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FUNGICIDAL EFFECT OF SOLANACEOUS PLANTS AGAINST ROOT ROT DISEASES AND ON THE GROWTH OF CROP PLANTS

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ABSTRACT

The present research work was conducted for the control of root pathogenic fungi and for the growth improvement of okra and cowpea crops by using medicinal plants belongs to family solanaceae. In the screen house experiment, okra and cowpea seeds were treated and soil was drenched with *Datura alba*, *Withania somnifera* and *Solanum nigrum* leaves extracts at 100% followed by 75% w/v improved the shoot (weight and height) and root (weight and height) of both crops but also suppressed the colonization of pathogenic fungi. Soil amended with 1% leaves extracts of *D. alba* and *W. somnifera* followed by *S. nigrum* were recorded as the best in the suppression of root infecting pathogens but 0.1% increased the height and weight of crops.

Key words: Concentrations, pathogenic fungi, solanaceous leaves extract and powder

INTRODUCTION

Fungal diseases in plants contribute heavy losses in agricultural economy and more than one million fungal species are considered as natural contaminants of agriculture (Kacaniova, 2003). Soil borne pathogens caused root deterioration regarded as destructive disease reported in many crops including *Fusarium* spp., responsible for wilting, rotting of diseases in root and stem of many monocotyledon and dicotyledon plants, *R. solani* produces rotting of root and seed, damping off of seedling and wilting while *M. phaseolina* produces charcoal rot and seedling blight) responsible for the decrease in yield production and decline the market values in different countries throughout the worldwide (Ghaffar, 1992; Wheeler and Rush, 2001; Tanina *et al*., 2004).

Plant pathogenic fungi are controlled by synthetic fungicides application (Harris *et al*., 2001). To overcome the economic losses and to protect the crops from the attack of pathogens various control measures are being used. Carbendazim and Bordeaux mixture are also the most commonly used chemicals but increasing use of hazardous impacts in soil and micro-organisms led to several environmental and health problems (Groenewald, 2005). Due to the extensive use of agro chemicals, researchers are now showing interest in protecting the environment and saving the human health from the consumption of toxicity by exploring the eco-friendly technique for disease management in the agriculture (Parka *et al.,* 2002; Kanwal *et al.,* 2010). Use of medicinal plants products is considered as substitute to chemical fungicides for the control of plant pathogens (Riaz *et al*., 2010; Jabeen *et al.,* 2011). Medicinal plants contain biochemical compounds that have biocidal properties and they have the potential to suppress rhizosphere fungal assemblages and the associated hematite populations (Shaukat *et al*., 2001; Abbasi *et al*., 2008). Medicinal plants extracts and essential oils shows antifungal activity in controlling large number of fungal diseases due to the presence of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids and glycosides (Shafique and Shafique, 2012; Javaid and Iqbal, 2014; Javaid and Rauf, 2015). In Pakistan, various research of using different plant part extracts used as a biological control against pathogenic fungi (Ahmed and Abdelgaliel, 2005; Zaheer *et al*., 2012). The solanaceous family comprises 3000–4000 species (Knapp *et al.,* 2004) are popularly known for possessing a broad range of alkaloids, including scopolamine, atropine and hyoscyamine and due to the presences of these alkaloids make this family medicinally important (Ansari, 2005).

Datura (Jimsonweed and Thorn apple) mainly used in medicinal herbs due to its anti-inflammatory property (Harbone, 1999), intoxicant and hallucinogen (Donatus and Ephraim, 2009). The entire plant has medicinal value, but leaves and seeds alone are recognized as an official medicinally used worldwide (Satyavati and Raina., 1977; Duke and Ayensu, 1984). Numerous studies have been carried out due to its antimicrobial activity against bacterial and fungal pathogens (Ali and Shuab, 1996; Sakthi *et al.,* 2011) due to the presence of the alkaloids containing hyoscyamine and hyoscine (scopolamine) and meteloidine are found in all *Datura* parts (Thakur *et al.,* 1989).

Withania somnifera (winter cherry) has immense medicinal significance being extensively used especially in Indian subcontinent as a therapy for treating various diseases in curing stress related disorders including hypertension, arthritis and diabetes (Alam *et al.,* 2012; Kaurav *et al.,* 2012). Its medicinal properties are due to

withanolides (secondary metabolite) which gives the characteristic of this plant species possesses antifungal activities (Ghosh, 2009).

Solanum nigrum (black night shade) contain medicinal properties like anti-microbial, anti-oxidant, cytotoxic and anti-ulcerogenic activity (Al-Fatimi *et al*., 2007; Jainu and Devi, 2006). *S. nigrum* is a potential herbal preference as anti-cancer, anti-malarial and anti-fungal activities (Mohamed *et al*., 1996; Gokhale and Purohit, 2002; Raju and Bird, 2007).

Present work was carried out to investigate the fungicidal activity by using the solanaceous plants to improve the growth of crop plants against the root pathogenic fungi.

MATERIALS AND METHODS

A. Collection of plant parts and extracts preparation

Leaves of *Withania somnifera* (L.) Dunal, *Solanum nigrum* (L.) and *Datura alba* (Ness) were collected from different campus of University of Karachi. After collection, plant leaves were dried and by using an electric grinder make into fine powder, respectively and each plant powder separately stored in the glass jar. 10g of tested solanaceous plant parts (leaves and stem) were soaked in 90 mL sterilized distilled water, separately and left it for at least 24 hours, filtrate and the concentration of the extract was used as a stock solution (100% v/w) and was further diluted with sterilized distilled water to prepare 75 and 50% v/w concentrations.

