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MANAGEMENT OF ROOT ROT FUNGI BY *GREWIA ASIATICA* L. LEAVES AND ON THE GROWTH OF CROP PLANTS

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Abstract

Plant pathogenic fungi deteriorate roots leading immense losses to agricultural economy of Pakistan, for this reason research was conducted to investigate the fungicidal potential of *Grewia asiatica* leaves powder against infectious fungi to ameliorate the cowpea and bottle gourd growth. *In vitro*, 100% concentration of *G. asiatica* leaves (aqueous extract) showed prodigious growth inhibition against tested fungi (*Rhizoctonia solani, Fusarium oxysporum* and *Macrophomina phaseolina*) recorded in paper disc and well methods, while 75% concentration controlled both *F. oxysporum*and *M. phaseolina* but 50% only suppressed the mycelium of *F. oxysporum* observed in both methods as compared to control. *In vivo*, leaves powder of *G. asiatica* used as organic amendment at 0.5 and 0.1% showed effectual enhancement on plant growth, where 1.0% suppressed the *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium* spp. colonization notably. Leaves extract of *G. asiatica* at 100% recorded greater suppression of pathogenic fungi colonization significantly when drenched in soil, while 75% showed maximum inhibition of root decay pathogens but highest plant growth found by 50% concentration. Furthermore, when tested seeds treated with 100% extract of *G. asiatica* leaves showed elevation in the growth on both hosts and controlling the colonization of pathogenic fungi as compared to 75 and 50% concentrations.

Key words: *G. asiatica* leaves, Methods, Concentrations, Growth and root rotting fungi.

Introduction

Root infecting fungi considered as the imperative pathogens of plant as they have ability to survive in the debris and infested soil (Berg & McClaugherty, 2014) by producing various diseases such as softening and browning of root tips, lesions, wilting of leaves and retarded growth reported in vegetables, fruits, cereals, legumes and other economically important crops (Fravel, 2005; Gonzalez *et al*., 2011) creating huge economic losses (Hamon *et al*., 2011). *Fusarium* spp. produces toxin in plant causes wilting by the blockage of xylem vessels along with disrupting cell membrane permeability and metabolism reported in many crop plants (Garces de Granada *et al*., 2001; Pawar & Thaker, 2007). *R. solani* inhibit the plant growth due to rotting of roots caused by minerals and water uptake blockage (Wallwork, 1996). *M. phaseolina* causes charcoal rot on plant by producing toxic metabolite, phaseoline which caused vascular blockage leading to the death of plants (Smith & Carvil, 1997; Santos *et al.*, 2016). Medicinal plant extracts possess potent control by exhibiting anti-microbial activity, non-phytotoxicity, systemicity, biodegradability against plant pathogens (Mahesh & Satish, 2008; Talibi *et al*., 2012). Recently, investigations on the application of medicinal plant parts and explicate their phytochemicals properties representing antifungal properties against the plant pathogens could be used due to the environment friendly mode of disease management (Matthiessen & Kirkegaard, 2006; Jasuja *et al*., 2012) which gained the attention of researchers from the use of hazardous agrochemicals (Parekh & Chanda, 2007).

Grewia asiatica (Family-Tiliaceae) known as 'Phalsa'about ≥150 species comprises trees and shrubs which are distributed worldwide. In Pakistan, more than 10 species belongs to this genus have been reported (Ali, 1974). The fruits of *G. asiatica*are beneficial for disorder of heart, liver, blood along with indigestion, thirst, stomatitis, anorexia, toxaemia, asthma, diarrhoea, fever but also used for curing throat infection, tuberculosis and sexual problems (Sharma & Sisodia,2009). Phytochemical studies revealed the presence of mineral, vitamin, fibre, carbohydrate, alkaloid, protein, glucosides and amino acid (Yadav, 1999; Tripathi *et al.*, 2010). *G. asiatica* also contains citric acid trimethyl ester (5.10%), stigma sterol (1.23%), canpesterol (2.15%), α-methyl-1-sorboside (11.52%) and methyl ester (0.10%) exhibiting antimicrobial activities (Nair & Panikkar, 1990).The leaves of *G. asiatica* used to heal the skin wounds, relieve painful rashes/irritation, treatment of diabetes, pustular eruptions and also used as cattle fodder (Bhangale *et al*.,2010; Zi-ul-Haq *et al*., 2012).

