (ANOVA) for the CRD technique. The means were separated using the Least Significant Difference (LSD) method.

Re Isolation of F. Solani from Contaminated Seedlings

Okra seedlings infected by F.solani were sent to the Department of Plant Pathology. The infected parts were cut into pieces and surface sterilized by 0.1 percent (HgCl2) solution before being placed on Petri dishes containing PDA medium. The pathogen was identified using the Nalson and Toussoun key (1976).

Effect of Lemon Grass Oil on Inoculum of F. Solani in Soil

A small pot experiment was setup to determine and quantify the Fusarium solani inoculum in the soil. The soil (clay, FYM and silt) was sterilized and placed in small pots and seeded with Okra seeds. A suspension of Fusarium solani spores was applied to the pots at a concentration of 2.0×105 spore/ml (Jain and Sharma, 2001). As previously described, lemon-grass oil was applied to the soil. A total of 5 replications were maintained with a Completely Randomized Design (CRD) which was selected for the study. At 14 days after seedlings, Fusarium solani was again isolated and quantified. Quantitatively determining the pathogens per gram of soil was done using the dilution plating technique with 1 gram of soil collected in 0.05% water agar (WA). Water agar was prepared using 0.5 grams of agar mixed with one litre of water. Weautoclaved (121ºC for 15 minutes), a hundred ml of the solution in a glass bottle to prepare soil suspensions of various dilutions (Burgess et al., 1994). I prepared a ten-fold dilution suspension. In each dilution, one ml of soil suspension was uniformly plated over PPA (peptone PCNB agar), which is selective medium for colonies per plate. PPA allows for the selective isolation of F. solani from the dilution of soil (Nash and Synder, 1962). Table 3.2.

Table: Experimental layout for *in vivo* study.

T_1R_1	T_6R_2	T_2R_1	T_8R_3	T_8R_5	T_5R_1	T_6R_4	T_7R_2
T_3R_1	T_1R_2	T_8R_1	T_3R_2	T_2R_4	T_6R_3	T_4R_1	T_4R_3
T_2R_2	T_7R_3	T_1R_3	T_7R_5	T_4R_5	T_3R_3	T_8R_4	T_6R_1
T_4R_2	T_4R_4	T_5R_4	T_1R_4	T_8R_2	T_5R_2	T_3R_4	T_6R_5

T_5R_5	T_7R_4	T_2R_3	T_5R_3	T_1R_5	T_2R_5	T_7R_1	T_3R_5

Table: Experimental layout for small pots.

T_1R_1	T_6R_2	T_2R_1	T_8R_3	T_8R_5	T_5R_1	T_6R_4	T_7R_2
T_3R_1	T_1R_2	T_8R_1	T_3R_2	T_2R_4	T_6R_3	T_4R_1	T_4R_3
T_2R_2	T_7R_3	T_1R_3	T_7R_5	T_4R_5	T_3R_3	T_8R_4	T_6R_1
T_4R_2	T_4R_4	T_5R_4	T_1R_4	T_8R_2	T_5R_2	T_3R_4	T_6R_5
T_5R_5	T_7R_4	T_2R_3	T_5R_3	T_1R_5	T_2R_5	T_7R_1	T_3R_5

RESULTS

Distribution of Okra Root Rot in the District Charsadda

A survey was conducted throughout the district of Charsadda, in fifteen okra growing areas. In each location, five fields were each assessed to track seedling mortality. Each field collected data from five separate locations for each individual field. The total percentage of the seedlings beyond trace mortality was calculated using the following formula:

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T_3R_1	T_1R_2	T_8R_1	T_3R_2	T_2R_4	T_6R_3	T_4R_1	T_4R_3
T_2R_2	T_7R_3	T_1R_3	T_7R_5	T_4R_5	T_3R_3	T_8R_4	T_6R_1
T_4R_2	T_4R_4	T_5R_4	T_1R_4	T_8R_2	T_5R_2	T_3R_4	T_6R_5
T_5R_5	T_7R_4	T_2R_3	T_5R_3	T_1R_5	T_2R_5	T_7R_1	T_3R_5