B. *In vivo*

Soil obtained from Department of Botany (University of Karachi) was sandy loam and was sieved (2mm) to discard stones and filled pots containing 300g soil. The soil contain pH 7.3 with moisture holding capacity 48% (Keen and Rakzowski, 1922), 6-8 sclerotia/g of *M. phaseolina* (Sheikh and Ghaffar, 1975), 12-15% of *R. solani* (Wilhelm, 1955) and *Fusarium* spp., 2700 CFU/g (Nash and Synder, 1962). Cowpea and okra seeds were surface sterilized in 1.0% calcium hypochlorite for 3-4 minutes, washed thoroughly with water twice. Both seeds (okra and cowpea) were treated with 100 and 75% v/w extracts of tested solanaceous plant leaves, respectively for 5-10 minutes, while untreated seeds soaked in sterilized distilled water regarded as control and were kept in laminar flow hood for one hour to dry aseptically, while untreated seeds served as control. For soil drenching method, plant extracts of solanaceous leaves (100 and 75 % v/w concentrations) were drenched separately in each pot (approximately 20 mL), soil without leaves extracts taken as control. In soil amendment method, tested solanaceous leaves powder (0.1 and 1.0 % w/w) were mixed in the soil and leave for one day for the decomposition before sowing seeds. Soil without amendment served as control and each treatment replicated thrice. Five seeds in each pot were sown and kept in the screen house at the Department of Botany (KU) randomly. After one month, plants were uprooted carefully and different parameters of growth were recorded (shoot length and weight, root length and weight, number of nodules). Roots were surface sterilized by 1.0% calcium hypochlorite for 3 minutes, than washed twice with water and dried aseptically. Each root was cut into five pieces and placed on poured PDA (Potato Dextrose Agar) supplemented with antibiotics. Petri plates were incubated at room temperature (32-36^{0}C) for one week. After the incubation period, colonization percentage of root rot fungi was calculated (Short *et al*., 1980). Data were analyzed as per experimental design and two way analysis (ANOVA) was carried out by using Duncan's Multiple Range Test (DMRT) at $P<0.05$ using "Statistica" software (Sokal and Rohlf, 1995).

RESULTS

1. Okra

Soil amended with *Withania somnifera, Solanum nigrum* and *Datura alba* at 0.1% w/w showed highest shoot length and weight as compared to 1.0 w/w which increased the root length and weight. Root decay pathogens were completely suppressed by *S. nigrum* and *D. alba* leaves extracts at 100% concentration ($P<0.001$), however 75% showed greater suppression of colonization of pathogenic fungi. Shoot weight and root weight were improved at 100% w/v, when tested seeds were treated and soil drenched with *S. nigrum* and *D. alba* leaves extracts ($P<0.05$). However, best control of pathogenic fungi of *R. solani, Fusarium* spp. and *M. phaseolina* were noticed by 100% concentration as compared to 75% w/v (Fig. 1-3).

2. Cowpea

Growth of cowpea plant was enhanced and showed significant increase in shoot length, root length and numbers of nodules when leaves powder of *W. somnifera, S. nigrum* and *D. alba* amended in soil at 1.0% w/w, where as when soil was amended at 0.1% w/w increased the shoot weight and root length but also reduced the colonization of *M. phaseolina, Fusarium* spp. in both concentrations ($P<0.01$), while complete inhibition of *R. solani* colonization

(P<0.001) at 100% concentration was recorded. When leaves of solanaceous plants extract was used as seed treatment and soil drenching, growth parameters (shoot length and weight, root length and weight along with numbers of nodules) showed significant increase in cowpea plants. Leaves extracts of *W. somnifera*, *S. nigrum* and *D. alba* when used at 100% v/w recorded maximum significant (P<0.05) inhibition of *M. phaseolina* and *Fusarium* spp. colonization, whereas complete suppression of *R. solani* colonization was recorded by both methods when *D. alba* leaves extract used at 100 and 75% v/w concentrations (Fig. 4-6).

