Int. J. Biol. Res., 12 (1-2): 50-63, 2024.

Fruit peels extract and microbial antagonists Improves the productivity of sunflower and chickpea plants in Greenhouse

Hira Sarfraz[1] and Shahnaz Dawar[1]

[1]-Department of Botany, University of Karachi, Karachi-75270, Pakistan
*Corresponding Author: hirasar93@gmail.com

Abstract

Agricullture is the backbone of developing countries and playing role in primary drivers of economy. Increasing use of chenical fertilizers and insecticides produces harzadous effect on human health, plant soils and environment. Biological agents for controlling root pathogens have gained more attention in recent years as substitutes in management of pest control and providing plant health management. Microbial antagonists including *Trichoderma harzianum*, *Paecilomyces lilacinus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* as seed treatment along with extract of *Momordica charantia* and *Citrus sinensis* peel powder as soil drenching were applied in soil. *P. lilacinus* in combination with 100% *M. charantia* peel extract gave excellent results in improvement of plant health and reduction in infection caused by plant pathogens.

Keywords: peel extract, pot experiment, root infecting fungi, soil amendment.

Introduction

Citrus sinensis L., locally called Mosambi (sweet orange), belongs to the Rutaceae family. It can be cultivated both in the subtropical and the tropical humid regions containing numerous bioactive phytochemical constituents e.g. polyphenols, flavone glycosides (hesperidin, pigments (anthocyanin, zeaxanthin), vitamins (B-complexes and C) and minerals (magnesium, calcium, iron, potassium, zinc and phosphorus) (Chopra, 2002). It also possesses anti-oxidant activity due to presence of flavonoids constituention (Guarnieri *et al.,* 2007). The essential oils of *C. sinensis* peels contain suih antimicrobial properties such as antiviral, antibacterial, antifungal and antiparasitic activities (Rehman *et al.,* 2007).

Momordica charantia L. locally known as bitter gourd or karalla in Pakistan, belongs to the family Cucurbitaceae mostly found in tropical and subtropical areas. It is mostly distributed in Africa, China, Malaysia and India (Gupta *et al*., 2011). *M. charantia* possesses an array of compounds which contains alkaloids, triterpens, steroids, flavonoids, proteins and saponins. Due to the occurance of these compounds *M. charantia* investigated as anti-viral, anti-fungal, anti-bacterial, antitumorous, anti-fertility, antiparasitic properties (Beloin *et al*., 2005; Gordon *et al*., 2011; Ordonez *et al*., 2006; Battinelli *et al*., 2006).

The rhizosphere considered to be an important ecological niche of microbial biodiversity having a benificial effect on each other and wit roots of host plant. Rhizospheric microorganisms produce different effects on plants like some can impact good development on plant while others have bad influence in terms of their development (Klopper and Beauchamp, 1992). Beneficial microorganisms can secrete substances to inhibit the growth of phytopathogenic organisms termed as anatagonistic. Studies showed that 10% of soil isolates recorded to hve antagonistic potential in vitro while small number are able to inhibit a broad spectrum of pathogenic species and it was reported that biocontrol mechanisms include secretion of extracellular metabolites like hydrogen cyanide, siderophores, antibiotics, hydrolytic enzymes or competition for nutrients (Fernando *et al.*, 2005; Gupta *et al*., 2000; Jin *et al.*, 2011; Kumar *et al*., 2011; Lesel *et al*. 1986; Ngoma *et al*., 2013; Rakotoarimanga *et al.*, 2014). Moreover, beneficial microorganisms were increase plant development, soil improvement and suppressing diseases caused by pathogens (Whipps and Lumsden, 2001).

The present work aimed to select some fungal and bacterial plant growth promoting strains having antagonistic activity and combined with fruits peel extracts and record its effect on suppression of phytopathogens in soil.

Materials and Methods

Peels collection: *Citrus sinensis* and *Momordica charantia* peels were collected from local markets of Karachi, washed, dried in air under sunlight and made fine powder using an electric grinder.

Microbial Antagonists Collection: *Bacillus subtilis* (Bs-13), *Pseudomonas aeruginosa* (Pa-32), *Trichoderma harzianum* Rifai (Th-60), *Paecilomyces lilacinus* (GenBank with accessium numbers MW566158) were collected from Karachi University Culture Collection (KUCC) and further multiplied on their respective media.

Efficacy of microbial antagonists with peels extract: Peels powder of *Citrus sinensis* and *Momordica charantia* were extracted using distilled water by soaking method for 24 hours under room temperature (32°C). The peel extracts were prepared at the concentrations of 100, 75 and 50 % w/v. Pots were filled with sandy loam soil and placed at screen house of Department of Botany, University of Karachi. Seeds of chickpea (*Cicer arietinum* (L.) and sunflower (*Helianthus annuus* (L.) washed with distilled water and sterilized with sodium hypochloride (1-2 minutes). Gum arabic (2%) was used as sticking agent to coated the seeds with microbial antagonists seperately and dried under laminar air flow. These coated seeds are sown in soil filled pots (4 seeds/pot). Some of coated seeds was observed for the colony forming unit (CFU) of bacteria and fungi by using serial dilution technique (Aneja, 2001; Yadav *et al.*, 2010). The soil was drenched with extract of *C. sinensis* and *M. charantia* peels. For comparision, control pots were also prepared where seeds were coated with distilled water and drenched with distilled water. Plants were uprooted after thirty days of germination and record the data and analysed.

