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MANAGEMENT OF ROOT DETERIORATING FUNGI BY THE APPLICATION OF SOLANACEOUS PLANTS

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Abstract

Solanaceous plant extract represent a potential source of antimicrobial properties that used as an alternative method for controlling root pathogens. In the present work, seed treatment and soil drenching methods with solanaceous plant extracts (*Withania somnifera* (L.) Dunal, *Solanum nigrum* (L.) and *Datura alba* Rumphius ex Nees showed positive effect on the soil environment, plant life and suppressed pathogenic fungi (*Fusarium* spp., *Rhizoctonia solani* and *Macrophomina phaseolina*). *In vitro* studies, *D. alba* and *W. somnifera* leaves extracts at 100 and 75% w/v concentrations recorded remarkable inhibition of tested pathogenic fungi by using well and paper disc methods followed by *S. nigrum* leaves extracts. In the screen house experiment, okra and cowpea seeds were treated with100% w/v leaves extract of *W. somnifera* and *D. alba* showed greater effect in suppressing root rot colonization but also increased the height and weight of plants followed by *S. nigrum*. Furthermore, when solanaceous leaves extract drenched in soil, it not only enhanced the growth of crop plants but also showed reduction in fungal colonization.

Key words: Solanaceous plants, Root rot pathogens, Seed treatment and soil drenching methods.

Introduction

Medicinal plants extracts mostly used in pharmaceutical studies, agriculture and industries (Bennett & Wallsgrove, 1994; Osbourne, 1996; Mahesh & Satish, 2008) because of possessing anti-microbial activity against pathogens namely fungi, virus and bacteria (Gómez *et al*., 1990; Talibi *et al*., 2012) as it produce bioactive constituents and secondary metabolites such as flavonoids, polyphenolic and tannins compounds (Mandalari *et al*., 2007). In addition, these plants contain saponins, terpenoids, nitrogen-containing alkaloids and sulphur-containing compounds (Funatogawa *et al*., 2004; Avato *et al*., 2006) and easily available in low price (Mann *et al*., 2008).

The family solanaceae is of medicinal importance which contains four thousand species with ninety genera of plants (Knapp *et al*., 2004). Solanaceae have ample range of alkaloids, including scopolamine, atropine and hyoscyamine and due to the presences of these alkaloids make this family medicinally important (Ansari, 2005). *Withania somnifera* (winter cherry) possess antifungal activity (Ghosh, 2009) due to the presence of withanolides (Matsuda *et al*., 2001). *Solanum nigrum* (black night shade) possess antifungal and hepatoprotective property (Jainu *et al*., 2006; Al-Fatimi *et al*., 2007; Harisankar *et al*., 2011). *Datura* (thorn apple) having anti-inflammatory and antimicrobial activity (Ali & Shuab, 1996; Harbone, 1999; Sakthi *et al*., 2011) due to the presence of alkaloids (hyoscyamine and scopolamine) which are found in the almost all parts of the plant (Thakur *et al*., 1989; Raju *et al*., 2003).

In Pakistan, plant pathogenic fungi on roots (*Fusarium* spp., *Rhizoctonia solani* and *Macrophomina phaseolina*) are most prevalent as these pathogens are a soil inhabiting fungus which survives in the soil for several years which affect the crop productivity by producing wilt and rotting diseases (Usman *et al*., 2013; 2014). Fastest way of controlling root rot pathogens is the use of agrochemicals, but it produced demerit effect in the soil environment of crop plants (Papavizas & Lumsden, 1980). Nowadays, many researchers exploit the application of medicinal plant extracts and their compounds as the cheapest way in controlling fungal pathogens (Matthiessen & Kirkegaard, 2006; Babu *et al*., 2008). Therefore, present research was carried out to study the antifungal activity of solanaceous plants against root pathogens to improve the growth of plants.

Materials and Methods

Plant parts collection and extracts preparation: *Withania somnifera*, *Solanum nigrum* and *Datura alba* stem and leaves were collected from the different sites of Karachi University, Karachi (Pakistan). Stem and leaves of each plant were dried, then powdered and stored in the glass jar, respectively. For extract preparation, 10g of tested leaves and stem were taken separately and soaked in sterilized distilled water (90 mL) for at least 24 hours. The plant extract was filtered and was further diluted with sterilized distilled water to make 75 and 50% concentrations.