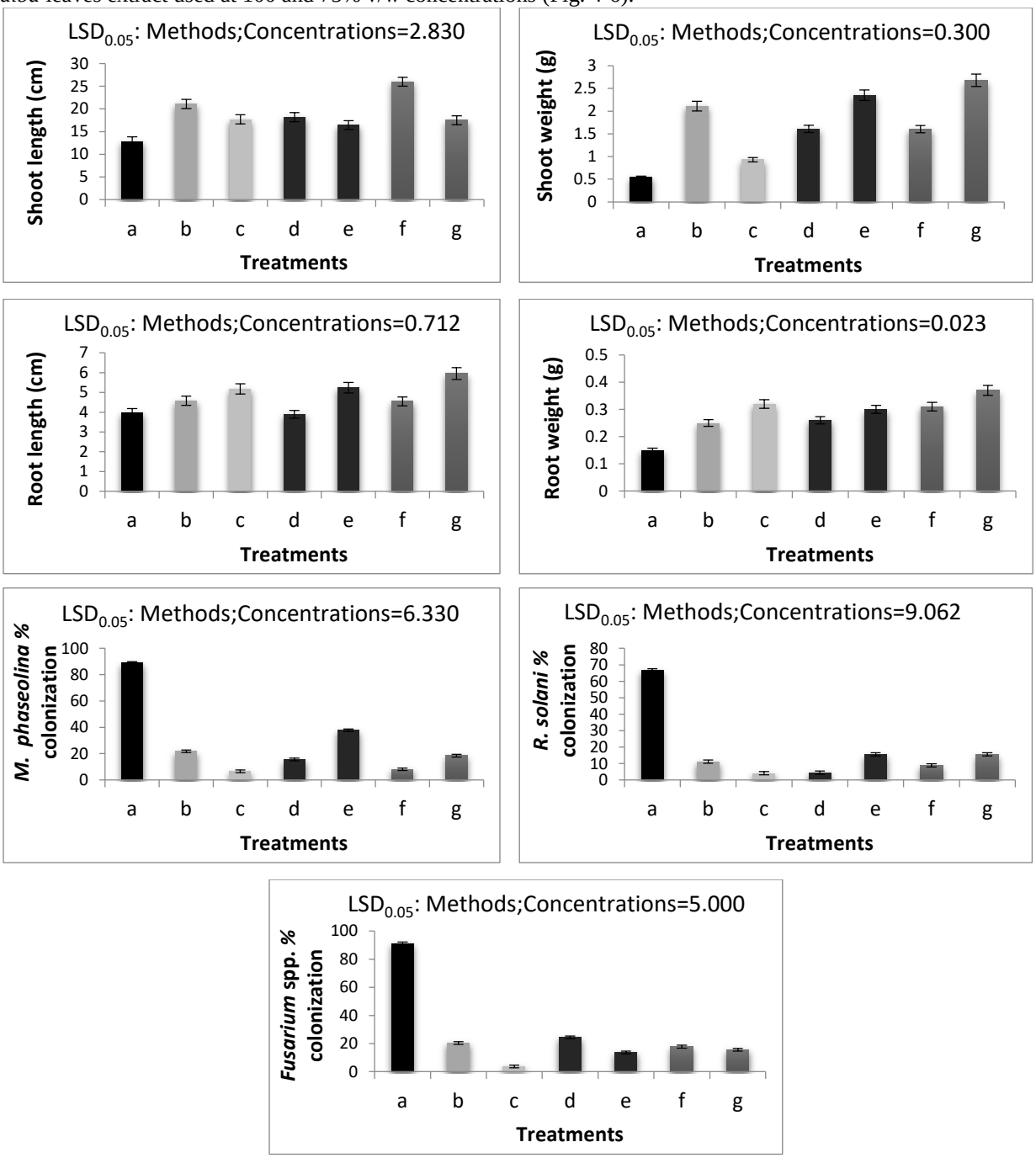

Fig.1. Formulation of *Withania somnifera* leaves against root rot pathogens on the growth promotion of okra plants.

Where; **a**= Control (Sterilized distilled water); **b**= soil amendment @ 0.1% concentration; **c**= soil amendment @ 1.0% concentration; **d**= seed treatment @ 100% concentration; **e**=seed treatment @ 75% concentration; **f**= soil drenching @ 100% concentration; **g**= soil drenching @ 75% concentration.