Results

Combination of *M. charantia* and *C. sinensis* peel extract as soil drenching with microbial antagonists as seed treatment were applied in soil. The colony forming unit of microbial antagonists were calculated as *Trichoderma harzianum* (65×10^{-6}), *Paecilomyces lilacinus* (76×10^{-6}), *Bacillus subtilis* (16×10^{-8}) and *Pseudomonas aeruginosa* (22×10^{-8}) respectively. The combination of peel extract and microbial antagonists showed reduction in infection of root rot fungi and increment of growth parameters. It was noticed that root length enhanced when soil was drenched with extract of *M. charantia* and seeds were coated with *P. lilacinus* followed by *C. sinensis* extract and *P. lilacinus* seeds coating ($P<0.001$). Soil drenching with *C. sinensis* extract and seed coating with *P. aeruginosa* maximized the root weight ($P<0.001$) followed by *C. sinensis* extract and *B. subtilis*. However, shoot length ($P<0.001$) increased in combination of soil drenching with *M. charantia* and seed coating with *P. lilacinus* whereas, seed coating with *P. lilacinus* in combination with soil drenching with *C. sinensis* extract improved the shoot weight followed by seed coating with *P. lilacinus* along with drenching of *M. charantia* extract

(P<0.001). Greater number of leaves (P<0.001) recorded when seed coating with *P. lilacinus* along with soil drenching of *C. sinensis* extract followed by soil drenched with *M. charantia* extract in combination with *P. aeruginosa* seed coating (Figure 1a). *C. sinensis* extract in combination with *P. lilacinus* seeds coating actively reduced colonization of *M. phaseolina* (P<0.001). The two most common root infecting fungi, *F. oxysporum* and *R. solani* significantly (P<0.001) reduced infection rate due to combination of *M. charantia* extract as drenching and seeds coating with *P. lilacinus* (Figure 1b).

In case of sunflower, root length was increased when soil drenched with *M. charantia* extract and seed coating with *T. harzianum* followed by seed coating with *P. lilacinus* and soil drenching with *M. charantia* extract (P<0.001). Maximum root weight (P<0.001) was noticed when soil was drenched with *M. charantia* extract and seed coating with *T. harzianum* while shoot length and weight optimally observed when seed coating with *P. lilacinus* in combination of soil drenching with *M. charantia* extract used followed by seed coating with *T. harzianum* and soil drenching with *M. charantia* extract (P<0.001). Combination of soil drenching of *M. charantia* extract along with seed coating of *B. subtilis* when applied to the soil improved number of leaves (P<0.001) while least number of leaves observed in combination of *M. charantia* extract and seed coating with *P. lilacinus* (Figure 2a). Colonization of sunflower roots with pathogenic fungi were markedly reduced when *P. lilacinus* in combination with *M. charantia* extract applied to soil significantly (P<0.001) reduced *F. oxysporum*. *M. phaseolina* (P<0.001) actively reduced infection of roots when *M. charantia* extract applied to soil and seed coating with antagonist *T. harzianum* followed by *P. lilacinus* in combination with *M. charantia* extract and other treatments. Another most common root infecting fungus *R. solani* (P<0.001) was actively managed when soil drenched with *M. charantia* extract and seeds coating with *P. lilacinus* followed by *M. charantia* extract and seed coating with *T. harzianum* (Figure 2b).

It was concluded that *M. charantia* and *C. sinensis* alone and combine with antagonists used as soil drenching and seed coating respectively gave productive results on growth as well as reduction in colonization and the combination revealed much better results. *M. charantia* in combination with *P. lilacinus* gave excellent results as compared to other treatments.