***In vitro*:** To study the growth inhibition of tested fungi (*M. phaseolina*, *R. solani* and *F. oxysporum*) by using the aqueous extracts of different concentrations of solanaceous plant parts, well and paper disc methods were used. In the agar well diffusion method, four wells (≈2.5mm deep) were made on the PDA medium in which three wells were filled with 50µL of 100, 75 and 50% concentrations of aqueous extracts of *W. somnifera*, *S. nigrum* and *D. alba* leaves and stem respectively, whereas forth well contained sterilize distilled water. In the same way, sterilized filter paper discs of 6mm were soaked in different concentrations (100, 75 and 50%) of leaves and stem extracts, respectively. Treated disc (100, 75 and 50% concentrations) were placed on three sides of Petri plate, respectively. While, the fourth disc was soaked in sterilize

distilled water (control). A disc (5mm) of each root rot fungus was placed in the center of each tested plates and replicated thrice (Nair *et al*., 2005). Petri plates were kept at room temperature (30-33^{0}C) for one week. After incubation period, measure the zone of growth inhibition (Lokesha & Benagi, 2007).

***In vivo*:** Screen house field experiment was prepared in properly leveled plots (4 × 4 feet), arranged in Complete Randomized Block Design (CRBD) at the Department of Botany (University of Karachi). Okra and cowpea seeds were treated with *W. somnifera*, *S. nigrum* and *D. alba* at 100% concentration, while untreated seeds taken as control. On the other set of an experiment, soil drenching (30mL extracts were drenched in each plot) was carried out by 100% of tested leaves extracts, while soil drenched with sterilized water was regarded as control. Treatment of each test was replicate thrice. Experiment was uprooted after four months and data of growth parameters such as root/shoot length (cm), root/shoot weight (g), numbers of nodules/leaves/pods and weight of pods/legumes (g) were recorded. The roots of control and treated plants of okra and cowpea after surface sterilized with sodium hypochlorite (1%) for 3-5 minutes, washed in running tap water (adhering soil particles completely removed) and dried in blotter paper. The roots were cut into small pieces and were placed on poured PDA plates having antibiotics to suppress the bacterial growth. Treated plates were kept at room temperature (28-33°C) for one week. After incubation period, fungus emerging from each root fragment was identified under microscope and colonization of root rot fungus was determined. The data were analyzed using two-way analysis (ANOVA) and estimated under DMRT (Duncan's Multiple Range Test) at $p<0.05$ where treatments and controls data were calculated using the LSD (Least Significant Difference) statistical test (Sokal & Rohlf, 1995).

Results

***In vitro*:** Leaves extracts of *W. somnifera* and *D. alba* at 100% concentrations showed highest mycelial suppression of *M. phaseolina*, *R. solani* and *F. oxysporum* followed by *S. nigrum* observed in both paper disc and well methods, where 75% concentration ($p<0.001$) also showed maximum zone of growth inhibition against tested pathogenic fungi (Tables 1-2). However, using well method was found best method as compared to paper disc method $p<0.05$). Part of leaves were considered most effective as compared to that of stem because of not inhibiting root rot fungi. No proper zone of inhibition was observed in stem extract when used at different concentrations ($p<0.001$).

Overall result showed that leaves extract like *W. somnifera* and *D. alba* at 100% concentrations were active and significantly controlled the root rot pathogenic fungi followed by *S. nigrum* at 100 and 75%. Therefore, leaves extract of solanaceous plants were selected for the screen house experiment.