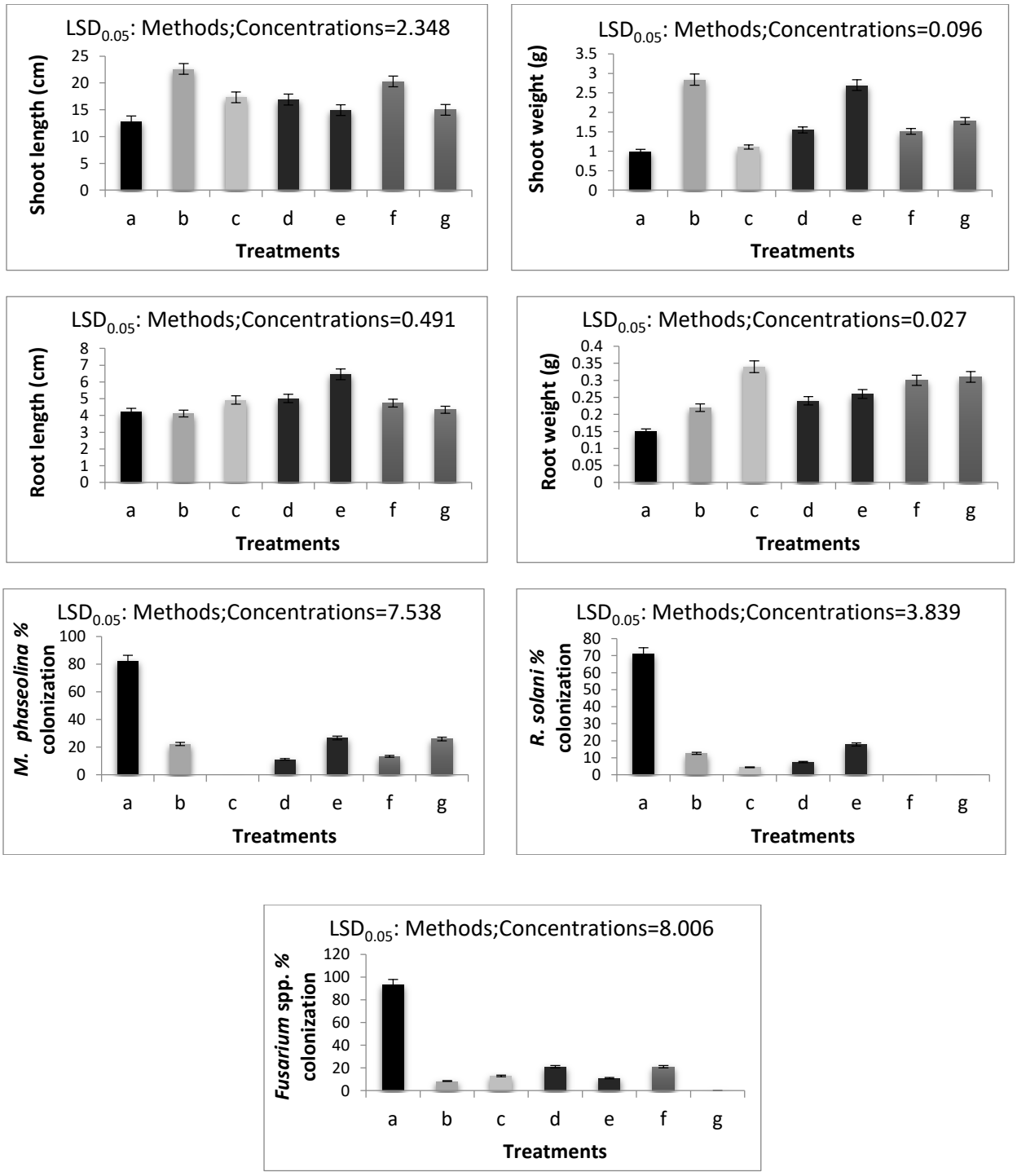

Fig.2. Formulation of *Solanum nigrum* leaves against root rot pathogens on the growth promotion of okra plants.

Where; **a**= Control (Sterilized distilled water); **b**= soil amendment @ 0.1% concentration; **c**= soil amendment @ 1.0% concentration; **d**= seed treatment @ 100% concentration; **e**=seed treatment @ 75% concentration; **f**= soil drenching @ 100% concentration; **g**= soil drenching @ 75% concentration.

