Research Article

Acacia nilotica Use as an Organic Amendment in Place of Chemicals

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Abstract | *Acacia nilotica* is one of the most popular plant having a strong antifungal activity for different fungal pathogens and have compounds are more effective to promote the growth of tomato plant. Tomato (*Lycopersicum esculentum*) plant is highly susceptible to root rotting fungi causing huge losses each year in Pakistan. *Acacia nilotica* used as a biofertilizer and Significant ($p< 0.05$) enhanced the growth of tomato plants. Infection % of all test fungi included *Fusarium solani, Fusarium oxysporum*, *Macrophomina phaseolina* and *Rhizoctonia solani* was significantly controlled by leaf powder (LP) and shoot powder (ShP) when applied alone and with combination. While combinations of 3% LP + 3% ShP, 3% LP + 5% ShP, 5% LP + 1% ShP, 5% LP + 3% ShP, 5% LP + 5% ShP completely controlled the infection of *Macrophomina phaseolina*. Similarly combination of 5% LP + 5% ShP completely control the infection of *Fusarium solani* While 5% LP + 3% ShP treatment completely controlled the infection of *F. oxysporum*.

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Introduction

Tomato (*Solanum lycopersicum* L), is commonly available crop, cultivated world wide. It can be grown in all most all climates i.e. from tropical to temperate. After potato, tomato is the second most consumed vegetable (Foolad, 2007). Production of this plant is 161.7 million metric tons with an approximate value of 59 billion dollars. Tomatoes are a useful fruit which can be consumed in fresh and processed forms. It is very rich in vitamin A and vitamin C containing enough antioxidant, lycopene pigment, which prevent the body against cancer and cardiovascular diseases (Bohm et al., 2012). The nutritional values and wide use make tomatoes valuable in the market. Therefore, its large scale production is crucial for fulfillment of the demand. However, the fungal, bacterial and viral diseases restrict commercial production of tomato (Adhikari et al., 2017). Although, synthetic chemical fungicides are available for efficient control of seed borne fungi. However, these chemicals are not applicable for the treatment of seeds because of its hazardous nature (Dukic et al., 2004). Therefore, search for harmless method is crucial for protection of crops. It was found that extracts of several higher plants have insecticidal, antifungal and antibacterial characteristics (Okigbo and Ogbonnaya, 2006; Ergene et al., 2006). Also, extracts obtained from some trees and crop residues play a major part in crop development and production

(Ahmed and Nimer, 2002; Farooq et al., 2008). A recent study revealed that seeds priming with plant extracts of *Acacia nilotica* can be used for the growth enhancement of plants (Rafi et al., 2015).

Acacia nilotica belongs to a family (*Leguminosae*, subfamily *Mimosoideae*) and widespread in Africa, Australia and Asia. It is profitable for nitrogen fixation and is beneficial for several other purposes. One of its accomplishment is that it can grow and survive in extreme temperature (> 50 °C) as well as dry air. However, it is frost sensitive in early stage. This plant exists over 2000 m from sea level (Bargali and Bargali, 2009).

Acacia nilotica is rich in many useful compounds such as volatile essential oil, phenol, resins, steroids, alkoloids, protocatechuicacid, tannins, terpenes, oleosins, phenolicglycosides, phlobatannins, gallicacid, pyrocatechol, (-) epi-gallocatechin-7-gallate, 7-digallate, (+)-catechin, and (-) epigallocatechin-5 (Ali et al., 2012). Antioxidant and antibacterial property of this plant observed by (Asma et al., 2019). *Acacia nilotica* has potential anti-oxidant activity and can be used for treating various serious diseases (Singh et al., 2009). This plant has anti-fungal, anti-microbial, anti-plasmodial and activity against HIV-I and HCV (Sultana et al., 2007; Thangum et al., 2018; Rao et al., 2018). Seeds, bark, gum, root, leaves, immature pods, flowers and fruits serve as a vasoconstrictor, anti-cancer, antipyretic, antimutagenic, spasmogenic, anti-diabetic, cytotoxic and anti-asthamatic (Ali et al., 2012). Bark of *Acacia nilotica* is used as styptic, leprosy, leucoerma, acrid, astringent, skin diseases, emollient, emetic, nutritive, in hemorrhage, anthelmintic, aphrodisiac, diuretic, expectorant, wound ulcers, seminal weakness, colds, bronchitis, biliousness, bleeding piles, diarrhea, leukoderma and dysentery (Baravkar et al., 2008; Del, 2009). While its twigs can be used as toothbrushes (Meena et al., 2006). Pods of *A. nilotica* have the ability to induce cythopathogenicity for inhibition of HIV-1 (Asres et al., 2005) while extract of its fresh roots known as Desisharab (local bear) is used as narcotic (Badshah and Hussain., 2011). The leaf extract of *A. nilotica* was found useful for anti-mutagenic and chemopreventive activities compared to its other parts (Kalaivani and Mathew, 2010a). All these characteristics make this plant applicable as a biocontrol agent in the field of plant pathology.