In vivo

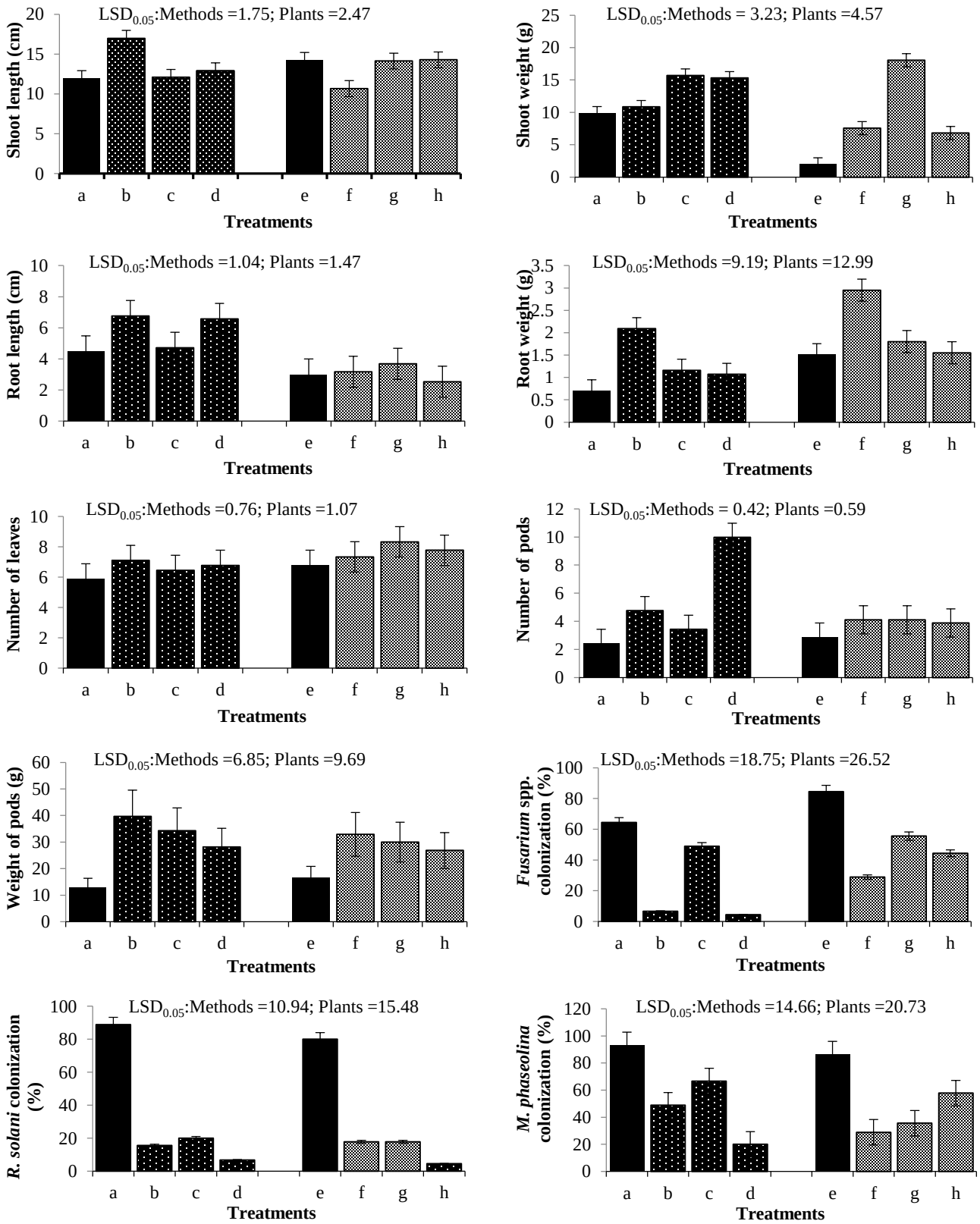
Okra plant: Okra seeds were treated, while soil was drenched with plant extracts of *W. somnifera, S. nigrum* and *D. alba* at 100% w/v improved the growth and controlled the root pathogens. Highest length and weight of shoot, root length, number and weight of pods were recorded when seeds were treated with plant extracts as compared to soil drenching method which increased the weight of roots and number of leaves. Maximum suppression of root pathogens was shown in the leaves of *D. alba* extracts at 100% concentration ($p<0.001$) followed by *W. somnifera* and *S. nigrum*. Shoot length was considerably increased when okra seeds were treated with *W. somnifera* leaves extract but weight of shoot and root along with number of leaves were increased when treated seeds along with drenched in soil with 100% leaves extract ($p<0.01$) of *S. nigrum* and *D. alba*. Best control of root pathogens (*Fusarium* spp., *R. solani* and *M. phaseolina*) were recorded by *D. alba* as compared to *W. somnifera* and *S. nigrum* leaves extracts (Fig. 1).