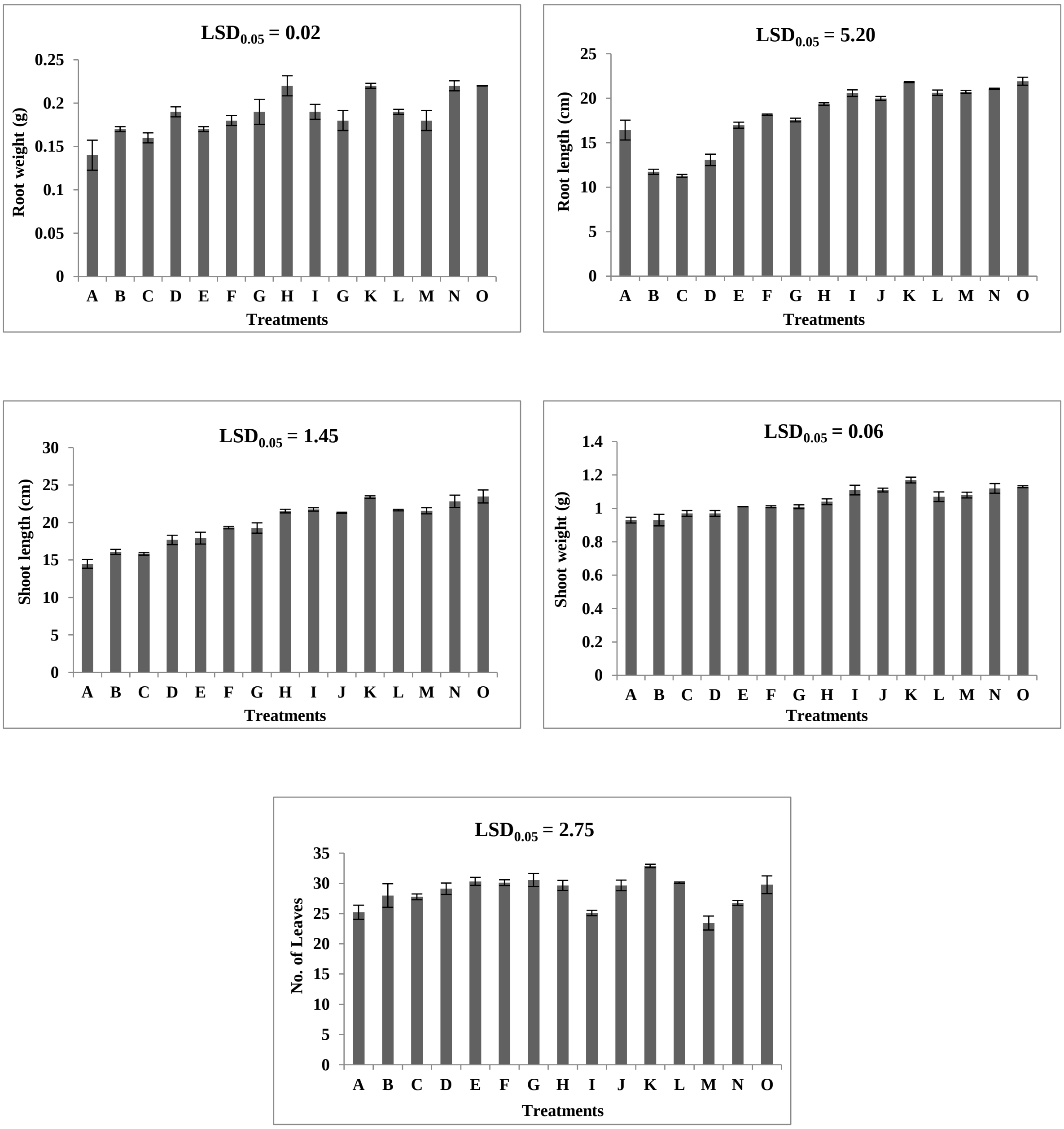


Figure 1a: Effect on microbial antagonists alone or in combination with extract of *C. sinensis* and *M. charantia* on growth parameters of chickpea plants. Values are mean and each column showed standard error of three replicates.

[A= Control; B = 100% *C. sinensis* peel extract; C = 100% *M. charantia* peel extract; D = *P. aeruginosa*, E = *B. subtilis*; F = *T. harzianum*; G = *P. lilacinus*; H = *C. sinensis* peel extract + *P. aeruginosa*; I = *C. sinensis* peel extract + *B. subtilis*; J = *C. sinensis* peel extract + *T. harzianum*; K = *C. sinensis* peel extract + *P. lilacinus*; L = *M. charantia* peel extract + *P. aeruginos;*, M = *M. charantia* peel extract + *B. subtilis*; N = *M. charantia* peel extract + *T. harzianum*; 0 = *M. charantia* peel extract + *P. lilacinus*].