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T_1R_1	T_6R_2	T_2R_1	T_8R_3	T_8R_5	T_5R_1	T_6R_4	T_7R_2
T_3R_1	T_1R_2	T_8R_1	T_3R_2	T_2R_4	T_6R_3	T_4R_1	T_4R_3
T_2R_2	T_7R_3	T_1R_3	T_7R_5	T_4R_5	T_3R_3	T_8R_4	T_6R_1
T_4R_2	T_4R_4	T_5R_4	T_1R_4	T_8R_2	T_5R_2	T_3R_4	T_6R_5
T_5R_5	T_7R_4	T_2R_3	T_5R_3	T_1R_5	T_2R_5	T_7R_1	T_3R_5

DISCUSSION

There are many soil borne pathogen fungi that attack okra plant. Fusarium solani (Martt.) Sacc.(1881), is considered to be one of the most detrimental. Okra plant is a summer crop and fungus like Fusarium solani loves warm soil, which causes the yield reduction. The root rot of okra is one out of many diseases that would harm crop yield and occurrence of such diseases ranges from 10-80 percent. The yield is greatly affected in the field (10 to 45%) (Zahoor et al., 2012). Many management techniques consisting of cultural, chemical and biological practices are performed to diminish harmful impacts of the pathogenic. Lemon grass oil as biocidal management, (F. solani) was used as the focus of the study. The biocidal management was easy, inexpensive, simple, and does not have na negative effect. (Josep

et al., 2008; Siriponivisal et al., 2009). The management of root rot (Fusarium spp) often using phytoextracts has been reported in okra plant. There has been reports from different parts of the word. Plant extracts reduced or inhibited the Fusarium solani growth by (0.76-56.17%) (Mamatha and Ravishankar, 2004).

In the present studies; a survey was done to see the prevalence of Fusarium solani in 15 different sites of district Charsadda, and an in-vitro and in-vivo efficacy of phytoextracts i.e. lemon grass oil was researched. Mithal (2006) also worked on Fusarium solani infestations and had similar findings when using phytoextracts. The treatment (T8) were maximum concentration (3%) was used gave the very promising results for all parameters under in-vitro studies. The colonies diameter was reduced by 6.15-76.10 % while biomass reduction was in range of 8.01- 76.60 %. Also the number of spore per ml were also reduced by 16.21-97.29 %. Other treatments such as T3 (0.5%) and T4 (1.5%) were least effective against the pathogen (F. solani). though it was reduced the colony diameter by 6.15 and 12.56 % respectively compared to control. Biomass reduction was 8.01 and 25.96 % when lemon grass oil was applied at (0.5%) and (1%) respective order. Kabadiya et al. (2014) had also seen similar results when they applied lemon grass oil to selle the fungal disease F. solani reported (62%) reduction in fungal growth (colony diameter). It is noted from Dominator (2012) that various plant extracts could be used to abstain spore germination or prect reduce the production of spores.

The data for okra seed germination rate and seedling mortality was collected from an in-vivo study. Responding relative to the untreated control, lemon grass oil promoted seed germination from 75.00-425.00 percent and reduced seedling mortality rate from 14.28-80.95 percent. Treated T3 (0.5%) had a seedling mortality rate reduced by 14.28 and promoted seed germination by 75.00. Similar finding were noted by Farag et al., (2011) . Abd el khair et al., (2011) filed experiment assessed lemon grass oil and reported a reduction of fungal mycelial growth by 42.2% . Compared to untreated controls lemon grass oil application reduced fungal infestation symptomatic of soil by 16.41- 70.14%.

The active biocidal constituent was extracted from the fractions of the lemon grass leaf (Govindachari et al., 1998). The lemon grass oil consist of antifungal constituents like ethyl acetate; ethanolic constituents, etc, that can destroy the structural and functional aspects of the fungal cell wall and disturb enzymatic bioactivity physiology. The lemon grass oil constituents penetrates the fungal cell altering the overall metabolism and functions by delocalization of pathogen enzymatic activity (Zambonelli et al., 1996). Also, the lemon grass oil disrupts the respiration of the fungal cells (Inouye et al., 1988).

