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ASSESSMENT OF EXTRACELLULAR METABOLITES FROM *BACILLUS* SPECIES AGAINST ROOT-KNOT NEMATODES AND ROOT-INFECTING FUNGI IN *ABELMOSCHUS ESCULENTUS* (L.) MOENCH

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Abstract

Bacillus species are well known rhizobacteria for their potential to improve plant growth and control of plant pathogens. *Bacillus* species were isolated from rhizosphere of different crop plants in lower Sindh. Secondary metabolites of *Bacillus cereus* (KUB-15), *B. coagulans* (KUB-20) and *B. cereus* (KUB-27) were extracted in ethyl acetate and hexane. Two concentrations (500 and 1000 ppm) of these extracts were applied to Okra (*Abelmoschus esculentus* [L.] Moench) seeds for the assessment of a) plant growth promoting ability b) root-knot nematode parasitism and c) infection of root-infecting fungi. Both extracts at 1000 ppm concentration showed significantly higher plant height and weight compared to lower concentration (500ppm). Root-knot nematode parasitism was measured in term of number of galls, egg masses and eggs/egg mass. Lower concentrations of the extracts showed greater parasitism of nematodes compared to higher concentration of both the solvents. Least root-knot nematode parasitism was observed in plants treated with ethyl acetate extract of KUB-20 at 1000 ppm. Colonization of three root-infecting fungi namely, *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium* species was recorded. Lowest infection of *M. phaseolina* (8.86%) and *R. solani* (17.71%) was observed with ethyl acetate extracts of KUB-20 and KUB-27, respectively. However, colonization of *Fusarium* species was greatly reduced by both ethyl acetate and hexane extracts of KUB-20 at 1000 ppm.

Keywords: Biocontrol, soil borne pathogens, solvent extracts

Introduction

Bacillus species are widely distributed plant growth promoting organisms, commonly associated with plant roots and rhizosphere and increase plant growth by vanishing plant pathogens due to unique antimicrobial activities, including, production of antibiotics, toxins and out-compete with the pathogenic organisms (Falardeau *et al.*, 2013; Liu *et al*., 2014). Some strains are known to induce systemic resistance in plants against a variety of pathogens. Application of *Bacillus* species as biocontrol agents against different plant pathogens has received increasing attention (Liu *et al*., 2009, Yánez-Mendizábal *et al*., 2012, Abbasi *et al*., 2013 & 2014; Zeriouh *et al*., 2014; Torres *et al*., 2017). *Bacillus* species are effective in the management of plant pathogens owing to their ability to produce antimicrobial compounds (Mannanov & Sattarova, 2001) and plant growth promoting ability, especially by increasing the performance of plant roots (Compant *et al*., 2005), production of metabolites that increase the mineral availability (Ongena & Jacques, 2007) to plants by solubilization of inorganic phosphate and mineralization of organic phosphate, which makes phosphorous available to the plants (Saharan & Nehra, 2011). Apart from antibiotic excretion by *Bacillus*, under conditions of low iron, they produce chelators that make iron unavailable to other microorganisms resulting in the lyses of fungal mycelium. There are reports that *Bacillus* species invade plant roots and produce different metabolites like phytohormones and enzymes that modulate plant hormones (Selvadurai *et al*., 1991; Goswami *et al*., 2015).

Plant pathogens cause severe damage to different crops resulting in reduction of yield and quality of bio-products. The soil-borne plant pathogens like nematodes, fungi and some bacteria infect plant roots cause disruption in absorption and translocation of nutrients and water from soil, leads to mortality of plants. Root-knot (RK) nematodes (*Meloidogyne* spp.) are the most destructive and widely distributed nematodes, cause disease on over 2,000 species of plants (Sasser & Freckman, 1987). Changes in root anatomy, disruption of vascular system, reduction of uptake of water and nutrients (Williamson & Hussey, 1996; Abad *et al*., 2003). Root-rot disease causing fungi are *Macrophomina phaseolina* (Tassi) Goid., *Rhizoctonia solani* Kühn., *Fusarium solani* (Mart) Appel & Wollenw. emend. Snyd. & Hans., *Fusarium oxysporum* Schltdl., *Fusarium moniliforme* Sheld., and *Pythium* species. *M. phaseolina* causes rotting of roots, stem and pods on hundreds of cultivated plant species (Dhingra & Sinclair, 1978). Pathogen remains present in soil as mycelium or sclerotia or in diseased plant debris. *R. solani* enters the host tissues either mechanically by producing infection pegs from the hyphal tip or through natural openings and wounds (Back *et al*., 2002) while *Fusarium* species are also very common plant pathogens reported from rhizosphere, cause root and stem rot and wilt diseases on a variety of crop plants causing yellowing of lower leaves, wilting and xylem vessels show red coloration or vascular discoloration (Lagopodi *et al*., 2002).