Cowpea plant: Leaves extract at 100% concentration of *W. somnifera*, *S.nigrum* and *D. alba* showed significant inhibition of pathogenic fungi colonization ($p<0.01$). Length and weight of shoot/ root, number of nodules and weight of pods significantly ($p<0.001$) increased when treated cowpea seeds at 100% extracts of *W. somnifera* and *D. alba*. Similarly, growth parameters such as plant weight, number of leaves and pods were also increased when soil was drenched at 100% *S. nigrum* extract. Significant ($p<0.001$) inhibition of *Fusarium* spp., *R. solani* and *M. phaesolina* were recorded when *W. somnifera* drenched in soil (Fig. 2). Moreover, seeds treated with *S. nigrum* and *D. alba* leaves extracts showed maximum suppression of root rot fungi colonization as compared to control.

Overall field results indicated that when both methods applied (seed treatment and soil drenching) at 100% concentrations of leaves extracts with *W. somnifera* and *D. alba* showed better results as compare to *S. nigrum* because medicinal solanaceous leaves extracts have antifungal properties against root decay pathogens which elevated the growth of okra and cowpea plants.

Discussion

Family solanaceae is distributed throughout the world (Griffin & Lin, 2000) due to its ecological aptitude (Fukuhara *et al*., 2004). Due to complex compounds isolated from solanaceous plants, they are used to treat various plant diseases caused by pathogenic microbes (Okrslar *et al*., 2002; Kone´*et al*., 2004). Currently, medicinal plants with beneficial and positive effect concerned the researchers are using alternate approach for controlling plant diseases (Jensen *et al.*, 1996) which are environmentally friendly as compared to the use of agrochemicals (Kerr, 1980).