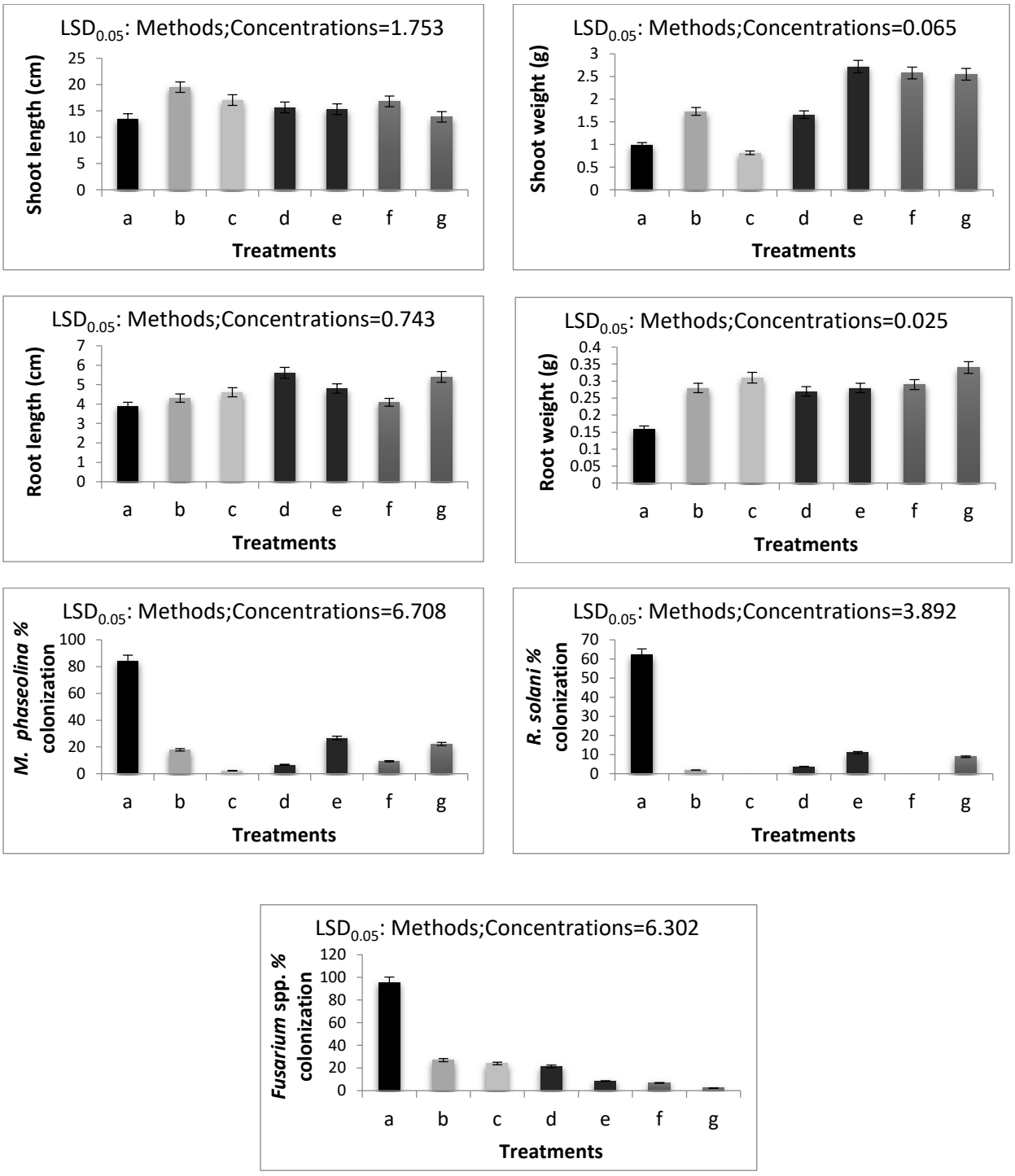

Fig.3. Formulation of *Datura alba* leaves against root rot pathogens on the growth promotion of okra plants.

Where; **a**= Control (Sterilized distilled water); **b**= soil amendment @ 0.1% concentration; **c**= soil amendment @ 1.0% concentration; **d**= seed treatment @ 100% concentration; **e**=seed treatment @ 75% concentration; **f**= soil drenching @ 100% concentration; **g**= soil drenching @ 75% concentration.

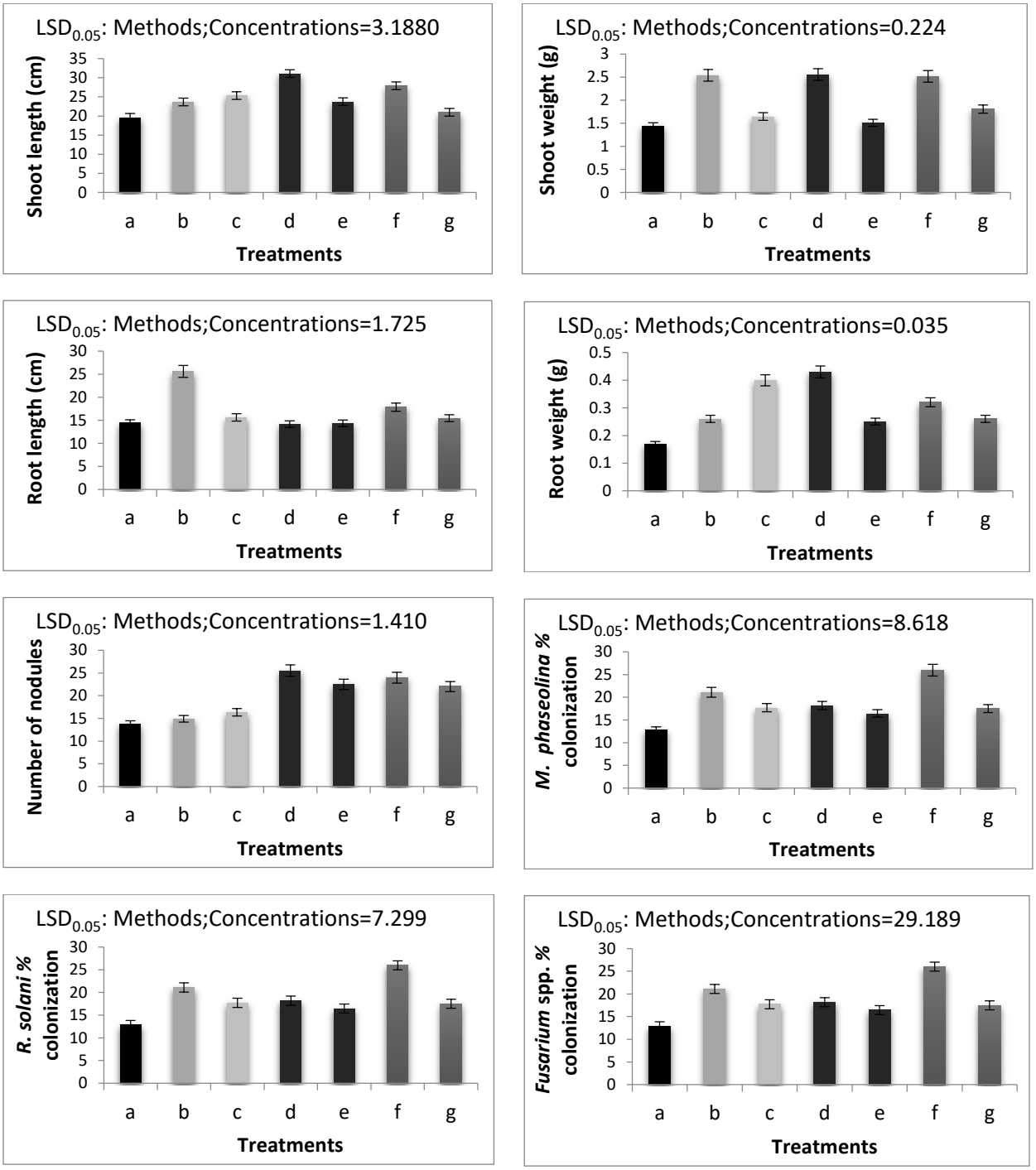

Fig.4. Formulation of *Withania somnifera* leaves against root rot pathogens on the growth promotion of cow pea plants.