The objective of present research was to explore the fungicidal efficacy of *G. asiatica*leaves against root pathogenic fungi and on the growth of cowpea and bottle gourd plants.

Materials and Methods

Extract preparation: Leaves of *G. asiatica* were collected from nursery of University of Karachi, washed thoroughly and dried under shade. Dried leaves were pulverized in to fine powder by electric grinder and stored in plastic jar. *G. asiatica* leaves powder (10g) were soaked in 90mL of sterilized distilled water and left it for 12 hours. The concentration of extract was filtered (100%) and was diluted with sterilized distilled water to prepare further different concentrations.

***In vitro* experiments:** Agar well and filter paper disc methods were used to study the inhibition of root rot fungi with aqueous concentrations by using *G. asiatica*

leaves extract. Petri-plates poured with Potato Dextrose Agar (PDA) medium supplemented with antibiotics (Penicillin and Streptomycin @ 250mg/L) to avoid bacterial growth. In the centre of the poured Petri-plates, a disc of each root rot fungus was inoculated. For paper disc method, sterilized Whatman filter paper disc (6mm) was soaked in respective concentrations (100, 75, 50%) for half an hour. Three different concentrations of discs were placed on three different sides of Petri-plates. Similarly, in case of agar well method, three wells filled (100uL) with three tested concentrations of leaves extracts. The fourth well and disc respectively, was poured with sterilized distilled water was taken as control. Each root rot fungus replicated thrice and was incubated for one week at room temperature (26-32ºC). After the completion of incubation period, zone of growth inhibition was measured (Espinel-Ingroff *et al*., 2002).

***In vivo* experiments:** Sandy loam soil was collected from Department of Botany (KU) with pH ranged from 7.2-7.6 having 40% moisture holding capacity observed by the Keen & Rakzowski (1922) method, 7≈8 sclerotia/g of *M. phaseolina* estimated by Sheikh & Ghaffar (1975) protocol while, 12≈13% of *R. solani* was calculated by Wilhelm, (1955) procedure and *Fusarium* spp., was estimated at 2700 CFU/g by Andrew & Pitt (1986) modification method. Soil (300g) was filled in a pot which was placed randomly in the screen house of the Department of Botany, then amended with powdered of *G. asiatica* leaves at 0.1, 0.5 and 1.0% w/w concentrations, respectively. After one day of amendment, seeds of bottle gourd and cowpea after treatment with 1.0% calcium hypochlorite (surface sterilization) for 2-3 minutes were sown in each pot (five seeds/pot), respectively. Un-amended soil taken as control was also placed for comparison. For soil drenching, different concentrations at 100, 75 and 50% (w/v) with leaves extract of *G. asiatica* was poured separately in each pot and five seeds of cow pea and bottle gourd were sown in each pot, while 25mL of sterilized distilled water was poured in pot regarded as control. Replicated thrice for each treatment. In seed treatment, cowpea and bottle seeds were treated with different concentrations (100, 75 and 50% w/v) of *G. asiatica* extract respectively, for ten minutes and then dried for 2-3 hours aseptically, five treated seeds were sown in each pot separately, while untreated seeds taken as control. Replicates were made thrice for each treatment and data were recorded after full growth of plants. After one month of germination, plants were uprooted and parameters of growth were recorded. Roots of each treatment was washed in sterilized distilled water (thrice) and then treated with 1% calcium hypochlorite for at least three minutes to remove soil particles and each root was cut into small pieces. Each root pieces were placed on poured Potato Dextrose Agar (PDA) supplemented with antibiotics to inhibit the growth of bacteria. Incubate tested plates for one week at room temperature (35-38°C) and after incubation period, colonization percentage of each root pathogenic fungi was recorded. The data were calculated by two way of analysis (ANOVA) at $p<0.05$ using 'Statistica' software (Sokal&Rohlf, 1995) analysed through Duncan's Multiple Range Test (DMRT).