Keeping in mind the importance of *Bacillus* species, their diversity, unique biochemical properties and matchless outstanding characteristics among the bacteria, particular attention was focused on *Bacillus* spp. The purpose of this study was to characterize the crude extracellular metabolites of indigenous *Bacillus* species against RK nematodes and root-rot fungal pathogens.

Materials and Methods

Culture conditions: Rhizosphere soils were collected from different crops at the depth of 10 to 25 cm. Soil samples were transferred to polythene bags and stored at -20ºC in the laboratory. *Bacillus* species were identified by phenotypic characterization according to Bergey's manual of determinative bacteriology (Holt *et al*., 1994). Identified strains were grown in Luria-Bertani (LB) broth in 20% glyceraldehydes for 24 hours at 37ºC and stored at -20ºC.

Roots of infested eggplants were collected and root-knot (RK) nematode species were identified by observing perennial pattern in adult females as described by Taylor & Netscher (1974). *Meloidogyne javanica* (Treub) Chitwood, root-knot nematodes were cultured on eggplant seedlings in pots containing autoclaved soil under greenhouse conditions from a single egg mass. At the time of application, RK nematode parasitized eggplant roots were collected. After washing, egg masses were collected directly from the parasitized roots in cavity blocks containing sterile water. Egg masses were incubated for 72 h at 28±2°C for emergence of juveniles from the eggs. Subsequently, second stage juveniles (J2) were collected and suspended in sterile water. The number of juveniles per mL was counted using a counting chamber (Hussey & Barker, 1973).

For the isolation of root-infecting fungi different techniques were used including serial dilution technique for *Fusarium* species, baiting technique for *Rhizoctonia solani* (Wilhelm, 1955) and wet sieving and dilution technique for *Macrophomina phaseolina* (Sheikh & Ghaffar, 1975). Fungal cultures were maintained in potato dextrose agar (PDA).

Organic solvent extraction and application: *Bacillus* isolates were grown on LB broth for 48h at 37°C in shaking incubator. Broth was centrifuged at 4000 X *g* for 15 min cell free supernatant was separated. Organic solvent (ethyl acetate or hexane) was added into cell free filtrates of selected *Bacillus* isolates in the ratio of 1:1 and thoroughly shaken for 2 h in a shaker. The solvent extract was separated from cell free filtrate by separatory funnel. The procedure repeated twice by adding solvent which was subsequently evaporated under reduced pressure in a rotary evaporator. After complete evaporation of organic solvent the remaining material was collected and weighed. 1000 and 500 ppm solutions were prepared in absolute ethanol and used for the study. Plant growth experiment was performed by treating okra seeds with extracts. Seeds were washed with sodium hypochlorite (2%) solution for 2 minutes and then with sterile distilled water before extract treatment. Seeds treated with absolute methanol considered as controls. Seeds were air dried in laminar flow and sown in pots containing 300g soil. Nematode suspension (containing about 1000 J2) was inoculated around the roots after 7 days of seedling emergence. Soil had natural infestation of root-infecting fungi (1-3 sclerotia/ g of *M. phaseolina*, $3x10^3$ cfu/g soil of *Fusarium* species and about 10 % of *R. solani* on sorghum seeds used as baits). Experiment was terminated after 45 days of nematode inoculation. Data on plant growth, root-knot parasitism and root-rot infection were recorded.

RK nematode parasitism was estimated by counting number of galls and egg masses under stereomicroscope (6x). Egg masses were randomly collected from roots for the count of eggs/ egg mass. Eggs released from egg masses when treating with 2% sodium hypochlorite were counted in compound microscope.

Infection of root-infecting fungi was quantified by root-plating method (Newsham *et al*., 1995). Infected roots were washed with sterile distilled water and surface sterilized with sodium hypochlorite (2%) for 1 minute. Roots were cut into small pieces and plated on PDA for 4 days at 30°C. Colonization percentages were calculated and data were subjected to arcsine transformation before statistical analysis (Zar, 2009).

Statistical analysis: Experiment was designed according to completely randomized design in screen house conditions. Data were subjected to three-factor analysis of variance (ANOVA), followed by Fisher's least significant test (Zar, 2009). Standard error of means were also calculated and shown in bar graphs.