The objective of this work was to introduce harmless method, use of *Acacia nilotica* as organic amendment, to reduce the infection of pathogenic fungi and ultimately enhance the growth of tomato plants.

Materials and Methods

Collection of plant materials

The *Acacia nilotica* plant was collected from surrounding areas of Women University, Swabi and the specie was identified.

Preparation of plant extract

Ethanolic extracts of shoot and leaves of *Acacia nilotica* were developed following the tailored procedure of Okogun (2000). Shoot (150 g) of the plant was dried in air and powdered in electric grinder. This powder was put in a beaker and 95 % ethanol (500 ml) was added at room temperature (28 ± 2°C). Afterwards, this mixture was agitated using rotary shaker for 24 hours to evaporate the solvent and get the crude extract. Same procedure was followed for obtaining extract of leaves of Acacia plant.

Isolation of root infecting fungi

Soil dilution was used for getting Fusarium specie (Nash and Snyder, 1962) which was identified by Booth (1971) and Nelson et al. (1983) methods. Baiting procedures was used for the isolation of *Rhizoctonia solani* as reported by Wilhelm, 1995. Moreover, dilution and wet sieving procedures were used for the isolation of *Macrophomina phaseolina* (Sheikh and Ghaffar, 1975).

Antifungal activity

Solution (100 mg/ml) of plant extracts was applied on sterilized discs prepared from Whatman No. 1 filter paper at 20 microliter per disc. They were placed on one side of Czapek's Dox agar petri dishes whose pH was maintained at 7.2. While 5mm discs of fresh and actively growing culture of test fungi i.e. *Fusarium solani*, *Macrophomina phaseolana*, *F.oxysporum*, *Rhizoctonia solani* were inoculated at the other side of the petri dishes. These systems were incubated at 28 °C for five days. All the treatments were performed in triplicate using 10%, 20% and 50% solutions of shoot and leaves. The zone of inhibition between fungal colony and the disc was measured in mm.

Field experiment

Field experiments were carried out in the Botanical

Garden of Women University Swabi, Pakistan in randomized block pattern. This soil has natural invasion of 0 to 6 percent colonization of *R. solani*, 1 to 7 sclerotia per gram of soil of *M.phaseolina* and 4500 cfu/g soil of *F.solani*. Different solutions (1%W/V, 3%W/V and 5%W/V) of the shoot and leaves of *Acacia nilotica* were put in sandy loam soil at 1000 ml per row rate and watered for two weeks after 2 to 3 days interval for the decomposition of organic matters. Afterwards, three weeks old equal sized tomato seedlings were planted at the at the edges of *Acacia nilotica* treated fields. Each treatment was done in triplicate with 12 seedlings per row. It was watered two times in a week depending upon the conditions, i.e. soil moisture and weather. Seedling planted in the untreated rows of experimental field were taken as control. After 45 days of plantation, five plants from each replicate were uprooted and examine the infection caused by pathogens and were subjected for further process.