Results

A. *In vitro*

When sterilized disc impregnated with 100% concentration of *G. asiatica* leaves extract (aqueous) showed highest zone of inhibition and significantly ($p<0.001$) controlled the mycelium growth of *R. solani, F. oxysporum*and *M. phaseolina* followed by well method. However at 75% concentration, suppressed the growth of *F. oxysporum*and *M. phaseolina* mycelium but failed to control *R. solani* recorded in both paper disc and well methods showing full growth. Whereas 50% concentration, significantly ($p<0.001$) inhibit the *F. oxysporum* only as compared to control (Fig. 1). The interaction between method and concentration was found significant ($p<0.01$) but paper disc was found better than well method (Table 1).

B. *In vivo*

i. Cowpea: *G. asiatica* leaves powder used as organic amendment with different concentrations increased the length and weight of shoot when soil was amended with 0.5% (w/w). However, greater root length and significant weight of root ($p<0.01$) was observed, when soil amended with 0.1% (w/w) as well as nodules were also increased in number in contrast to 1.0% (w/w)of leaves powder. However, 1.0% concentration of leaves powder reduced the colonization of pathogenic fungi remarkably followed by 0.5 and 0.1% (w/w) concentrations as compared to control. Among different concentrations of *G. asiatica* leaves extracts drenched in soil, 50% (v/v) showed maximum growth enhancement and significantly ($p<0.001$; $p<0.01$) reduced the *M. phaseolina, R. solani*and *Fusarium* spp. colonization. Shoot/root length and weight of root were increased when soil was drenched with 75% (w/v) leaves extract. While, shoot weight and number of nodules were increased at 100% (w/v) concentration of leaves extract but impressively suppressed the colonization of pathogenic root fungi as compared to other concentrations. Furthermore, when cow pea seeds were treated with *G. asiatica*leaves extract at 50 and 75% (v/v) showed better growth and remarkably reduced the plant pathogenic fungi. Shoot length and weight were increased when cowpea seeds were treated with 75% (w/v) leaves extract. Root length and weight were increased at 100% concentration of leaves extract along with number of nodules. Highest control of pathogenic fungi colonization on root was recorded when cowpea seeds were treated with 100% leaves extract followed by 50% (w/v) concentration (Fig. 2).