Results

Data indicated the improvement of plant height in all treatments compared to controls. At the time of measurements of total plant height, shoot and root lengths were measured as well (Fig. 1). Most significant increase in plant height (29.67cm) was noticed in plants treated with ethyl acetate extract of KUB-27 at 1000 ppm. Similarly, the shoot lengths were highest in this treatment (18 cm). However, maximum root length was measured in plants treated with hexane extract of KUB-27at 1000 ppm. It was also observed that ethyl acetate extracts improved plant height significantly ($P<0.001$) compared to hexane extracts, further higher concentrations of both the solvents gave better results ($P<0.001$). Higher plant weight was obtained in both the solvent extracts (ethyl acetate and hexane) when used at higher concentration (1000 ppm) ($P<0.01$). Maximum plant fresh weight was revealed in treatments with hexane extract of KUB-15 at 1000 ppm, followed by treatment of ethyl acetate extract of KUB-27at 1000 ppm (Fig. 1).

RK nematode parasitism was measured in term of galls and egg masses per root system. When compared to control all the treatments showed lesser root-knot nematode parasitism. Least RK nematode parasitism was observed in plants treated with ethyl acetate extract of KUB-20 at 1000 ppm ($P<0.001$). It was noted that lower concentrations showed greater parasitism of nematodes compared to higher concentration of both the solvents ($P<0.01$). Further, the ethyl acetate extract compared to hexane extract, showed decreased nematode parasitism. Number of eggs per egg mass is very important parameter, which gives the idea about the reproductive rate of adult female; moreover number of eggs per egg mass can be correlated with the production of second stage infectious juveniles in the rhizosphere. Maximum number of eggs per egg mass was recorded in controls, while least number was counted in treatments with KUB-27 extract of hexane at 500 ppm ($P<0.001$). Hexane extracts relative to ethyl acetate extracts revealed lesser eggs per egg mass count ($P<0.001$) (Fig. 2). *M. phaseolina* significantly varied after different treatments of solvent extracts. KUB-20 extract of ethyl acetate when applied at 1000 ppm on seeds showed lowest infection (8.86%) of *M. phaseolina,* followed by the treatment of

KUB-20 hexane extract at 1000 ppm (Fig. 3). Higher concentration of both solvent extract showed better results when comparing with lower concentrations ($P<0.05$). Maximum infection of *M. phaseolina* on roots of okra was observed in controls. *R. solani* colonization % reduced in all the treatments compared to control, which showed maximum infection (54.99%), lowest infection (17.71%) of *R. solani* revealed by the treatment with KUB-27 ethyl acetate extract at 1000 ppm (Fig. 4). *Fusarium* species colonization greatly reduced by KUB-20 ethyl acetate and hexane extracts at 1000 ppm, both extracts showed 17.71% colonization. Untreated control plants revealed higher colonization % of *Fusarium* species ($P<0.001$) (Fig. 5).