Isolation of root rotting fungi

Five plants rooted out from each treatment were washed under tap water and sterilized with 1% bleach. Five equal size root pieces (1cm length) were placed on potato dextrose agar (PDA) plats which were already treated with streptomycin (0.2g/ L) and penicillin (100,000 units / L). Fungi appeared after incubating period of five days at 28 °C, identified, infection and colonization percentage was calculated. Weight (in grams) of both root and shoot of tomato plant was also measured.

Statistical analysis

Data of growth parameters, infection and colonization percentage were statistically analyzed by one-way analysis of variance (ANOVA) which was followed by the least significant difference (LSD) test at $P<0.05$. All analysis was performed using IBM SPSS STATISTICS programe. (Sokal and Rohlf, 1995)

Results and Discussion

Invitro antifungal activity

Loaded discs of various concentrations (10, 20 and 50 µl) of leaf and shoot extract of *Acacia nilotica* were used against test fungi i.e. *F.oxysporum, M.phaseolina, F.solani, R.solani* and inhibition zone was recorded. Maximum zone against all four test pathogenic fungi was produced at 50 µl solution of both shoot and leaf (Table 1).

Table 1: *In vitro growth inhibition of root infecting fungi M. phaseolina, R. solani, F. solani, F. oxysporum by fungicidal activity of Acacia nilotica.*

Treatments	*Zone of inhibition (mm)*							
	M.Phaseolina		*F.solani*		*F.oxysporum*		*R.solani*	
	Extract of leaf	Extract of bark	Extract of leaf	Extract of bark	Extract of leaf	Extract of bark	Extract of leaf	Extract of bark
Control	0	0	0	0	0	0	0	0
10 µl	2	3	5	6	2.5	2	3	5
20 µl	4	2	10	8	3	4	2	4
50 µl	5	4	12	13	10	6	8	7

Field experiment

The field experiment was performed to observe the efficacy of *Acacia nilotica* leaf, shoot and mixed powder used to reduce the infection and enhance the growth of tomato plant. Significant ($p< 0.05$) tomato growth was increased by all treatments while maximum growth was observed at 5% ShP, 3% LP, and 5% LP (Table 2 and Figure 1). Infection % of all test fungi included *F. solani, R.solani, F. oxysporum* and *M.phaseolina* was significantly controlled by all treatments when applied while combination of 3% LP + 3% ShP, 3% LP + 5% ShP, 5% LP + 1% ShP, 5% LP + 3% ShP, 5% LP + 5% ShP completely controlled the infection and colonization of *M.phaseolina*. Similarly 5% LP + 3% ShP treatment completely controlled the infection of *F.oxysporum*. (Figures 2 and 3).

Figure 1: *Effect of treatments (leaf and shoot powder of Acacia nilotica) on the growth of Tomato plants. A: 5% Shoot powder; B: 3% Leaf powder; C: 5% Leaf powder.*

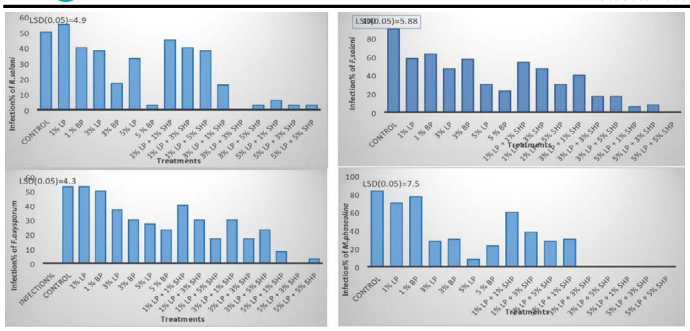

Figure 2: *Effect of Acacia nilotica leaf and shoot powder on the infection % of root infection fungi like, M. phaseolina, R. solani, F. solani and F. oxysporum under field conditionon tomao plant.*
(1)Mean values of treatments in graph show a different in LSD values that are significantly different at (p<0.05).