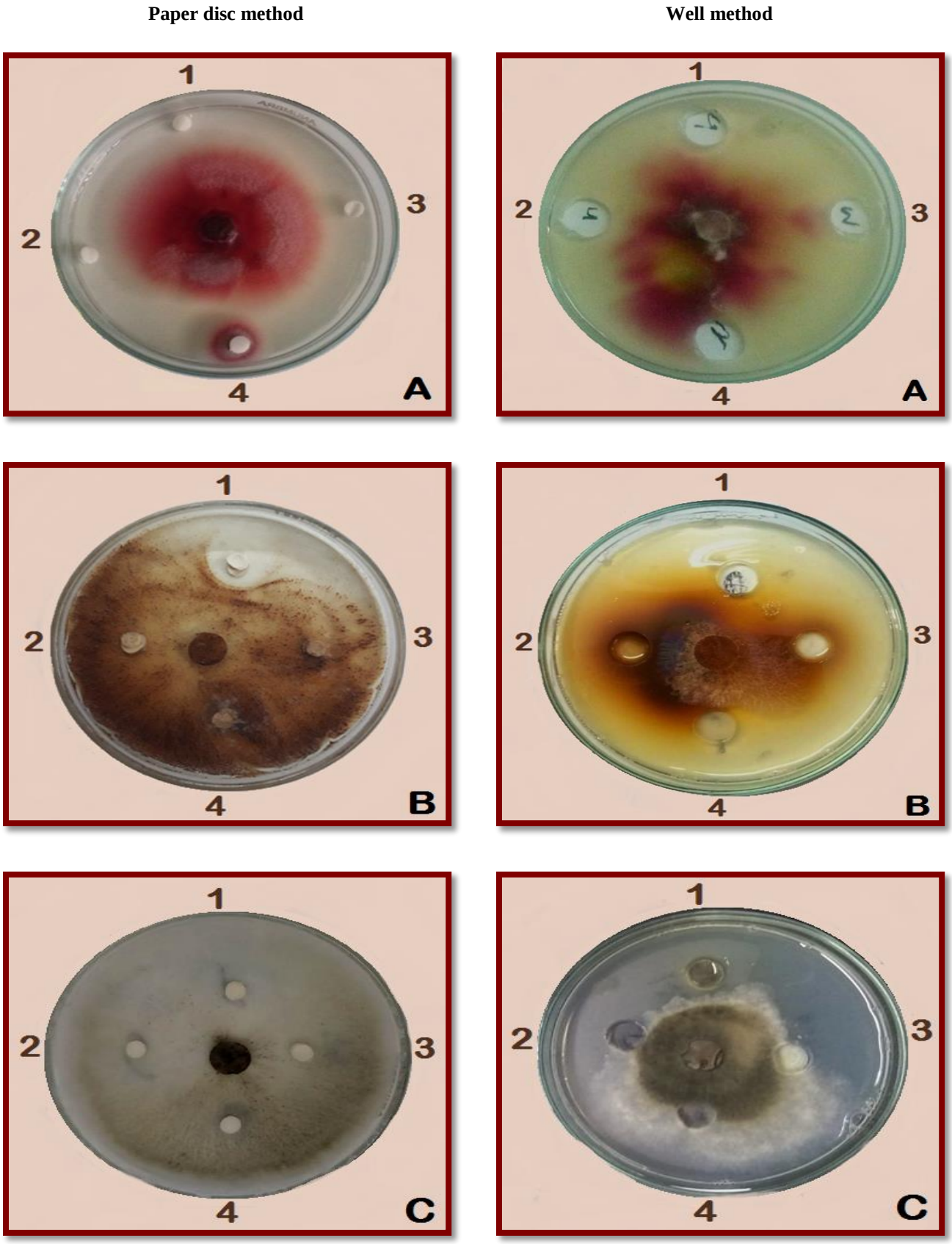

Fig. 1. Inhibition of root pathogenic fungi by using *G. asiatica* leaves aqueous extract at different concentrations.

where,
A = *Fusariumoxysporum*; **B** = *Rhizoctoniasolani*; **C** = *Macrophominaphaseolina*
1 = 100% concentration, **2** = 75% concentration, **3** = 50% concentration, **4** = Control contains disc soaked or well inoculated with sterilized distilled water