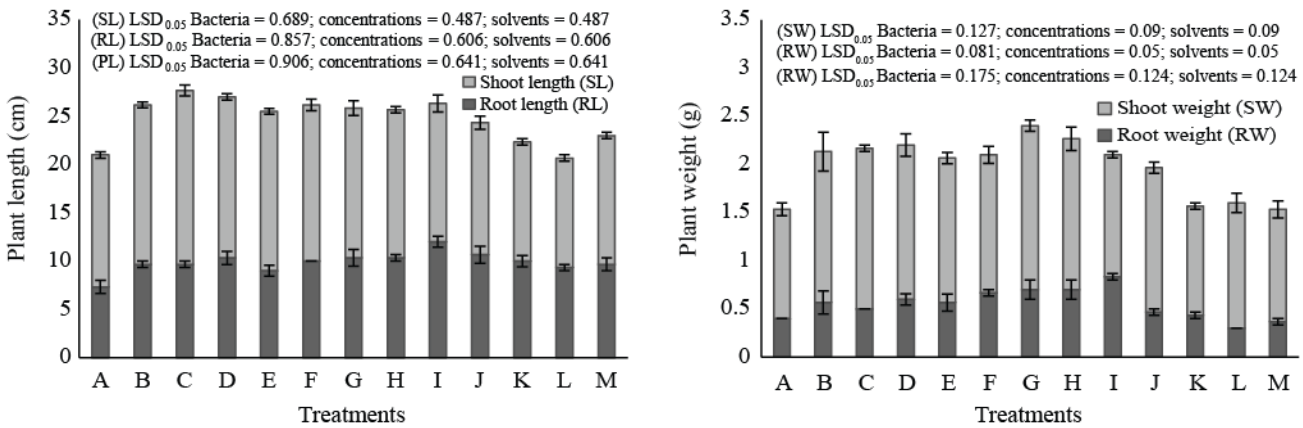

Fig. 1. Effect of solvent extracts of cell free filtrate from *Bacillus* species at different concentrations on growth of okra.
A= control, B= ethyl acetate extract (EA) of KUB-15 1000ppm, C= EA of KUB-27 1000ppm, D=EA of KUB-20 1000ppm, E= EA of KUB-15 500ppm, F= EA of KUB-27 500ppm, G=EA of KUB-20 500ppm, H=hexane extract (H) of KUB-15 1000ppm, I=H of KUB-27 1000ppm, J=H of KUB-20 1000ppm, K=H of KUB-15 500ppm, L=H of KUB-27 500ppm, M=H of KUB-20 500ppm

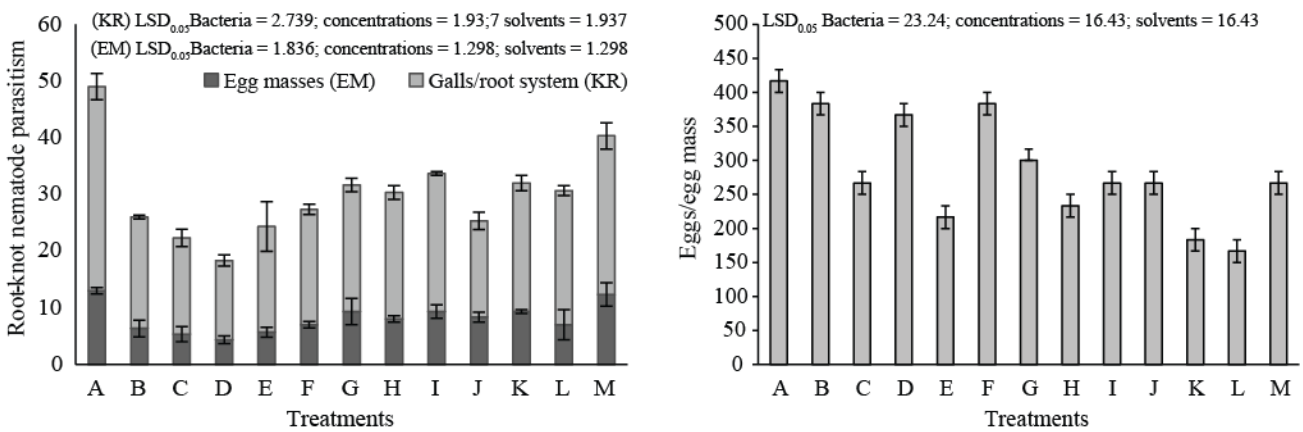

Fig. 2. Effect of solvent extracts of cell free filtrate from *Bacillus* species at different concentrations on root-knot nematode parasitism in okra. See figure 1 for treatment design.

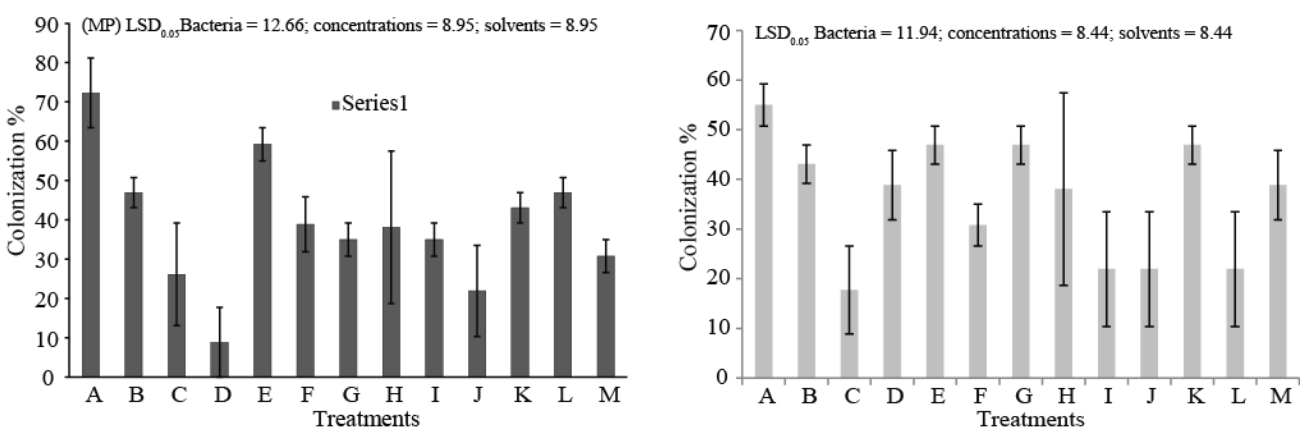

Fig. 3. Effect of solvent extracts of cell free filtrate from *Bacillus* species at different concentrations on colonization of *M. phaseolina* in okra. See figure 1 for treatment design.