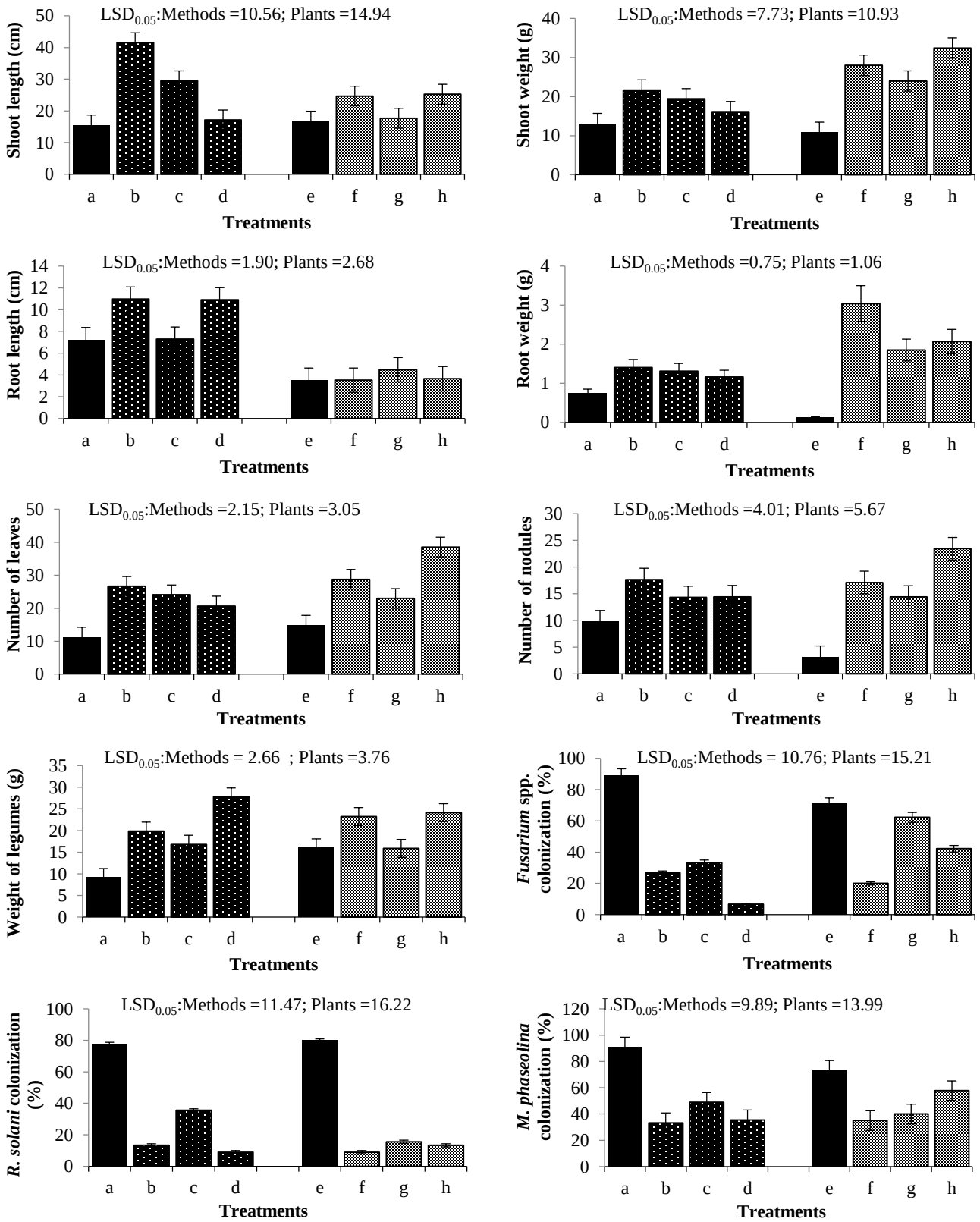


Where;

Seed treatment method
a = Control (sterilized distilled water)
b = *W. somnifera* @ 100% leaves extract
c = *S. nigrum* @ 100% leaves extract
d = *D. alba* @ 100% leaves extract

Soil drenching method
e = Control (sterilized distilled water)
f = *W. somnifera* @ 100% leaves extract
g = *S. nigrum* @100% leaves extract
h = *D. alba* @ 100% leaves extract

Fig. 1. Effect of seed treatment and soil drenching methods with medicinal solanaceous leaves extract in the management of root rot fungi on okra plants.



Where;

Seed treatment method
a = Control (sterilized distilled water)
b = *W. somnifera* @ 100% leaves extract
c = *S. nigrum* @ 100% leaves extract
d = *D. alba* @ 100% leaves extract

Soil drenching method
e = Control (Sterilized distilled water)
f = *W. somnifera* @ 100% leaves extract
g = *S. nigrum* @ 100% leaves extract
h = *D. alba* @ 100% leaves extract

Fig. 2. Effect of seed treatment and soil drenching methods with medicinal solanaceous leaves extract in the management of root rot fungi on cowpea plants.

Table 1. ***In vitro,*** **using extracts of solanaceous plants with different concentrations against growth inhibition of pathogenic fungi by using paper disc method**

Treatments	Growth inhibition											
	Paper disc method											
	Fusarium oxysporum (mm ± SD)				*Rhizoctonia solani* (mm ± SD)				*Macrophomina phaseolina* (mm ± SD)			
	0%	50%	75%	100%	0%	50%	75%	100%	0%	50%	75%	100%
	Withania somnifera											
Leaves extract	0.00 ± 0.00	3.66 ± 1.69	2.66 ± 1.24	3.66 ± 1.24	0.00 ± 0.00	0.00 ± 0.00	1.00 ± 0.00	1.33 ± 0.47	0.00 ± 0.00	0.00 ± 0.00	1.33 ± 0.47	1.66 ± 0.47
Stem extract	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
$LSD_{0.05}$												
(Conc.) =			1.29				0.24				0.35	
(Treatments) =			0.91				0.17				0.24	
	Solanum nigrum											
Leaves extract	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.33 ± 0.47	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.33 ± 0.47
Stem extract	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
$LSD_{0.05}$												
(Conc.) =			0.24				0.00				0.24	
(Treatments) =			0.17				0.00				0.17	
	Datura alba											
Leaves extract	0.00 ± 0.00	1.00 ± 0.00	3.66 ± 0.47	4.66 ± 3.29	0.00 ± 0.00	0.00 ± 0.00	2.00 ± 0.00	2.33 ± 0.47	0.00 ± 0.00	0.00 ± 0.00	1.66 ± 0.47	2.33 ± 0.47
Stem extract	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
$LSD_{0.05}$												
(Conc.) =			1.76				0.24				0.35	

Table 2. ***In vitro*****, growth inhibition of root rot fungi by extracts of solanaceous plants with different concentrations by using agar well diffusion method.**