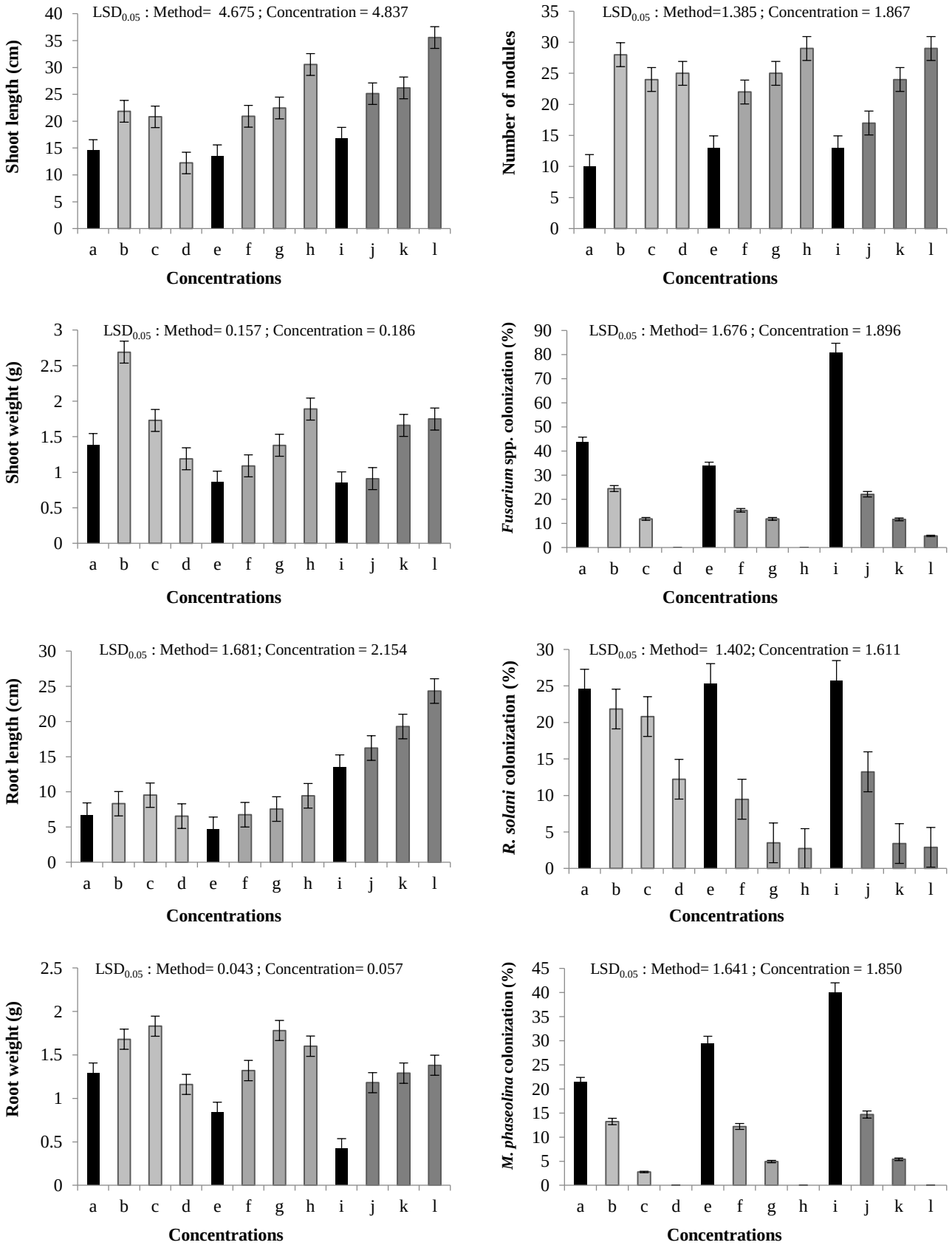

Fig. 2. Application of *G. asiatica* leaves against root rot fungi and on the growth of cow pea plants

where;
Soil amendment: a= Control; b= 0.1%; c= 0.5%; d= 1.0% (w/v) concentrations
Soil drenching: e= Control; f= 50%; g= 75%; h= 100% (v/v) concentrations
Seed treatment: i= Control; j=50%; k=75%; L= 100% (v/v) concentrations