Fig. 4. Effect of solvent extracts of cell free filtrate from *Bacillus* species at different concentrations on colonization of *R. solani* in okra. See figure 1 for treatment design.

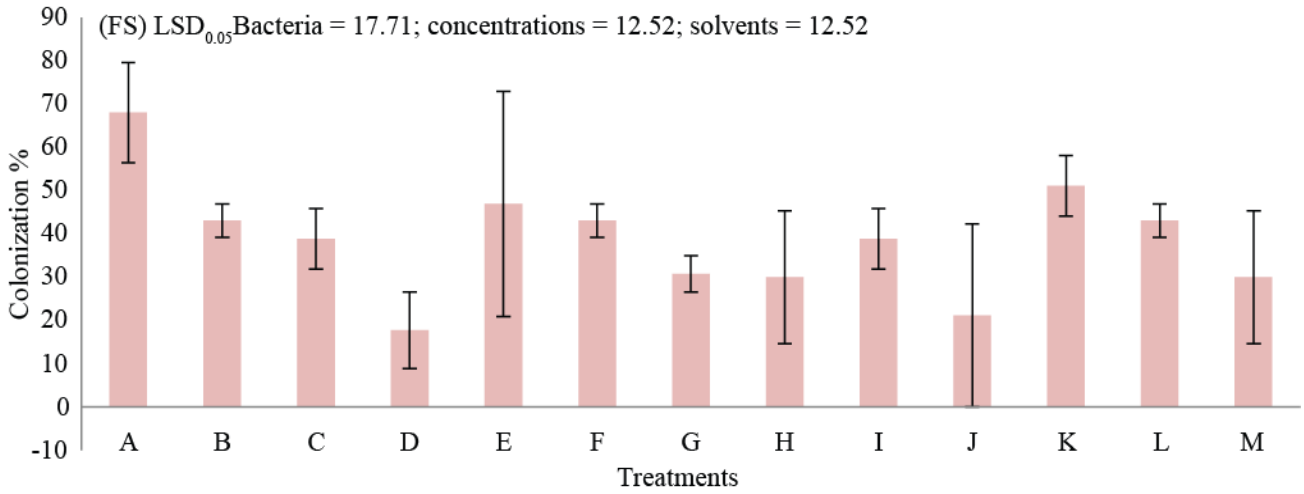

Fig. 5. Effect of solvent extracts of cell free filtrate from *Bacillus* species at different concentrations on colonization of *Fusarium* spp., in okra. See Figure 1 for treatment design.

