AWARDS AND HONOURS

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Seed bio-priming in the management of seed- and soil-borne diseases*

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ABSTRACT: The use of pesticides in agriculture is restricted due to environmental and health hazards. However, pests need to be managed to harvest healthy crops. Environmental Protection Agency (EPA) in the developed countries encourages the management of the plant diseases and pests through biological control by using the appropriate, efficient and quality bioagents. The bioagents viz., *Trichoderma harzianum*, *T. viride*, *T. virens*, *T. atroviride*, *T. koningii*, *Paecilomyces lilacinus* and *Pseudomonas fluorescens*, etc have been proved effective for the management of the seed- and soil borne plant pathogens/ diseases. Different workers have worked out the mode of action of the bioagents. Molecular studies have shown that different genes in the bioagents are responsible for their efficiency to manage the pathogens and enhancing the plant growth. The bioagent like *T. asperellum* has been found to be endophyte in the plant system and perform very well for all these characters. Therefore, a large number of rhizospheric bioagent should be isolated and studied for useful characters of eco-friendly management of biotic and abiotic stresses. The method of use of the bioagents in plant health management should also be studied thoroughly, as seed bio-priming of the bioagents particularly *Trichoderma* spp. have been found quite effective to manage the plant pathogens and enhancing germination, seedling vigour, plant growth, etc. In the wake of climate change, the bioagents are the hope to overcome the biotic and abiotic stresses and ecofriendly and sustainable management of diseases and plant health. An attempt has been made to summarize the work carried out on seed priming by a different method in general and seed bio-priming in particular and has been presented in this article.

Keywords: Bioagents, chitinases, seed bio-priming, seed priming, *Trichoderma* spp., *T. asperellum*

Biological control of plant diseases involves the use of organism(s) to inhibit the pathogen and reduce disease. The fungal bioagents viz., *Trichoderma asperellum*, *T. harzianum*, *T. viride*, *T. virens*, *T. atroviride*, *T. koningii* etc. occupy the major share of fungal biocontrol agents in biopesticide industry (Sharma *et al.*, 2014; Woo *et al.*, 2014) and bacterial bioagents viz. *Pseudomonas fluorescens*, *P. putida*, *Enterobacter cloacae*, *Bacillus subtilis*, etc. (Chaur-Tsuen, 1998) are doing very well in the management of biotic as well as abiotic stresses viz., plant pathogens, salinity, drought, etc.

Mechanisms of biocontrol

The mechanisms of biocontrol have been discussed in several papers and books (Adams, 1990; Chaur-Tsuen, 1998; Fravel, 1988; Ghisalberti and Sivasithamparam, 1991; Handelsman and Parke, 1989; Kessmann *et al.*, 1994). The bioagents mechanisms mainly include mycoparasitism, antibiosis, competition, cell wall

degrading enzymes and induced resistance. *Trichoderma* spp. attack target fungi due to chitinases, glucanases, proteases and N acetyl β -D-glucosaminidase (Harman *et al.*, 2004). The bioagents produce low molecular weight diffusible compounds i.e. antibiotic e.g. phenazine-1-carboxylic acid, anthranilic acid, pyoluteorin, gliotoxin, gliovirin, akyl pyrones, peptaibol having antimicrobial properties which restrain the growth of the pathogens (Wiest *et al.*, 2002). *Trichoderma* spp. are having the high competitive saprophytic ability and best colonizer of the rhizosphere ecosystem, besides producing siderophores, therefore, efficiently utilize the available nutrients from the soil leading to starvation of the other fungi (Benitez *et al.*, 2004). Besides, *Trichoderma* spp. produces hydrolytic enzymes viz., cellulases, glucanases, pectinases, cutinases, chitinases, proteases and lipases which play a key role in the degradation of cell wall components of plants and the fungi (Woo and Lorito, 2007). The bioagents induces systemic resistance in plants as they associate with plant roots and produce the compounds that stimulate growth and plant defense mechanism.