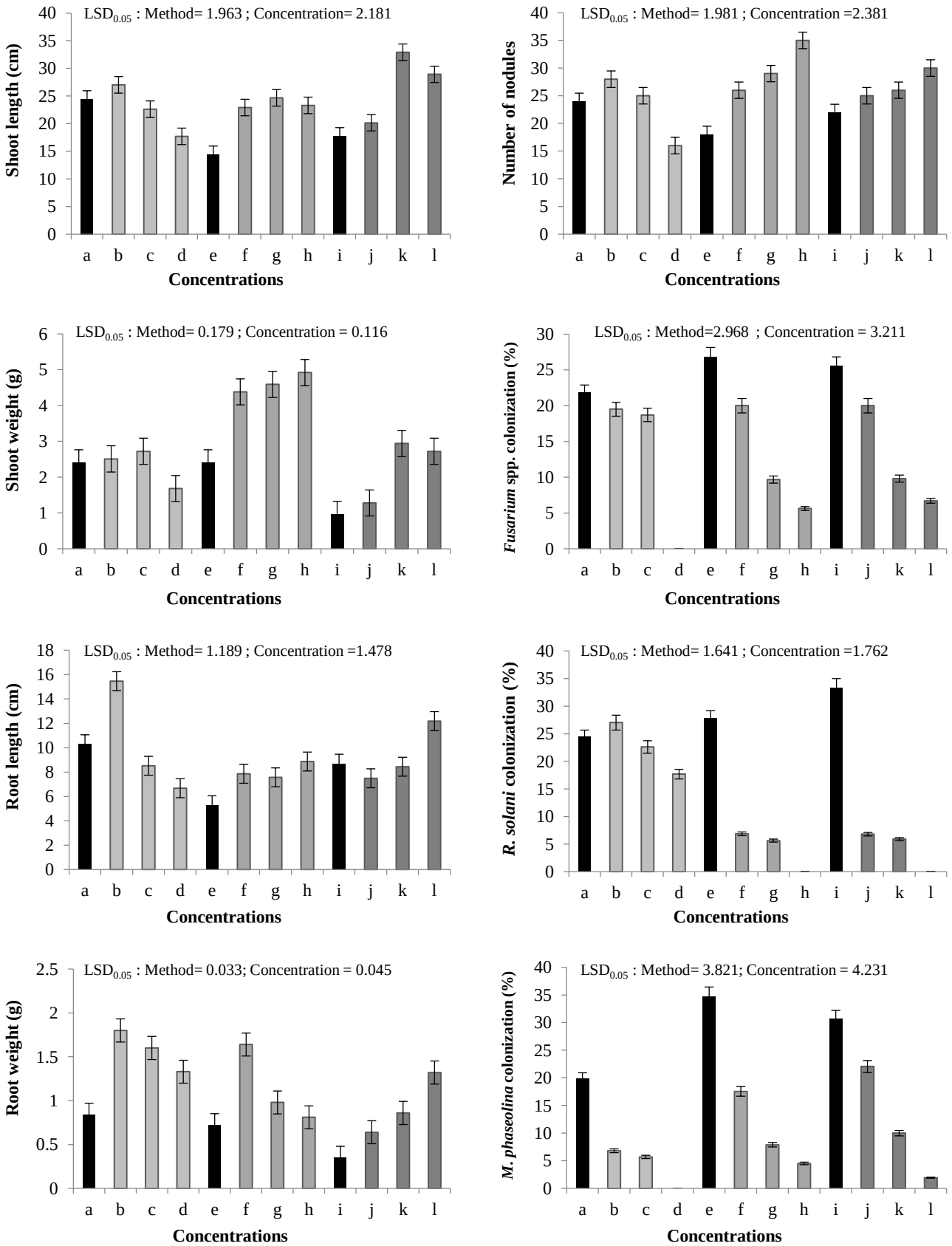

Fig. 3. Application of *G. asiatica* leaves against root rot fungi and on the growth of bottle gourd plants.

where;
Soil amendment: a= Control; b= 0.1%; c= 0.5%; d= 1.0% (w/v) concentrations
Soil drenching: e= Control; f= 50%; g= 75%; h= 100% (v/v) concentrations
Seed treatment: i= Control; j=50%; k=75%; L= 100% (v/v) concentrations

Table 1. *In vitro* studies showing antifungal activities of *G. asiatica*leaves extract by using agar well diffusion and paper disc methods.

Pathogenic fungi	Growth inhibition (%)			
	Concentrations of *G. asiatica* leaves extract in µg/mL			
	Paper disc method			
	Control ± SD	100 ± SD	75 ± SD	50 ± SD
F. oxysporum	0.00 ± 0.00	86.70 ± 2.00	77.80 ± 2.08	66.00 ± 2.00
R. solani	0.00 ± 0.00	62.90 ± 1.53	0.00 ± 0.00	0.00 ± 0.00
M. phaseolina	0.00 ± 0.00	81.60 ± 4.16	61.60 ± 2.08	0.00 ± 0.00
	Agar well diffusion method			
F. oxysporum	0.00 ± 0.00	80.00 ± 2.64	63.78 ± 2.52	44.44 ± 3.00
R. solani	0.00 ± 0.00	65.11 ± 2.08	0.00 ± 0.00	0.00 ± 0.00
M. phaseolina	0.00 ± 0.00	71.10 ± 1.08	58.40 ± 1.53	0.00 ± 0.00