Discussion

Two solvent extracts (hexane and ethyl acetate) of cell free filtrates from three *Bacillus* species (KUB-15, KUB-20, KUB-27) when applied to seeds revealed the improvement of plant height in all treatments compared to controls. Most significant increase in plant growth was noticed in plants treated with ethyl acetate extract of KUB-20 at 1000 ppm. It was also observed that ethyl acetate extracts improved plant height significantly compared to hexane extracts, further higher concentrations of both the solvents gave better results. *Bacillus* species are able to produce a variety of bioactive metabolites (Bacon *et al*., 2015) for many biotechnological applications, some of which are potentially plant growth promoting compounds. For instance, *B. subtilis* 155 produces extracellular water soluble antibiotics, antifungal proteins, and volatile organic compounds that may be associated with induction of plant resistance (Leelasuphakul *et al*., 2008). Al-Saraireh *et al.,* (2015) observed active antibacterial activity of *Bacillus* sp. 7B1 against gram positive bacteria namely *Bacillus subtilis, Micrococcus luteus* and *Staphylococcus aureus* which is due to production of large number of antimicrobial peptides having different chemical structures. Some volatile organic compounds of *B. subtilis* have been associated with increased plant growth and the induction of plant systemic resistance mechanisms (Compant *et al*., 2005; Tahir *et al*., 2017). Ethyl acetate extracts of cell free filtrates from *Bacillus* and *Pseudomonas* species when applied as seed treatment enhanced flavonoids in roots, which might be an additional factor in nodule promotion by the rhizobacteria. Further, bacteria also produce some fluorescent compounds similar to those of plant flavonoids (Parmar & Dadarwal., 1999). When compared to control all the treatments showed lesser RK nematode parasitism. Least RK nematode parasitism was observed in plants treated with ethyl acetate extract of *B. coagulans* (KUB-20) at 1000 ppm. KUB-20 extracts of both organic solvent (ethyl acetate and hexane 1000 ppm) when applied on seeds showed lowest infections of *M. phaseolina* and *Fusarium* species. Lowest infection of *R. solani* revealed by the treatment with KUB-27ethyl acetate extract at 1000 ppm. Higher concentration of both solvent extract showed better results as compared to lower concentrations. Lower concentrations showed greater parasitism of nematodes and infection of root-rot fungi compared to higher concentration of both the solvents. Further, the ethyl acetate extract compared to hexane extract, showed decreased nematode parasitism. Previously, *B. firmus* showed systemic nematicidal and biocontrol activity against *Meloidogyne incognita* in pot experiments on tomato (Xiong *et al*., 2015). Secondary metabolites produced by *Bacillus* species are ideal chemical products for biological control of phytopathogens (Sharga & Lyon 1998; Brader *et al*., 2014; Oliveira *et al.,* 2014). Crude organic solvent extracts (diethyl ether and ethyl acetate) of *Bacillus amyloliquefaciens* inhibited the growth of pathogenic microorganisms (Bhoonobtong *et al*., 2012). Different solvent extracts including ethyl acetate, hexane and chloroform from *Pseudomonas* species significantly induced J2 mortality of RK nematodes, additionally, ethyl acetate extract caused highest mortality among all the organic solvents used (Ali *et al*., 2002). Bioactive secondary metabolites of *B. firmus* produced during fermentation, significantly reduced egg hatch of *M. incognita* and showed significant rates of paralysis and mortality of nematodes (Mendozaa *et al*., 2008). *B. subtilis* produced volatile compounds causing up to 70% inhibition of fungal growth, furthermore, ethanol extract of cell free filtrate referred to as secondary metabolite caused inhibitory effect on mycelial growth and spore germination of *Penicillium digitatum* pathogen, a cause of citrus fruit rot disease (Leelasuphakul *etal*., 2008). Some studies also reported the antifungal activities of proteins and ethanol extracts against *R. solani* (Kavitha *et al*., 2005). Lipopeptide antibiotics of *B. subtilis* effectively suppressed the growth of plant pathogenic fungi including *Fusarium* and *Rhizoctonia* species (Nagorska *et al*., 2007).

Conclusion

Bacillus species are well known rhizobacteria for their potential to synthesize metabolites for commercial importance. Secondary metabolites of *Bacillus cereus* (KUB-15), *B. coagulans* (KUB-20) and *B. cereus* (KUB-

27) showed improved plant growth at higher tested concentration. Ethyl acetate extract of KUB-20 at 1000 ppm concentration greatly reduced nematode parasitism. Similarly, ethyl acetate extracts of KUB-20 and KUB-27 revealed lowest colonization of root-infecting fungi in okra roots.

References

Abad, P., B. Favery, M. Rosso and P. Castagnone-Sereno. 2003. Root- knot nematode parasitism and host response: molecular basis of a sophisticated interaction. *Mol. Plant Pathol.,* 4: 685 217–224.

Abbasi, M.W., M.J. Zaki and M. Anis. 2013. Application of *Bacillus* species cultured on different low cost organic substrates for the control of root-knot nematode infection on okra (*Abelmoschus esculentus* Moench). *Pak. J. Bot.*, 45(3): 1079-1084.

Abbasi, M.W., N. Ahmed, M.J. Zaki, S.S. Shuakat and D. Khan. 2014. Potential of *Bacillus* species against *Meloidogyne javanica* parasitizing eggplant (*Solanum melongena* L.) and induced biochemical changes. *Plant & Soil*, 375: 159-173.

Ali, N.I., I.A. Siddiqui, S.S. Shaukat and M.J. Zaki. 2002. Nematicidal activity of some strains of *Pseudomonas* spp. *Soil Biol. & Biochem.*, 34(8): 1051-1058.

Al-Saraireh, H., W.A. Al-Zereini and K.A. Tarawneh. 2015. Antimicrobial activity of secondary metabolites from a soil *Bacillus* sp. 7B1 isolated from South Al-Karak, Jordan. *Jordan J. Biol. Sci.,* 8(2): 127-132.