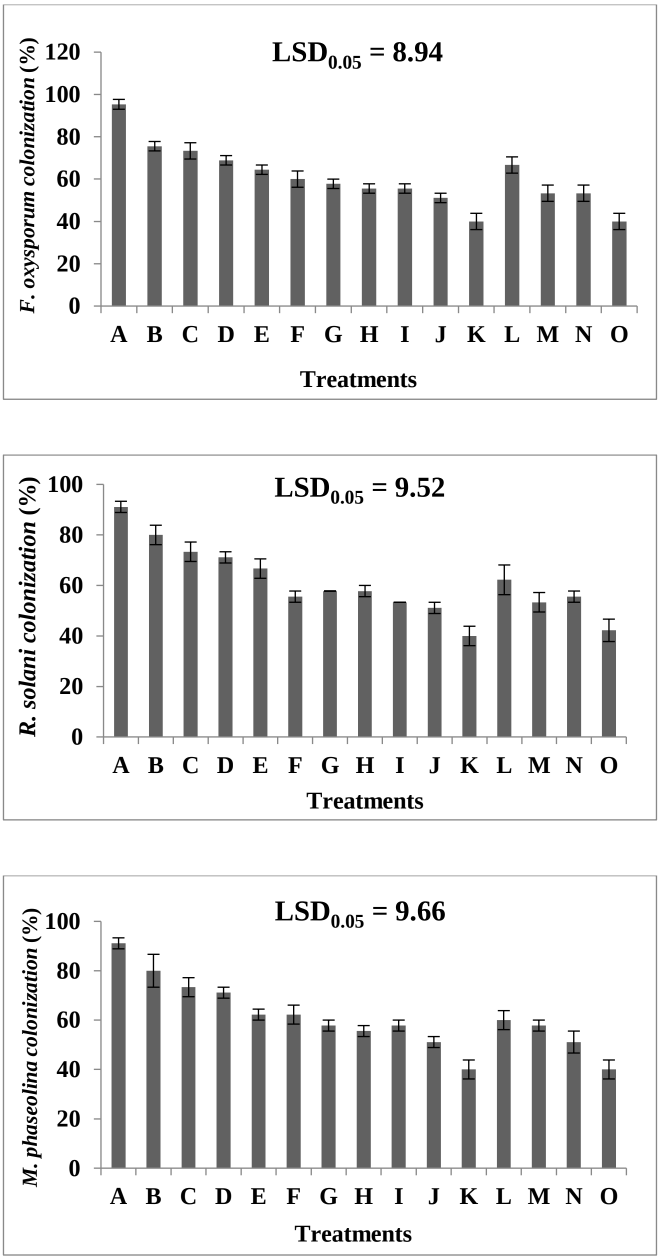


Figure 1b: Effect on microbial antagonists alone or in combination with extract of *C. sinensis* and *M. charantia* on colonization % of chickpea plants. Values are mean and each column showed standard error of three replicates.

[A= Control; B = 100% *C. sinensis* peel extract; C = 100% *M. charantia* peel extract; D = *P. aeruginosa*, E = *B. subtilis*; F = *T. harzianum*; G = *P. lilacinus*; H = *C. sinensis* peel extract + *P. aeruginosa*; I = *C. sinensis* peel extract + *B. subtilis*; J = *C. sinensis* peel extract + *T. harzianum*; K = *C. sinensis* peel extract + *P. lilacinus*; L = *M. charantia* peel extract + *P. aeruginos;*, M = *M. charantia* peel extract + *B. subtilis*; N = *M. charantia* peel extract + *T. harzianum*; 0 = *M. charantia* peel extract + *P. lilacinus*].