$LSD_{0.05}$: Concentrations = 1.114; Fungi = 0.965; Methods = 0.788
where; SD= Standard deviation

ii. Bottle gourd: When *G. asiatica*leaves powder at 0.1% (w/w) amended in soil showed highest growth and suppressed root rot fungi colonization considerably. Shoot length and weight ($p<0.05$) along with number of nodules greatly increased when soil was mixed at 0.5 and 0.1% (w/w) leaves powder but length and weight of roots showed greater at 0.5% concentration of leaves powder. 1.0 and 0.5% concentrations of leaves powder significantly ($p<0.05$) reduced the colonization of root decay fungi followed by 0.1% leaves powder. In soil drenching, shoot length and weight were increased at 75 and 100% leaves extract, while root length/weight and number of nodules were amplified when 50% leaves extract drenched in soil. Colonization of root pathogenic fungi greatly reduced at 50 and 75% of leaves extract; whereas 100% leaves extract showed complete inhibition of pathogenic fungi. When bottle gourd seeds treated with 100% (w/v) extract of *G. asiatica*leaves gave better enhancement of growth parameter and control root rot fungi colonization followed by 75% (w/v) concentration. However, seeds treated at 50% also increased the plant weight and height as compared to control. 100% extract effectively reduced the colonization of plant pathogenic fungi significantly ($p<0.05$) as compared to other concentrations (Fig. 3).

Overall results showed that soil drenching with *G. asiatica*leaves extract were more effectual for the growth and obtained best result in controlling root rot fungi colonization as compared to other treatments, but use of soil drenching and soil amendment in commercial scale was rather difficult to apply as compared to seed treatment method which can easily be applicable. However, these two methods were recommended on low organic farming scale.

Discussion

G. asiatica is widely cultivated in many countries (Sasteri, 1992) where its young leaves and seeds of plants are regarded as nutritional power house (Grene, 2002). *G. asiatica* leaves exhibiting antimicrobial properties due to the presence of flavonoids, saponins, phenolic and tannins compounds (Sato *et al.*, 2004) having therapeutics effect which have been used to treat many plant diseases throughout the world (Elevitch, 2011). Plants with therapeutic effect have received the attention of scientists using alternate method when used to control plant diseases (Jensen *et al.*, 1996) which also protect the safety of an environment from the use of agrochemicals (Kerr, 1980).

Present research *In vitro* showed best result by 100% concentration of *G. asiatica* leaves extract was tested against root pathogenic fungi by paper disc and well methods. According to Nayan & Shukla (2011), plants rich in phenolic compounds and tannins have been shown to possess antimicrobial activity against wide number of harmful microbes. However, *in vivo* experiments showed that when soil was amended with three different concentrations (0.1, 0.5 and 1.0%) respectively, revealed increment in the growth parameters on the contrary to control, whereas leaves powder at 0.5% was found best on both tested plants. Colonization of root rotting fungi (*M. phaseolina, R. solani* and *Fusarium* spp.) effectually controlled when *G. asiatica* leaves powder applied at 1.0% as compared to control. Surprisingly, 1.0% with leaves powder does not give better growth of plant length and weight. Similar result had been represented on *Moringa oleifera* leaves powder (Ejaz *et al*., 2014). Soil amendment with plant parts producing antimicrobial compounds during decomposition (Brown & Morra, 1997; Tenuta & Lazarovits, 2002). *M. phaseolina, R. solani* and *Fusarium* spp. inhabitant in the soil for longer period and during favourable conditions, pathogens invade inside the roots and block the xylem cells which affect the physiological process by closing the stomata present in the leaves results in the wilting, ultimately the plant die (Agrios, 1988). It was reported that diseased plant contain toxins produced by pathogenic fungi and bacteria which alter the function of stomata disturbing the transpiration process which results in wilting of plants (Aducci *et al.*, 1995). Soil amendment gives a potential tool to control root diseases through chemical producing anti microbial compounds during biological and decomposition activity in soil protecting roots from the invasion of pathogens (Chen *et al*., 1998; Ikram & Dawar, 2016).