Back, M.A., P.P.J. Haydock and P. Jenkinson. 2002. Disease complexes involving plant parasitic nematodes and soil-borne pathogens. *Plant Pathol.*, 51: 683-697.

Bacon, C.W., E.R. Palencia and D. M. Hinton. 2015. Abiotic and biotic plant stress-tolerant and beneficial secondary metabolites produced by endophytic *Bacillus* species. In: *Plant Microbes Symbiosis: Applied Facets*. Springer India, pp. 163-177.

Bhoonobtong, A., S. Sawadsitang, S. Sodngam and W. Mongkolthanaruk. 2012. *Characterization of endophytic bacteria, Bacillus amyloliquefaciens for antimicrobial agents production*. Proceedings of 2012 International Conference on Biological and Life Science (ICBLS), 23-24th July, Singapore.

Brader, G., S. Compant, B. Mitter, F. Trognitz and A. Sessitsch. 2014. Metabolic potential of endophytic bacteria. *Curr. Opinion in Biotechnol.*, 27: 30-37.

Compant, S., B. Duffy, J. Nowak, C. Clement and E. Barka. 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. *App. & Environ. Microbiol.*, 71: 4951–4959.

Dhingra, O.D. and J.B. Sinclair. 1978. *Biology and Pathology of Macrophomina phaseolina*. Imprensa Universitaria, Universidade Federal Devicosa, Vicosa-Minas Gerais-Brasil, pp. 166.

Falardeau, J., C. Wise, L. Novitsky and T.J. Avis. 2013. Ecological and mechanistic insights into the direct and indirect antimicrobial properties of *Bacillus subtilis* lipopeptides on plant pathogens. *J. Chem. Ecol.*, 39(7): 869-878.

Goswami, D., J.N. Thakker and P.C. Dhandhukia. 2015. Simultaneous detection and quantification of indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA) produced by rhizobacteria from l-tryptophan (Trp) using HPTLC. *J. Microbiol. Methods.*, 110: 7-14.

Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staaley and S.T. Williams. 1994. *Bergey's manual of determinative bacteriology, 9th Edition*. The Williams and Wilkins Company, Baltimore.

Hussey, R.S. and K.R. Barker. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Disease Reporter,* 57: 1025-1028.

Kavitha, S., S. Senthilkumar, S. Gnanamanickam, M. Inayathullah and R. Jayakumar. 2005. Isolation and partial characterization of antifungal protein from *Bacillus polymyxa*strain VLB16. *Process Biochem.*, 40: 3236-3243.

Lagopodi, A.L., A.F.J. Ram, G.E.M. Lamers, P.J. Punt, C.A.M.J.J. Van den Hondel, B.J.J. Lugtenberg and G.V. Bloemberg. 2002. Novel aspects of tomato root colonization and infection by *Fusarium oxysporum* f. sp. *radicis-lycopersici* revealed by confocal laser scanning microscopic analysis using the green fluorescent protein as a marker. *Molecular Plant-Microbe Interaction*, 15(2): 172-179.

Leelasuphakul, W., P. Hemmaneea and S. Chuenchitt. 2008. Growth inhibitory properties of *Bacillus subtilis* strains and their metabolites against the green mold pathogen (*Penicillium digitatum* Sacc.) of citrus fruit. *Postharvest Biol. & Technol.,* 48: 113-121.

Liu, B., H. Qiao, L. Huang, H. Buchenauer, Q. Han, Z. Kang and Y. Gong. 2009. Biological control of take-all in wheat by endophytic *Bacillus subtilis* E1R-j and potential mode of action. *Biological Control*, 49: 277-285.

Liu, J., I. Hagberg, L. Novitsky, H. Hadj-Moussa and T.J. Avis. 2014. Interaction of antimicrobial cyclic lipopeptides from *Bacillus subtilis* influences their effect on spore germination and membrane permeability in fungal plant pathogens. *Fungal Biol.*, 118(11): 855-861.

Mannanov, R.N. and R.K. Sattarova. 2001. Antibiotics produced by *Bacillus* bacteria. *Chemistry of Natural Compounds,* 37: 117-123.

Mendozaa, A.R., S. Kiewnickb and R.A. Sikora. 2008. *In vitro* activity of *Bacillus firmus* against the burrowing nematode *Radopholus similis*, the root-knot nematode *Meloidogyne incognita* and the stem nematode *Ditylenchus dipsaci*. *Biocontrol Sci. & Technol.*, 18(4): 377-389.