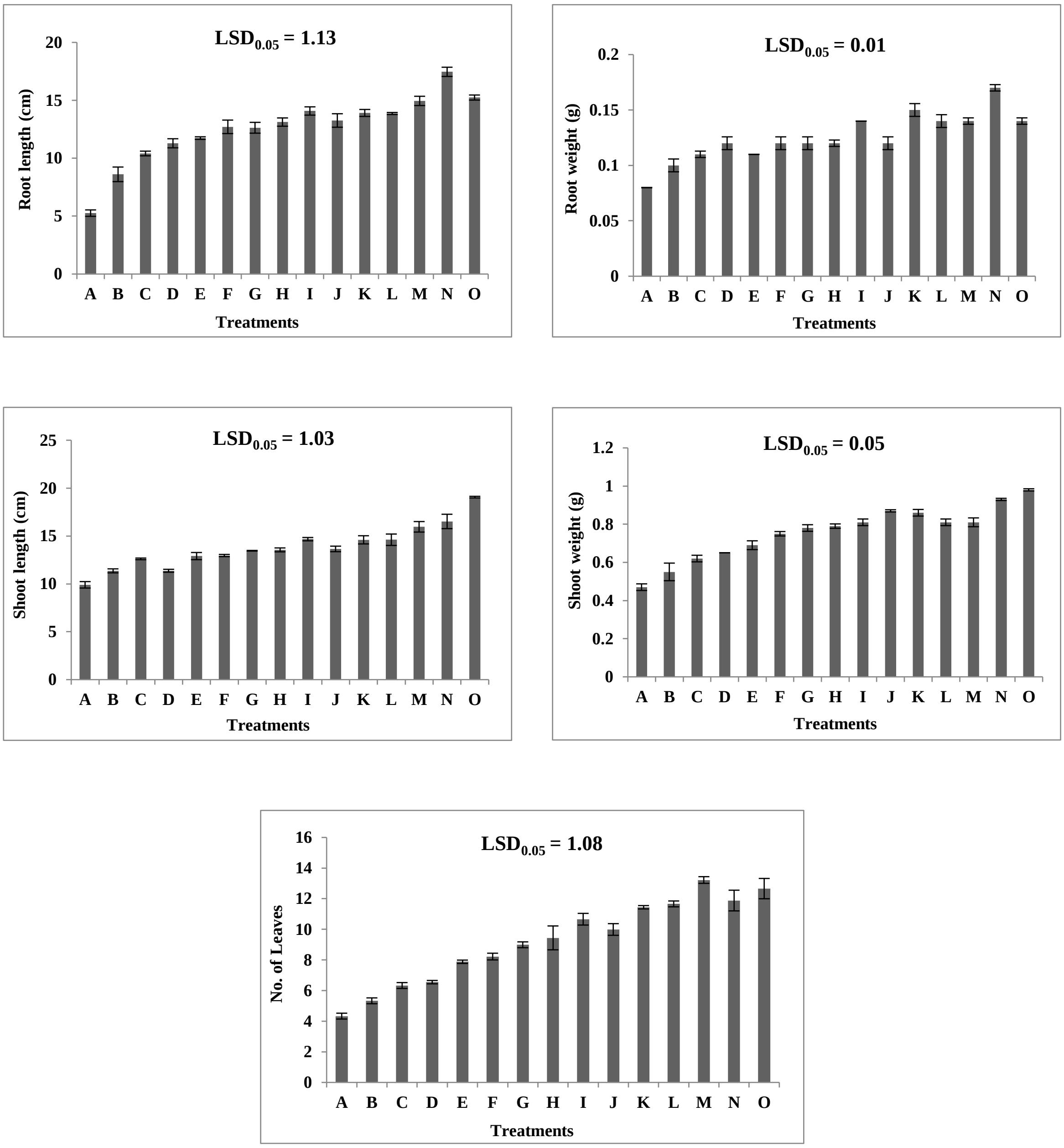
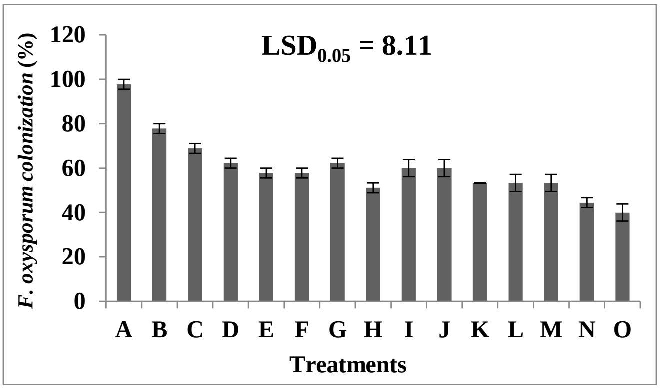
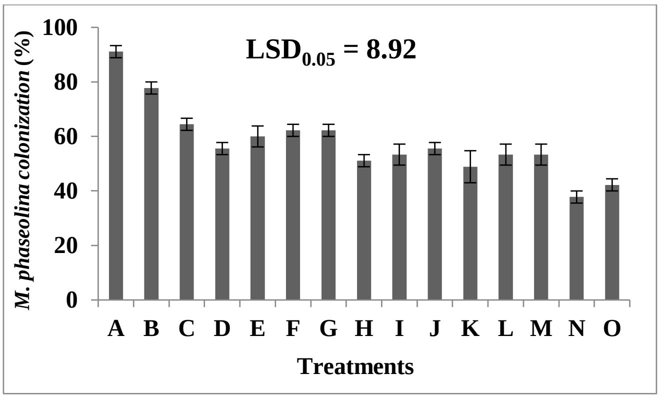


Figure 2a: Effect of microbial antagonists alone or in combination with peel extract of *C. sinensis* and *M. charantia* on growth parameters of sunflower plants. Values are mean and each column showed standard error of three replicates.

[A= Control; B = 100% *C. sinensis* peel extract; C = 100% *M. charantia* peel extract; D = *P. aeruginosa*, E = *B. subtilis*; F = *T. harzianum*; G = *P. lilacinus*; H = *C. sinensis* peel extract + *P. aeruginosa*; I = *C. sinensis* peel extract + *B. subtilis*; J = *sinensis* peel extract + *T. harzianum*; K = *C. sinensis* peel extract + *P. lilacinus*; L = *M. charantia* peel extract + *P. aeruginos;*, M = *M. charantia* peel extract + *B. subtilis*; N = *M. charantia* peel extract + *T. harzianum*; 0 = *M. charantia* peel extract + *P. lilacinus*]





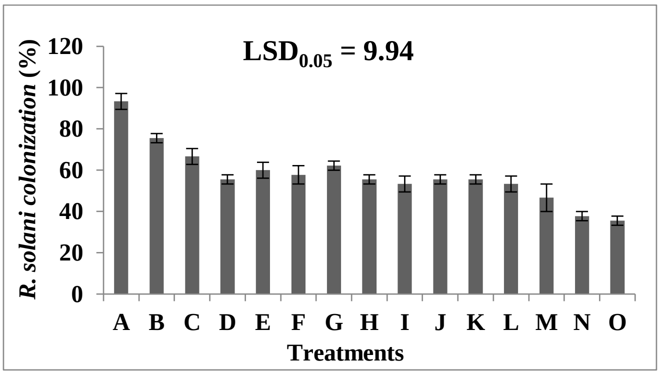


Figure 2b: Effect on fungal antagonists alone or in combination with peel extract of *C. sinensis* and *M. charantia* on colonization (%) of infecting fungi on sunflower plants. Values are mean and each column showed standard error of three replicates.