Responses of aqueous extract of *G. asiatica* leaves on the growth of tested crops were estimated by using seed treatment and soil drenching techniques. When soil was drenched with 100 and 75% concentrations of *G. asiatica* leaves, both concentrations showed maximum suppression of root rot fungi colonization and better growth of cowpea

and bottle gourd plants were observed. *G. asiatica* leaves extract at 50% improved the growth parameter and reduced root rot fungi colonization when drenched in soil. While, in seed treatment with *G. asiatica* leaves extract at 100% efficiently improved the plant growth of both crops and reduced the root infecting fungi colonization followed by 75 and 50% concentrations. Seed treatment considered as useful method for controlling soil and seed borne pathogens (Chang & Kommedahl, 1968). Leaves extract at 100% concentration gave maximum growth and inhibition of root rotting fungi on cowpea, whereas leaves extract at 75% concentration gave minimum growth as well as reduced root rot fungi on bottle gourd plants. Similar results had shown by Rafi *et al*., (2015) in the suppression of root rot fungi by priming seeds at 10 minutes intervals with plants extract (*A. nilotica* and *S. mukorossi*) was found effective and showed healthy growth of sunflower, okra, peanut and chickpea plants. Zia-ul-Haq *et al.,* (2012) recorded significant results when *G. asiatica* leaves (ethanolic extract) tested against bacteria including; *Escherichia coli*, *Proteus mirabilis*, *Citrobacter* spp., *staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis* and fungi such as; *Aspergillus* spp. (*A. niger*, *A. effusus*, *A. parasiticus*), *Candida albicans*, *Saccharomyces cerevisiae* and *Fusarium solani* exhibiting antibacterial and antifungal activities. Patil *et al.,* (2010) reported that using extracts of leaves of *G. asiatica* either in ethanol, chloroform and petroleum ether respectively, showed anti-hyperglycaemic activity. Main objective to control the plant parasitic pathogens and improved the growth and quality of plants can be achieved by the suppression of soil borne and seed borne pathogens which reduced the growth and yields causing heavy economic losses. The leaves extract of *G. asiatica* containing anti-ulcer, anti-inflammatory, anti-tumour (Pal *et al*., 1995; Makonnen *et al*., 1997) control of cholesterol and diabetes, anti-oxidant, anti-hypertensive (Mehta *et al*., 2003) anti-fungal and anti-bacterial activities (Nickon *et al*., 2003) due to the presence of 4-benzyl glucosinolate,4-benzyl isothiocyanate, benzyl isothiocyanate, pterygospermin and niazimicin (Napoleon *et al*., 2009). Majority of the anti-oxidant activity is due to the isoflavones, flavones, flavonoids, lignin, anthocyanin, coumarinisocatechin and catechin present in the leaves of *G. asiatica* (Gupta *et al*., 2007) and are used for the curing of different diseases such as alzheimer's disease, cancer, stroke, atherosclerosis and diabetes etc. which may be arise due to oxidative damage by free radicals (Kumar & Kumar, 2009). The phytochemical study of the leaves of *G. asiatica* in the extract of petroleum ether contains glycoside, fats and diterpenes. In addition, *G. asiatica* leaves extract in chloroform contains glycoside and alkaloids, while leaves extract in ethanol contains triterpenoids, flavonoids, sterols, tannins and saponins (Gupta *et al*., 2008; Patil *et al*., 2011). Other compounds, kaempferol, quercetion and a mixture of their glycoside were also isolated from the leaves extract of *G. asiatica* (Ali *et al*., 1982) which also contains citric acid trimethyl ester, campesterol and stigmasterol which are main compounds (Gupta *et al*., 2012). The aqueous leaves extract contains methyl thiazolyltetrazolin which reported anti-cancer activity against lungs, breast, cervical and kidney cancer (Marya *et al*., 2011). The ethanolic leaves extract showed anti-fungal and anti-bacterial activities (Zia-ul-Haq *et al*., 2011). The leaves extract also showed the anti-viral activity against mash bean leaf crinkle virus (Sangita *et al*., 2009). *G. asiatica* showed highly potent plant with remarkable range of medicinal benefits and high nutritional value (Sharma & Sisodia, 2009).

The purpose of present investigation was to indicate the potentiality of *G. asiatica* leaves in the suppression of root decay fungi which help in the better growth of crop plants on cheaper bases can be applied on agricultural field.

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