Chitinases: Endophytic strains of *T. asperellum*, *T. viride*, *T. harzianum*, etc are reported to harbours highest

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number of chitinases (Seidl, 2008). Chitinases of these species has so many advantages over the chitinase present in plants, insects, bacteria and others, as they are antagonistic and having the nutritional role too and act upon both the soft, *i.e.* hyphal tip and hard structures *viz.*, mature hypha, chitin, conidia, chlamydospore and sclerotia. The chitinases are having high antifungal activity over a wide range of pathogens and are non toxic to the hosts *i.e.* plants even at high concentrations. Attempts have been made to develop transgenic plants of tobacco, potato, cotton, tomato, having endo-chitinase gene (*ech42/chit42*) from different - *Trichoderma* spp. (Emani *et al.*, 2003; Saiprasad *et al.*, 2009; Shah *et al.*, 2010).

Seed treatment and seed priming with bioagents

Seed-applied bioagents can be considered either as a direct alternative to chemical seed treatment or as part of an integrated system, combining both microorganisms and chemical pesticides. The various mechanisms of seed priming have been found quite beneficial for the management of the biotic and the abiotic stresses. It is well accepted fact that priming improves germination, reduces seedling emergence time and improves plant stand establishment (Javid *et al.*, 2013; Yadav *et al.*, 2013). Seed priming is an ancient technique of quick and uniform seed germination. It plays an important role in increasing the yield of different crops in relation to enhancing 37, 40, 70, 22, 31, 56, and 20.6% yield in wheat, barley, rice, maize, sorghum, pearl millet and chick pea, respectively (Harris *et al.*, 2005).

Germination of seeds has three distinct phases *viz.*, Phase I: seed imbibitions *i.e.* hydration process related to passive imbibition of dry tissues associated with water movement first occurring in the apoplastic spaces; Phase II: Metabolism activation phase associated with the re-establishment of metabolic activities *i.e.* energy metabolism, regulation of oxidative status, DNA repair, cell cycle activation, reserve mobilization, modification of hormonal status and repairing processes at the cell level; and Phase III: initiation of growing processes associated with cell elongation and leading to radical protrusion. Phases I and III both involve an increase in the water content while hydration remains stable during Phase II. It is commonly considered that before the end of Phase II, germination remains a reversible process: the seeds may be dried again and remain alive during storage and able to subsequently re-initiate germination under favorable conditions (Stanley *et al.*, 2016). Seed-priming is a pre sowing treatment of seeds in an osmotic solution that allows them to imbibe water. During this phase, if the bioagents are subjected to the germinating seeds, it facilitates the bioagents to establish well with the seedlings in improving the health of plants.

Seed-priming *i.e.* halopriming refers to soaking of seeds in the solution of inorganic salts *i.e.* NaCl, KNO₃, CaCl₂, CaSO₄, etc. A number of studies have shown a significant improvement in seed germination, seedling emergence and establishment, and final crop yield in salt affected soils in response to halopriming. In osmopriming or osmotic conditioning, seeds are soaked

for a certain period in solutions of sugar, polyethylene glycol (PEG), glycerol, sorbitol, or mannitol or inorganic salts NaCl, NaNO₃, MnSO₄, MgCl₂, K₃PO₄, followed by air drying before sowing. Hormonal priming is the pre-seed treatment with different hormones *i.e.* salicylic acid, ascorbate, kinetin, etc. which promote the growth and development of the seedlings. Solid matrix priming using vermiculite, diatomaceous earth, clay beads; hydropriming involves soaking the seeds in water before sowing to hydrate seeds to the desired moisture of 25-30% and may or may not be followed by air-drying of the seeds (Pill and Necker, 2001). Matrix priming, osmopriming, and hydropriming methods have all been employed to increase beneficial microbial populations on the seed (Harman and Taylor, 1988; Ramón *et al.*, 2015).