The data of okra seed germination rate and seedling mortality was generated from an in-vivo experiment. Lemon grass oil promoted seed germination from 75.00-425.00 percent and lowered seedling mortality rate from 14.28-80.95 percent relative to the untreated control. Treated T3 (0.5%) was reduced from 15.38 to 14.28 and promoted seed germination from 75.00 similar finding have been report by Farag et al., (2011). Abd el khair et al., (2011) field trial studied the effects of lemon grass oil and reported a reduction of fungal mycelial growth by 42.2% . Lemon grass oil application, relative to untreated controls, reduced fungal infestation related to soil from 16.41-70.14%.

The active biocidal constituent was recover from fractions of lemon grass leaf (Govindachari et al., 1998). Lemon grass oil contains antifungals like ethyl acetate; ethanolic constituents, etc., which can damage the structural- and functional- aspects of the fungal cell wall and interfere in the physiology of enzymatic bioactivity itself. The constituents of lemon grass oil penetrates the fungal cell, the metabolism and functions were altered by delocalization of the pathogen enzymatic activity (Zambonelli et al., 1996). Inouye et al., (1988), described this process, lemon grass oil alters the respiration of the fungal cell.

The efficacy of lemon grass oil in limiting Fusarium solani was assessed in vitro. The experiment utilized six (6) different treatments of lemon grass oil (T3 @ 0.5%, T4 @ 1%, T5 @ 1.5%, T6 @ 2%, T7 @ 2.5% and T8 @ 3.00%) against F. solani. One treatment (T1) is kept as a negative control, while (T2) is a positive check for comparison. The poisoned food method was used to manage F. solani using lemon grass oil. The parameters used to track the efficacy of lemon grass oil on Fusarium solani was biomass, colony diameter and spore concentration/ml.

The data for colony diameter and fresh biomass was collected at intervals of 4, 8 & 12 days. The results

suggest the highest concentration T8 (3%) was the most effective. Treatments T3 (0.5%) and T4 (1%) had a small impact on mycelial growth. Data for spore concentration/ml was recorded after 12 days incubation temperature of 25^{oC}. When compared to the highest concentration treatment T8 (3%) of lemon grass oil, the fungal culture spore count was severely decreased to 0.20 $\times 10^5$.

A pot study was conducted at Screen House of the Malakander Farm, Department of Plant Pathology, University of Agriculture, Peshawar. Silt, Clay and farm yard manure (1:1:1 by volume) was put in forty pots. Each pot was sown with ten seeds (treatment with lemon grass oil). The pots were then covered with soil. The pots were checked for germination . At twenty two days, percent germination and percent mortality of okra seedlings were determined. Treatments T3 (0.5%) and treatment T4 (1%) produced least number of seed germination while percent mortality of seedlings was high. Whereas maximum treatment, T8 (3%) increased seed germination percentage by 84% and decreased seedling mortality percentage by 16%. Treatment T8 (3%) produced the maximum reductions in number of colonies (12.00) of F. solani and treatment T3 (0.5%) produced minimum reduction in number of colonies (33.60) respectively.

CONCLUSIONS AND RECOMMENDATION

Conclusions

1. Okra root rot was prevalent in district Charsadda (9.00 to 20.14%).
2. The caused organism (Fusarium solani) was sensitive to the maximum concentration of lemon grass oil (3.00%) used in a in-vitro study.
3. Minimum concentrations (0.5 and 1%), produced the least effect on the pathogen (F. solani).

Recommendations

1. . Lemon grass oil at a concentration of 3% can be used as part of an IDM program in the integrated management of F. solani.
2. It is also recommended that research be conducted on different formulation and combinations of bio extracts to increase its effectiveness against the pathogen (F. solani).

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