Where; **a**= Control (Sterilized distilled water); **b**= soil amendment @ 0.1% concentration; **c**= soil amendment @ 1.0% concentration; **d**= seed treatment @ 100% concentration; **e**=seed treatment @ 75% concentration; **f**= soil drenching @ 100% concentration; **g**= soil drenching @ 75% concentration.

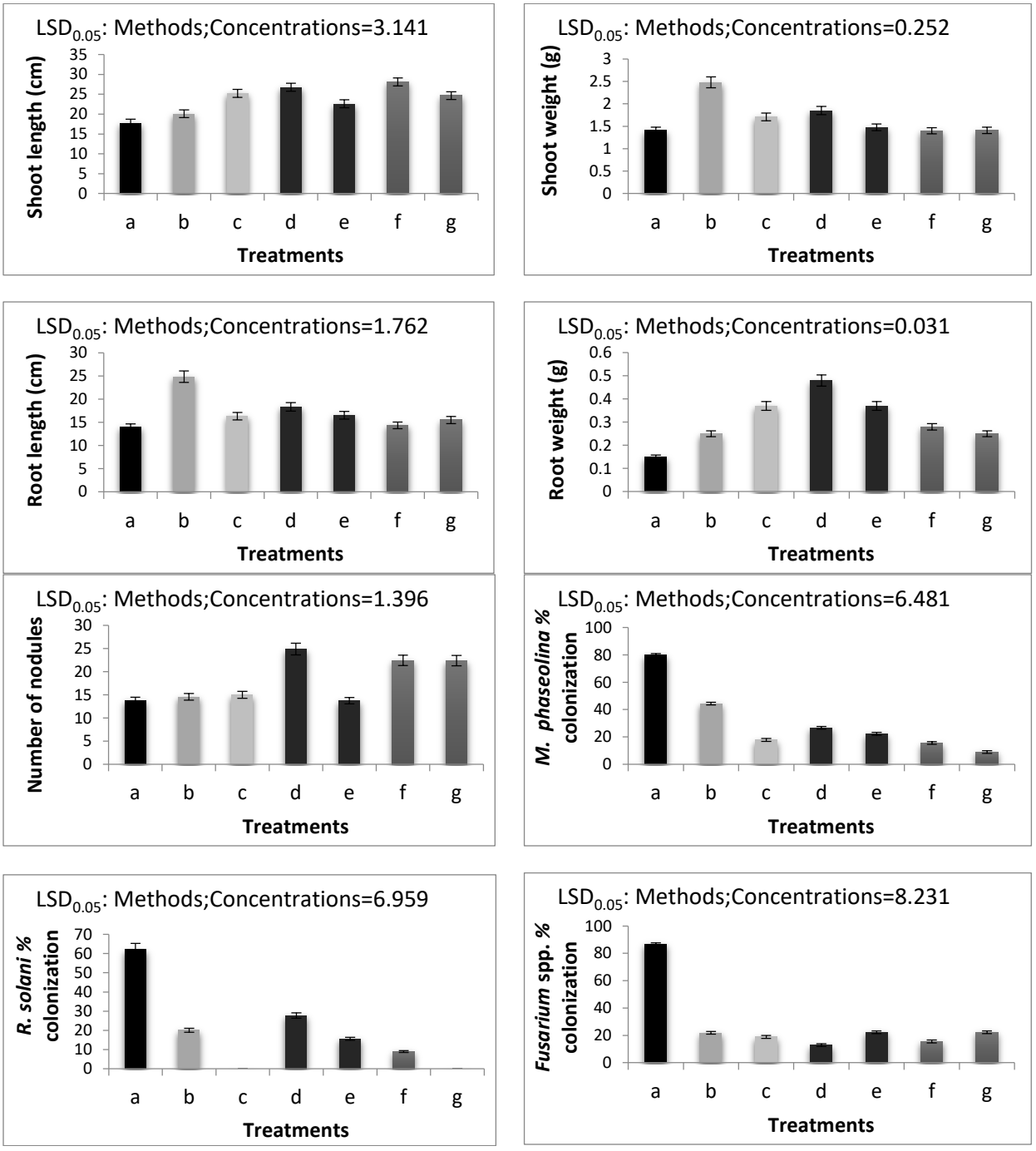

Fig.5. Formulation of *Solanum nigrum* leaves against root rot pathogens on the growth promotion of cow pea plants.

Where; **a**= Control (Sterilized distilled water); **b**= soil amendment @ 0.1% concentration; **c**= soil amendment @ 1.0% concentration; **d**= seed treatment @ 100% concentration; **e**=seed treatment @ 75% concentration; **f**= soil drenching @ 100% concentration; **g**= soil drenching @ 75% concentration.