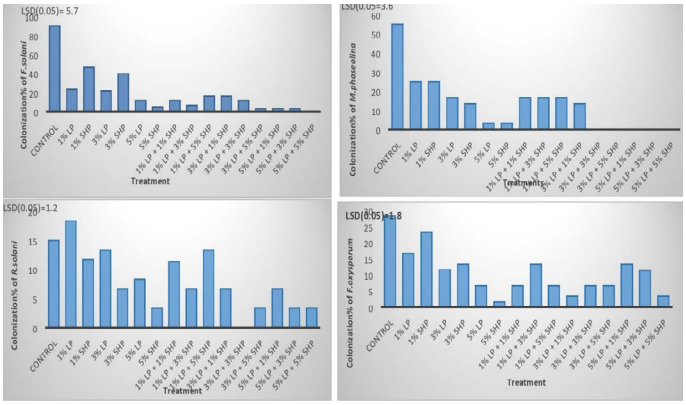

Figure 3: *Effect of Acacia nilotica leaf and shoot powder on the colonization % of root infection fungi like, M. phaseolina, R. solani, F. solani and F. oxysporum under field conditionon tomao plant.*
(1)Mean values of treatments in graph show a different in LSD values that are significantly different at (p<0.05).

To control plant root rotting fungi by use of chemicals is very effective but very expensive method and also caused soil pollution (Sukul and Spiteller, 2001). In the replacement of pesticides to control the root pathogenic fungi the use of biocontrol agent is an alternate and very effective method (Ehteshamul-Haque et al., 1995, 1996). One of several non-chemical control measures are plants extracts currently being intensively researched for prevention of plant diseases and it has been testified that extracts of plant have antimicrobial property against a wide range of fungi and bacteria (Lakshmi et al., 2014; Yildiz et al., 2018). Plants extracts prevent development of spores, its germination and mycelial growth in vitro, hence provide protection against diseases (Plaza et al., 2004; Lee et al., 2007). Patnaik et al. (2012) used different plant extracts to enhanced the growth and control the diseases in tomato plant. In the present study, extract of *Acacia nilotica* revealed excellent antifungal activity which significantly reduced the infection of pathogenic fungi and hence enhanced the growth of tomato plants.

Table 2: *Effect of Acacia nilotica leaf and shoot powder on different growth parameters of tomato plants under field condition.*

Treatments	Length (cm)		Weight (g)	
	Root	Shoot	Root	Shoot
Control	5.7	8.4	0.53	3.69
1% Leaf Powder	4.6	9.6	0.688	2.71
1% Shoot Powder	4.3	9.8	1.162	6.27
3% LP	6.4	13.6	1.864	14.13
3% ShP	5.6	16.8	1.622	11.23
5% LP	8.6	18	2.6	27.94
5% ShP	8.3	15.8	3.148	23.49
1% LP + 1% ShP	4.1	9.2	0.8	5.4
1% LP + 3% ShP	4.0	9.4	0.7	7.0
1% LP + 5% ShP	5.0	10	1.3	11.0
3% LP + 1% ShP	6	10.	1	10.7
3% LP + 3% ShP	4.2	10.3	0.88	9.5
3% LP + 5% ShP	3.9	9.6	0.76	11.5
5% LP + 1% ShP	8.0	9.2	0.66	11
5% LP + 3% ShP	7.5	9.4	0.76	13.2
5% LP + 5% ShP	7.7	9.8	1.5	18.5
LSD (0.05)	0.43	0.76	0.18	1.7

(1)Mean values of treatments in columns show a difference in LSD values that are significantly different at (p<0.05).

Conclusions and Recommendations

This study reveal that *A.nilotica* is a very cheap and tremendous agent for growth promotion. It exhibits strong antifungal activity. The application of root and shoot powder to the plant infected with root rotting pathogen, promoted growth and reduced the effect of pathogen. So the recent study reveals that *Acacia nilotica* due to its dual beneficial property can be use as biofertilizer in replace of chemicals

Author's Contribution

Gulnaz Parveen: Conceived the idea, Research work and Wrote abstract, Methodology, Result and discussion, Data collection.
Salma Gul: Preparation of plant extracts and help in writing of paper Introduction and arranged references.
M Ather Rafi: Overall Management of the article.
Ashfaq Ali Khattak: Data entry in SPSS analysis.
Hikmatulla Jan: Technical Input at every step.

Conflict of interest
There is no conflict of interest.

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