Treatments	Agar well diffusion method											
	Fusarium oxysporum (mm ± SD)				*Rhizoctonia solani* (mm ± SD)				*Macrophomina phaseolina* (mm ± SD)			
	0%	50%	75%	100%	0%	50%	75%	100%	0%	50%	75%	100%
	Withania somnifera											
Leaves extract	0.00 ± 0.00	1.00 ± 0.00	3.66 ± 1.69	4.00 ± 1.41	0.00 ± 0.00	0.00 ± 0.00	1.00 ± 0.00	1.66 ± 0.94	0.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.66 ± 0.94
Stem extract	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
$LSD_{0.05}$												
(Conc.) =		1.17				0.49				0.49		
(Treatments) =		0.82				0.35				0.35		
	Solanum nigrum											
Leaves extract	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.66 ± 0.47	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.33 ± 0.47	0.00 ± 0.00	0.00 ± 0.00	1.00 ± 0.00	1.33 ± 0.47
Stem extract	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
$LSD_{0.05}$												
(Conc.) =		0.24				0.24				1.24		
(Treatments) =		0.17				0.17				0.17		
	Datura alba											
Leaves extract	0.00 ± 0.00	2.00 ± 0.81	2.33 ± 0.47	2.66 ± 0.47	0.00 ± 0.00	3.00 ± 1.41	3.66 ± 0.47	3.33 ± 1.24	0.00 ± 0.00	0.00 ± 0.00	3.66 ± 0.47	3.33 ± 1.24
Stem extract	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
$LSD_{0.05}$												
(Conc.) =		0.55				1.03				0.70		
(Treatments)=		0.39				0.72				0.49		

Where; C = Control, Conc. = Concentrations, mm = Millimeter, %= Percentage and ± SD = Standard deviation

Present *In vitro* results showed that the antifungal activity of *W. somnifera, S. nigrum* and *D. alba* leaves were found effective as compared to stem parts against tested fungi namely; *M. phaseolina, R. solani* and *F. oxysporum* at 100 and 75% concentrations were found best by using both paper disc and well methods. Furthermore, when 100% leaves extract of tested solanaceous plants were carried out in the field experiment to investigate the antifungal activity against root rot fungi on the growth of cowpea and okra plants. Results of tested plant extracts showed positive effect against pathogenic fungi and improved the growth as compared to control. Similar results by Rafi *et al.*, (2015) who demonstrated the suppression of root rot fungi colonization by using seeds priming (okra, sunflower, peanut and chickpea) at 10 minutes time interval with plants extracts (*Acacia nilotica* and *Sapindus mukorossi*). Extracts of *W. somnifera, S. nigrum* and *D. alba* leaves when drenched in the soil at 100% concentrations suppressed the colonization of root pathogens which gave healthy growth of okra and cowpea plants. Leaves and seeds extract of *Carica papaya* inhibit *Colletotrichum gloeosporiodies* possessing antifungal activity (Banos *et al.,* 2002). Treatment of seeds with plant extracts showed effective method in various crop plants and this treatment found to be easy, low price and applied easily in agricultural field (Thanaboripat, 2003; Harris, 2006). Aqueous extract of *Cynodon dactylon* and *Datura alba* drenched in soil with okra and cowpea seeds treated by *P. variotii* suppressed the root rot pathogens significantly (Dawar *et al.,* 2010). Aqueous extracts of *Prosopis juliflora* leaves using two methods (seed treatment and soil drenching) at 100 and 50% concentrations showed admirable effect in the control of pathogenic fungi on roots and improved the cowpea and mung bean growth (Ikram & Dawar, 2014).When okra and cowpea seeds were treated with solanaceous leaves extract at 100%, efficiently improved the growth parameters and reduced root rot fungi colonization. Seed treatment is a successful method for controlling both soil/seeds borne pathogens allowing seed to germinate as a vigorous seedling (Chang & Kommedahl, 1968). Ethanolic extract of *G. asiatica* leaves were tested against bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and fungi (*Aspergillus niger* and *Fusarium solani*) showed significant results exhibiting antibacterial and antifungal activity (Zia-ul-Haq *et al.,* 2011; Dawar *et al*., 2020). Many researchers worked on the various medicinal parts of plants by using seed treatment and soil drenching methods in the management of root pathogens and for the better growth of crop plants (Dawar *et al*., 2007; Ikram & Dawar, 2016).

Present study confirmed the antifungal activity in the solanaceous leaves against root infecting fungi which enhanced the growth of crops. Control of plant pathogens through fungicide proved positive but these agrochemicals are expensive and demerits to the soil environment by killing beneficial micro-organisms inhabitant in the soil. Therefore, by using plant leaves extract as a seed treatment and soil drenching methods can easily be used as antifungal and recommended as affordable to agricultural field due to beneficial effect on the growth of plants.

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