Nagorska, K., M. Bikowski and M. Obuchowski. 2007. Multicellular behavior and production of a wide variety of toxic substances support usage of *Bacillus subtilis* as a powerful biocontrol agent. *Acta Biochimica Polonica,* 54: 495-508.

Newsham, K.K., A.H. Fitter and A.R. Watkinson. 1995. Arbuscular mycorrhiza protect an annual grass from root pathogenic fungi in the field. *J. Ecol.*, 83: 991-1000.

Oliveira, D.F., H.M.D.S. Junior, A.S. Nunes, V.P. Campos, R.S.C. De Pinho and G.C. Gajo. 2014. Purification and identification of metabolites produced by *Bacillus cereus* and *B. subtilis* active against *Meloidogyne exigua,* and their *in silico* interaction with a putative phosphoribosyI transferase from *M. incognita. Anals of the Brazilian Academy of Sciences,* 86(2): 525-538.

Ongena, M. and P. Jacques. 2007. *Bacillus lipopeptides*: versatile weapons for plant disease biocontrol. *Trends in Microbiology,* 16: 115-125.

Parmar, N. and K.R. Dadarwal. 1999. Stimulation of nitrogen fixation and induction of flavonoid like compounds by rhizobacteria. *J. App. Microbiol.,* 86: 36-44.

Saharan, B.S. and V. Nehra. 2011. Plant Growth Promoting Rhizobacteria: A Critical Review. *Life Sciences and Medicine Research* (LSMR), 21:1-30.

Sasser, J.N. and D.W. Freckman. 1987. A world prospective on nematology: the role of the society, *In: Vistas on Nematology*, edited by J.A. Veech and D.W. Dickson. Society of Nematologists, Hyattsville, MD. pp. 7–14.

Selvadurai, E.L., A.E. Brown, J.T.G. Hamilton. 1991. Production of indole-3-acetic acid analogues by strains of *Bacillus cereus* in relation to their influence on seedling development. *Soil Biol. & Biochem.*, 23: 401-403.

Sharga, B.M. and G.D. Lyon. 1998. *Bacillus subtilis* BS 107 as an antagonist of potato blackleg and soft rot bacteria. *Can. J. Microbiol.*, 44: 777-783.

Sheikh, A.H. and A. Ghaffar. 1975. Population study of the sclerotia of *Macrophomina phaseolina* in cotton fields. *Pak. J. Bot.*, 7: 13-17.

Tahir, H.A.S., Q. Gu, H. Wu, W. Raza, A. Hanif, L. Wu, M.V. Colman and X. Gao. 2017. Plant growth promotion by volatile organic compounds produced by *Bacillus subtilis* SYST2. *Front*. *Microbiol.*, 8:171.

Taylor, D.P. and C. Netscher. 1974. An improved technique for preparing perennial pattern of *Meloidogyne* spp. *Nematologica,* 20: 268-286.

Torres, M. J., C.P. Brandan, D.C. Sabaté, G. Petroselli, R. Erra-Balsells and M.C. Audisio. 2017. Biological activity of the lipopeptide-producing *Bacillus amyloliquefaciens* PGPBacCA1 on common bean *Phaseolus vulgaris* L. pathogens. *Biol. Cont.*, 105: 93-99.

Wilhelm, S. 1955. Longevity of the *Verticillium* wilt fungus in the laboratory and field. *Phytopathology*, 45: 180-181

Williamson, V.M. and R.S. Hussey. 1996. Nematode pathogenesis and resistance in plants. *Plant Cell,* 8*:* 1735-1745.

Xiong, J., Q. Zhou, H. Luo, L. Xia, L. Li, M. Sun and Z. Yu. 2015. Systemic nematicidal activity and biocontrol efficacy of *Bacillus firmus* against the root-knot nematode *Meloidogyne incognita*. *World J. Microbiol. & Biotechnol.*, 31(4): 661-667.

Yánez-Mendizábal, V., H. Zeriouh, I. Viñas, R. Torres, J. Usall, A. de Vicente, A.P. García and N. Teixidó. 2012. Biological control of peach brown rot (*Monilinia* spp.) by *Bacillus subtilis* CPA-8 is based on production of fengycin-like lipopeptides. *European J. Plant Pathol.*, 132: 609-619.

Zar, J.H. 2009.*Biostatistical Analysis*, 5th edn. Prentice-Hall, Englewoods Cliff.

Zeriouh, H., A. Vicente, A. Pérez- García and D. Romero. 2014. Surfactin triggers biofilm formation of *Bacillus subtilis* in melon phylloplane and contributes to the biocontrol activity. *Environ. Microbiol.*, 16(7): 2196-2211.

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