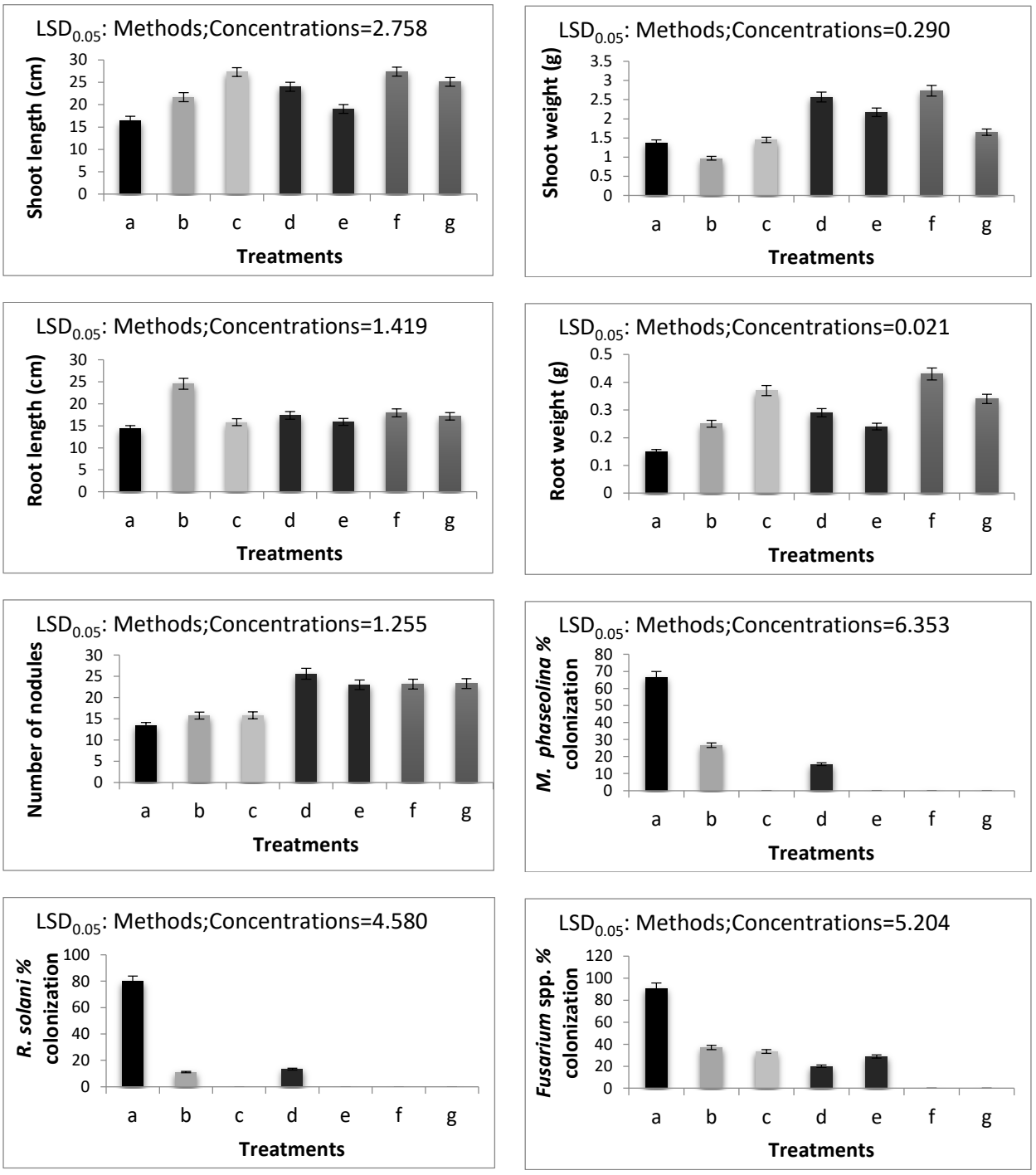

Fig.6. Formulation of *Datura alba* leaves against root rot pathogens on the growth promotion of cow pea plants.

Where; **a**= Control (Sterilized distilled water); **b**= soil amendment @ 0.1% concentration; **c**= soil amendment @ 1.0% concentration; **d**= seed treatment @ 100% concentration; **e**=seed treatment @ 75% concentration; **f**= soil drenching @ 100% concentration; **g**= soil drenching @ 75% concentration.

Overall result showed excellent control of pathogenic fungi on both crops (cowpea and okra) when 100% concentration of leaves extract of *D. alba* drenched in soil followed by *W. somnifera* and *S. nigrum* of 75% concentration, leaves powder and extract showed anti-fungal activity against root rot fungi but also improve the growth of plants. Application of leaves powder as soil drenching and soil amendment not applicable for commercial scale since huge amount of plants extracts and powder required but can be applicable on low scale. Furthermore, seed treatment method considered as the best method which is reasonably priced, undisruptive and can easily be applied on large agricultural scale.

DISCUSSION

The solanaceae family is broadly distributed throughout the world (Griffin and Lin, 2000) regarded as significant from the commercial and ecological viewpoint (Fukuhara *et al*., 2004). It possesses steroidal glycoalkaloids used as defense allelochemicals in controlling enormous number of fungal infection (Weissenberg *et al*., 1998). Furthermore, glycoalkaloids contains anti-tumor, anti-cancer, and anti-herpes activity (Ikeda *et al*., 2003) as well as teratogenic, embryotoxic, anti-fungal and molluscicidal properties (Kim *et al*., 1996). They are also source of raw materials for the semi synthesis of pharmaceutical industries (Okrslar *et al*., 2002). Due to the significant variety of compounds obtained from the solanaceous plants they are used to treat different types of plant diseases against bacterial/fungal pathogen (Kinghorn *et al*., 1999; Kone´ *et al*., 2004). Medicinal plants with therapeutic effect have received the attention of researchers using alternate method in terms of controlling plant diseases (Jensen *et al.,* 1996) which also protect the safety of an environment from the use of agrochemicals (Kerr, 1980).