[A= Control; B = 100% *C. sinensis* peel extract; C = 100% *M. charantia* peel extract; D = *P. aeruginosa*, E = *B. subtilis*; F = *T. harzianum*; G = *P. lilacinus*; H = *C. sinensis* peel extract + *P. aeruginosa*; I = *C. sinensis* peel extract + *B. subtilis*; J = *sinensis* peel extract + *T. harzianum*; K = *C. sinensis* peel extract + *P. lilacinus*; L = *M. charantia* peel extract + *P. aeruginos*; M = *M. charantia* peel extract + *B. subtilis*; N = *M. charantia* peel extract + *T. harzianum*; 0 = *M. charantia* peel extract + *P. lilacinus*].

Discussion

Various microorganisms like fungi and bacteria used as antagonists to soil borne pathogens causing reduction in disease management sighted high significance in recent years (Mohamed *et al*., 2021; Abo-Elyousr, 2019; Ashmawy, 2020). The main purpose was to reduce chemical inputs, energy saving with new opportunities for sustainable fertilization and disease management due to readily available (Sánchez-Montesinos, 2021; Jardin *et al.*, 2016). Some bacteria (*Bacillus subtilis* and *pseudomonas aeruginosa*) and fungi (*Trichoderma harzianum* and *Paecilomyces lilacinus*) significantly proved in terms of growth characteristics and decrease in colonization of root pathogenic fungi. Seed treatment encourages seedling establishment, ensure yield and lowers quality losses brought by numerous pathogens. Control of root rotting fungi by using numerous antagonists and phyto-constitutes offer an alternate mechanism to the prevalent use of synthetic products. Seed treatment controls the pathogenicity of fungi both on the seed surface and inside the seed showed effectiveness against pathogenic fungi that are soil borne and cause damping off, seed rot, and other root rot diseases (Martha *et al.,* 2003). *Pseudomonas fluorescens* assessed to have high efficiency in reducing the pathogen growth due to rapid growth and sporulation resulting in higher competition success for space and available sources in contrast to other species (Wang *et al*., 2021; Ganeshan and Kumar, 2005). Combined effect of *C. sinensis* and *M. charantia* peel extract has tremendous effect on plant growth and in the management of root pathogenic fungi. Present research revealed that *P. lilacinus* in combination with *C. sinensis* on chickpea plants gave significant growth and reduction in colonization of pathogenic fungi. Previously, agro industrial waste in combination with antagonistic agents including *Lactobacillus plantarum*, *L. casei, Rhodobacter sphaeroides*, *Rhodopseudomonas palustris*, *Saccharomyces* sp., *Streptococcus lactis* and *Streptomyces* sp. was used and resulted a promising strategy against *Verticilium* wilt of olive (Varo-Suarez *et al*., 2018; Montes-Osuna and Mercado-Blanco, 2020; Fernández-González, 2020). According to Noreen *et al.* (2015), antagonistic bacteria tends to increase plant growth parameters either by inhibition of phyto-pathogens or by direct stimulation. Among various rhizospheric bacteria, fluorescent pseudomonades (Rahman *et al.*, 2016) are aggressively colonized and have large antagonistic properties against the phyto-pathogens on different plant crops. *M. charantia* alone and in combination with antagonist *P. lilacinus* used as soil drenching and seed coating not only gave excellent results on growth as well as in infection of root pathogenic fungi as compared to control. The reason may be that plant *M. charantia* contains biologically active compounds including steroids, alkaloids, saponins and flavonoids due to occurance of these plant contains antimicrobial properties (Scartezzini and Speroni, 2000). Similar applications observed by (Korejo *et al.,* 2019) on endophytic

Pseudomonas aeruginosa in soil treated with *L. nudicaulis* significantly controlled *R. solani*, *F. solani* and *M. phaseolina* on sunflower crops. Studies have already proved that active compounds played an essential role in survival mechanism of plants towards various pathogenic fungi (Akila *et al.*, 2011) and it was responsible for antifungal properties (Sharma and Kumar, 2009; Glazer *et al.*, 2012).

Refrences

Abo-Elyousr, K.A., H.M. Khalil Bagy, M. Hashem, S.A. Alamri and Y.S. Mostafa. 2019. Biological control of the tomato wilt caused by *Clavibacter michiganensis* subsp. michiganensis using formulated plant growth-promoting bacteria. *Egypt. J. Biol. Pest Cont.*, *29*(1): 1-8.