Bio-priming and its benefit over dry seed treatment

It involves seed imbibition together with bioagents inoculation of seed (Callan *et al.*, 1990). It is the addition of beneficial rhizosphere microorganisms in the priming process, either as a method for efficient delivery to the crop rhizosphere or to control pathogen (Callan *et al.*, 1997; Jensen *et al.*, 2004). Seed bio-priming involves seed coating with a myriad of beneficial agriculturally important microbes leading to rapid and uniform seed colonization. When seeds of oilseed rape were bio-primed with *P. chlororaphis* in different bio-priming solutions for four hours, maximum CFU per seed was observed for MgSO₄ solution. Bio-primed seeds of pea with *T. asperellum* BHUT8 isolate were evaluated for plant growth promotion effect (Singh *et al.*, 2016). The results showed enhancement in plant growth in the treated plants as compared to control. There was an increase in shoot length, root length, the number of leaves, shoot fresh weight, root fresh weight, shoot dry weight and root dry weight by 35.29, 96.49, 28.13, 36.10, 146.26, 30.17 and 77.20%, respectively, as compared to the control. Bio-priming of sunflower seeds and hot pepper seeds with bioagents significantly reduced *Colletotrichum capsici* and *Alternaria* blight (Rao *et al.*, 2009). Similarly, bio-priming of carrot seeds, naturally infected with *Alternaria radicans*, *A. dauci* and *A. alternata*, with *Chlonostachys rosea* IK726 at 15°C, significantly reduced the occurrence of this spp. (Jensen *et al.*, 2004). Different strains of *P. fluorescens* as seed bio-priming was found effective for the control of pre-emergence damping-off, of *sh2* sweet corn, caused by *Pythium ultimum* (Callan *et al.*, 1990). Seed bio-priming of *B. subtilis* significantly reduced pre-emergence damping-off caused by *Fusarium solani*, *Rhizoctonia solani* and *Sclerotium rolfsii* in pea as compared to seed coating with same bacteria and fungicide treatment. Combined application of *Rhizobium* spp. and *Pseudomonas* spp. as seed priming significantly reduced collar rot (*S. rolfsii*) in chickpea. Seed bio-priming of *T. viride* along with soil application of FYM enriched *T. viride* @ 100g/m² resulted in better seedling emergence (97.0%) and highest vigour index (3496.0), followed by seed biopriming application of *T. viride* alone with seedling emergence (96.0%) and high vigour index of 3235.2 (Jaisani *et al.*, 2016).

Mass production of *Trichoderma* spp. through liquid fermentation: *Trichoderma* spp. are multiplied on sorghum grains and through liquid fermentation in PDB or any other suitable media. There are advantages of liquid fermentation as it is quick in the duration time of production and volume of the product, besides adding enzymes and antibiotic in culture media. Therefore, protocols for mass production of *Trichoderma* in Potato Dextrose Broth (PDB) has been developed using 20 litres vessel of laboratory scale Cleaver Scientific Fermentor with pre-standardized parameters viz. aeration @ 1vvm, agitation @ 140 RPM, pH 7.0 and temperature of 28°C. The multiplied mass culture was kept for 12 hours in pre-sterilized vessels having PDB medium in BOD incubator at $28 \pm 2^\circ\text{C}$ for sporulation. Entire liquid fermented biomass of the bioagent was mixed with 300 mesh talc powder in a blender of packing machine. Talc-based formulation of the bioagent was prepared by mixing the fermented *Trichoderma* biomass with double the quantity of talc and shade dried. Thereafter, 0.1% carboxy methyl cellulose powder was added as sticker to enhance the shelf life of *Trichoderma*. Colony forming units (cfu)/g of the bioagents in the formulation was determined by serial dilution method. This formulation was used for the evaluation of the efficacy of the bioagents.

Seed bio-priming and soil application of bioagents in the management of the diseases of pulse crops