The present results showed that solanaceous plant powder and extract revealed pronounced effect by using different methods and possess antifungal activity against root pathogenic fungi when soil was incorporated with tested solanaceous leaves powder at 0.1% concentration significantly improved the growth of cowpea and okra plants on the contrary to control, whereas root pathogenic fungi colonization of *M. phaseolina*, *R. solani* and *Fusarium* spp. remarkably reduced when leaves powder amended at 1.0% as compared to control. Similar result had been achieved by *Moringa oleifera* leaves powder controlled the colonization of root pathogenic fungi at 1% was reported by Ambreen *et al*., (2017). Pathogenic root rotting fungi inhabitant in the soil and invade the roots inside where it reaches in xylem cells produce toxins which disrupt the growth, blockage water supply which eventually closes stomata present in the leaves, causing the wilt and plant die off (Agrios, 1988; Aducci *et al.,* 1995). Amendment of plant powder in soil to control root diseases through chemical producing anti microbial compounds during biological and decomposition activity in soil protect the roots from the invasion of root pathogens (Chen *et al*., 1998). Many researchers worked on the medicinal plant parts by using organic amendment method in the inhibition of root rot fungi for the elevation of plant growth (Dawar *et al*., 2007; Ikram and Dawar, 2016).

In vivo, extract of solanaceous leaves on the growth of tested crops were evaluated by using seed treatment and soil drenching methods with different concentrations (100 and 75%) were found to be more effective in the controlling of root rot fungi colonization and maximum enhancements on growth parameter were recorded by seed treatment method with solanaceous leaves extract at 100% concentration gave maximum growth and inhibition of root infecting fungi on cowpea and okra, whereas leaves extract at 75% concentration gave minimum growth as well as reduced root rot fungi on both tested plants. Similar results had shown by Rafi *et al*., (2015) in the suppression of root rot fungi by seed priming with plants extract (*A. nilotica* and *S. mukorossi*). Seed treatments allow the seed to germinate into healthy seedling (Chang and Kommedahl, 1968). Using both methods (seed treatment and soil drenching), all parts of solanaceous plant extracts have positive effect in improving the germination, growth of plant and suppressed the pathogenic fungi colonization. *W. somnifera* is attributed as antifungal due to the presence of withanolides (group of steroidal lactones) have been collected from the leaves reported a potent antimicrobial agent with antifungal and antibacterial activity (Matsuda *et al.,* 2001). *Semecarpus anacardium* Linn. extracts significantly inhibit the *Staphylococcus aureus* when used in both aqueous and organic solvent (Mohanta *et al.,* 2007). Mangang and Chhetry (2012) reported that cold water extracts of *Lantana camara, Artemisia vulgaris, coixlacrymajobi, Michelia champaka, Passiflora foetida, Punica granatum* and *Strobilanthes flaccidifolius* suppressed *R. solani* remarkably. *Avicennia marina* pellets (leaves and stem) amended in soil respectively, improved the growth of cowpea and brinjal plants but also reduced the root rot pathogens (Tariq and Dawar, 2011). Extracts of *Adhato davasica* and *Zingiber officinale*, *Piper betle*, *Azadirachta indica* and *Vinca rosea* in addition with cow dung and *Calotropis procera* (leaf) extract along with cow urine possess strong activity to suppress the conidial germination of *Bipolaris sorokiniana* (Akhter *et al.* 2006). Similarly, Suleiman and Emua (2009) reported that *Aloe vera* showed excellent inhibition against *Pythium aphanidermatum* followed by *Zingiber officinale, Garcinia kola* and *Azadirachta indica* extracts. Datar (1999) reported that *Polyalthia longifolia* extracts contain antifungal activity

against *Macrophomina phaseolina*. By using plant extracts with different methods of formulation possess antifungal activity in the management of soil borne pathogens (Muchovej and Pacovsky, 1997; Ahmed and Nimer, 2002).

Datura plant contain various number of constituents mainly alkaloids (hyoscyamine, hyoscine, littorine, acetoxytropine, valtropine, fastusinine, a number of withanolides and a variety of trigloyl esters of tropine, pseudotropine, calystegines) compounds have also been found in various *Datura* species (Ghani, 2003). Entire plant particularly the leaves and seed have anaesthetic, hallucinogenic, anti-asthmatic, anti-spasmodic, anti-tussive, narcotic, bronchodilator, hypnotic and mydriatic effects (Yusuf *et al*., 2009). Solvent extracts (hexane, chloroform, acetone and methanol) of *Datura metel* by using microbroth dilution and percent spore germination inhibition assays methods, significantly controlled the pathogenic species (*A. fumigatus, A. flavus* and *A. niger*) were reported for antifungal properties (Sharma, 2002).

Controlling of plant pathogens with chemicals showed positive results but these agrochemicals are very costly and contribute detrimental effects on the environment. Present research showed that used of solanaceous medicinal leaves control pathogenic fungi caused by *Fusarium* spp., *R. solani* and *M. phaseolina* remarkably but also showed better growth of okra and cowpea plants. Therefore, by using plant resources is a good choice for the enhancement in crop yield on cheaper bases. For that reason, it is recommended that solanaceous leaves needs to be applied into agricultural fields which showed successful approach for controlling root rot pathogens instead of using chemical fungicides. Furthermore, using the solanaceous aqueous leaves extract as a seed treatment can easily use as antifungal in place of using fungicides.

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