Akila, R., L. Rajendran, S. Harish, K. Saveetha, T. Raguchander and R. Samiyappan. 2011. Combined application of botanical formulations and biocontrol agents for the management of *Fusarium oxysporum* f. sp. Cubense (Foc) causing *Fusarium* wilt in banana. *Biol. Cont.*, 57: 175-183.

Aneja, K.R. 2001. *Experiments in Microbiology*, *Plant pathology* and *Biotechnology*. New age international publishers. 4: 157-162.

Ashmawy, N.A., M.Z.M. Salem, N. El Shanhorey, A.A. Al-Huqail, H.M. Ali and S.I. Behiry. 2020. Eco-friendly wood-biofungicidal and antibacterial activities of various Coccoloba uvifera L. leaf extracts: HPLC analysis of phenolic and flavonoid compounds. *Bioresour.*, 15: 4165–4187.

Battinelli, L., C. Daniele, M. Cristiani, G. Bisignano, A. Saija and G. Mazzanti. 2006. *In vitro* antifungal and antielastase activity of some aliphatic aldehydes from *Olea europaea* L. fruit. *Phytomed.*, 13: 558-563.

Beloin, N., M. Gbeassor, K. Akpagana, J. Hudson, K. de Soussa, K. Koumaglo and J.T. Arnason. 2005. Ethnomedicinal uses of *Momordica charantia* (Cucurbitaceae) in Togo and relation to its phytochemistry and biological activity. *J. Ethnopharmacol.*, 96: 49-55.

Chopra, R.N. 2002. *Glossary of Indian Medicinal Plants*, National Institute of Science Communication and Information Resources, Delhi, 6: 69.

Fernando, W.D., S. Nakkeeran and Y. Zhang. 2005. Biosynthesis of antibiotics by PGPR and its relation in biocontrol of plant diseases. In PGPR: Biocontrol and Biofertilization, Springer, Netherlands, pp. 67-109.

Fernández-González, A.J., M. Cardoni, C.G.-L. Cabanás, A. Valverde-Corredor, P.J. Villadas, M. Fernández-López and J. MercadoBlanco. 2020. Linking belowground microbial network changes to different tolerance level towards *Verticillium* wilt of olive. Microbiome., 8: 1–19.

Ganeshan, G. and A.M. Kumar. 2005. *Pseudomonas fluorescens*, a potential bacterial antagonist to control plant diseases. *J. Plant Interact*. 1: 123–134

Glazer, I., S. Masaphy, P. Marciano, I.B. Ilan, D. Holland, Z. Kerem and R. Amir. 2012. Partial identification of antifungal compounds from *Punica granatum* peel extracts. *J. Agric. and Food Chem.,* 60: 4841-4848.

Gordon, A., E. Jungfer, B.A. Silva, J.G.S. Maia and F. Marx. 2011. Phenolic constituents and antioxidant of four underutilized fruits from the Amazon Region. *J. Agric. and Food Chem.,* 59: 7688-7699.

Guarnieri, S., P. Riso and M. Porrini. 2007. Orange juice vs Vitamin C: Effect on hydrogen peroxide induced DNA damage in mononuclear blood cells. *B. J. Nutri.,* 97: 639-643.

Gupta, M., S. Sharma, A.K. Gautam and R. Bhadauria. 2011. *Momordica charantia* L. (karela): nature's silent healer. *Int. J. Pharm Sci. Rev. Res.,* 11(1): 32-37.

Gupta, A., M. Gopal, K.V. Tilak. 2000. Mechanism of plant growth promotion by rhizobacteria. *Ind. J. Exp. Biol*., 838: 856-862.

Jardin, P.D., F. Verheggen, B. Fassotte. 2016. Implementing plant biostimulants and biocontrol strategies in the agroecological management of cultivated ecosystems. *Biotechnol. Agron. Soc. Environ.* 20: 299–313.

Jin, F., Y. Ding, W. Ding, M.S. Reddy, W.G. Fernando, B. Du. 2011. Genetic diversity and phylogeny of antagonistic bacteria against *Phytophthora nicotianae* isolated from tobacco rhizosphere. *Int. J. Mol. Sci*., 12(5): 3055-3071.

Kloepper, J.W. and C.J. Beauchamp. 1992. A review of issues related to measuring colonization of plant roots by bacteria. *Can. J. Microbiol.*, 38: 1219-1232.

Korejo, F., F. Urooj H. Farhat, S. Moin, V. Sultana and A. Rahman. 2019. Effect of soil amendment with *launaea nudicaulis* L. on biocontrol potential of endophytic fluorescent *Pseudomonas* against root rot disease of sunflower. *Int. J. Biol. Res.,* 7(1): 33-38.

Kumar, A., A. Prakash, B.N. Johr. 2011. *Bacillus as PGPR in Crop Ecosystem. D.K. Bacteria. in Agrobiology*: Crop Ecosystems. Springer, Berlin, Heidelberg, pp. 37-59.

Lesel, R., C. Fromageot, M. Lesel. 1986. Cellulose digestibility in grass carp, Ctenopharyngodon idella and in goldfish, *Carassius auratus*. *Aquaculture,* 54(1- 2): 11-17.

Martha, M., J. Riesselman, D. Mathre, B. Johnston and S. Blodgett. 2003. *Revised by Bob Johnstan. In: Manual of Small seed grain treatment guide*. pp. 55.

Mohamed, A., M. Salah, M. El-Dein, M. El-Hefny, H. Ali, D. Farraj, A. Hatamleh, M.Z.M. Salem, N. Ashmawy. 2021. Ecofriendly bioagents, Parthenocissus quinquefolia, and Plectranthus neochilus extracts to control the early blight pathogen (*Alternaria solani*) in tomato. *Agron.*, 11: 911.

Montes-Osuna, N. and J. Mercado-Blanco. 2020. *Verticillium* wilt of olive and its control: What did we learn during the last decade? *Plants*, 9: 735.

Ngoma, L., B. Esau, O.O. Babalola. 2013. Isolation and characterization of beneficial indigenous endophytic bacteria for plant growth promoting activity in Molelwane Farm, Mafikeng, *S. Afr.J. Biotechnol*., 12(26): 4105-4114.

Noreen, R., S.A. Ali, K.A. Hassan, V. Sultana, J. Ara and S. Ehteshamul-Haque. 2015. Evaluation of biocontrol potential of fluorescent *Pseudomonas* associated with root nodules of mungbean. *Crop Prot.,* 75: 10-24.

Ordonez, R.M., A.A.L. Ordonez, J.E. Sayago, M.I.N. Moreno and M.I. Isla. 2006. Antimicrobial activity of glycosidase inhibitory protein isolated from *Cyphomandra betacea* Sendt. fruit. *Peptides,* 27: 1187-1191.

Rehman, S., S. Hussain and H. Nawaz. 2007. Inhibitory effect of *Citrus* peel essential oils on the microbial growth of bread. *Pak. J. Nutri.*, 6: 558-61.

Rahman, A., V. Sultana, J. Ara and S. Ehteshamul-Haque. 2016. Induction of systemic resistance in cotton by the neem cake and *Pseudomonas aeruginosa* under salinity stress and *Macrophomina phaseolina* infection. *Pak. J. Bot.*, 48: 1681-1689.

Rakotoarimanga, N., J. Zananirina, D. Ramamonjisoa, H. Ramanankierana. 2014. Lutte biologique antifongique: actinomycètes du sol rhizosphérique antagonistes de *Fusarium* isolé du fruit de tomate (*Solanum lycopersicum* L., 1753 pourri. Afr. Rev. Sci. Tech., 10(3): 244-254.

Sánchez-Montesinos, B., M. Santos, A. Moreno-Gavíra, T. Marín-Rodulfo, F. Gea, F. Diánez. 2021. Biological control of fungal diseases by *Trichoderma aggressivum* f. europaeum and its compatibility with fungicides. *J. Fungi,* 7: 598.

Scartezzini, P. and E. Speroni. 2000. Review on some plants of Indian traditional medicine with antioxidant activity. *J. Ethnopharmacol.*, 71: 23-43.

Sharma, B. and P. Kumar. 2009. *In vitro* antifungal potency of some plant extracts against *Fusarium oxysporum*. *Int. J. Green Pharm.*, 63-65.

Varo-Suárez, A., M. Raya-Ortega, C. Agustí-Brisach, C. García-Ortiz-Civantos, A. Fernández-Hernández, A. Mulero-Aparicio, A. Trapero. 2018. Evaluation of organic amendments from agro-industry waste for the control of verticillium wilt of olive. *Plant Pathol.*, 67: 860–870.

Wang, Z., T. Zhong, K. Chen, M. Du, G. Chen, X. Chen, K. Wang, Z. Zalán, K. Takács, J. Kan. 2021. Antifungal activity of volatile organic compounds produced by *Pseudomonas* fluorescens ZX and potential biocontrol of blue mold decay on postharvest citrus. *Food Contr.*, 120: 107499.

Whipps, J.M. and R.D. Lumsden. 2001. *Commercial use of fungi as plant disease biological control agents: status and prospects*. pp. 9-22. In Butt, T.M., Jackson, C., Magan, N. (eds.): Fungi as biocontrol agents: progress, problems and potential. CABI Publishing. Wallingford, UK

Yadav, R.K.P., K. Kakamanoli and D. Vokou. 2010. Estimating bacterial population on the phyllosphere by serial dilution plating and leaf imprint methods. *Ecoprint: An Int. J. Ecol.*, 17: 47-52.