Soybean root rot: Application of *T. viride* or *T. harzianum* (2×10^8 cfu/g - 1% WP) enriched FYM (10 kg bioagent/ton FYM) in furrow @ 1 ton/ha, followed by seed treatment with *T. viride* or *T. harzianum* (2×10^8 cfu/g) @ 10 g/kg seeds, respectively, at the time of sowing have been found effective for the management of charcoal rot of soybean (Pandey and Gohel, 2017). Significantly lowest incidence of charcoal rot (4.83%) and highest seed germination (95.98%), shoot length (11.30 cm), root length (8.86 cm), vigour index (1587) and grain yield (2233 kg/ha) was obtained in seed treatment with *T. viride*, which was at par with *T. harzianum* having low incidence of charcoal rot (5.21%) and higher seed germination (95.03%), shoot length (11.30 cm), root length (8.76 cm), vigour index (1552) and grain yield (2106 kg/ha). Whereas, check with *M. phaseolina* and *R. solani* and untreated check recorded highest incidence of charcoal rot i.e. 30.43, 28.85 and 26.50% with lowest grain yield of 1433, 1357 and 1457 kg/ha, respectively.

Bio control agents *T. harzianum*, *T. virens*, *T. atroviride* and *Pseudomonas fluorescens* were applied on soybean seed and their impact on seedling was evaluated (Entesari *et al.*, 2013). The bioagents increased seedling length, root length by 52% and 44% and their dry weight by 55 and 61% and total chlorophyll to 206% respectively, (compared with non-inoculated controls). *T. virens* As19-1(T.v7) increased Fe uptake to 77% and UTPF5 increased Zn uptake (90mg/kg), N (7.5%) and total protein (47.1 mg/kg) in comparison with other treatments and control.

Chickpea root rot: Biopriming of chickpea seeds were done by treating the seeds with the suspension of talc

based formulation of *T. viride*, *T. harzianum*, *T. virens* and *P. fluorescens* (2×10^8 cfu/g) @ 50 g in 250 ml of water/kg of seed for 10 hrs. The bio-primed seeds were shade dried and sown in the field. Simultaneously, the soil application of bioagents enriched FYM (10 kg/ 1 ton FYM) @ 1 ton/ha) was done. The pooled data of 2013-14 and 2014-15 revealed significantly lowest incidence of wilt and root rot (8.59%) and highest seed germination (96.69%), vigour index (2734) and grain yield (1535 kg/ha) in the seed bio-priming + soil application of *T. viride* enriched FYM, which was at par with *T. harzianum* having low incidence of wilt and root rot (9.78%) and higher seed germination (94.34%), vigour index (2552) and grain yield (1466 kg/ha) as compared to untreated check, which recorded highest incidence of wilt and root rot (38.53%) and lowest grain yield of 1117 kg/ha (Pandey *et al.*, 2017).

Green gram root rot: Seed bio- priming of green gram with bioagents i.e. *T. viride*, *T. harzianum*, *T. virens* and *P. fluorescens* were done individually and in combination for evaluating their efficacy against root rot and blight caused by *M. phaseolina* and *R. solani*. Combination of *T. viride* and *P. fluorescens* promoted seed germination (99.44% and 95.27%), vigour index (2832.91 and 1417.2), shoot length (18.93 and 11.67 cm), root length (14.13 and 6.63 cm), in rolled paper towel method and under sick soil with pathogen and fresh shoot weight (2.28 g), dry shoot weight (0.37g), plant height (27.93 cm) under sick soil with pathogen and found most effective in reducing root rot incidence (78.63%) of green gram as against control which exhibited vigour index (791.93 and 453.7) shoot length (8.43 and 5.83 cm), root length (5.77 and 2.13 cm), in rolled paper towel method and under sick soil with pathogen and fresh shoot weight (1.110 g), dry shoot weight (0.20 g), plant height (13.50 cm) under sick soil with pathogen, respectively (Meena *et al.*, 2016). The activity of defense-related enzymes viz., peroxidase, polyphenol oxidase, Phenylalanine ammonia lyase (PAL) was significantly greater in this treatment. Phenol content, total soluble sugar and protein contents also increased in the leaves of the plant under this treatment.

Fababean root rot: Faba bean seeds primed with *T. viride*, *B. subtilis*, *P. fluorescens* suffered less by root rot pathogens. Solid matrix biopriming of pea seeds with different strains of *T. harzianum* significantly increased final plant stand in sick soil with *P. ultimum*.

During the coating process, the spores are physiologically activated because of the presence of water and subsequently dried to return to a dry quiescent state. This adversely affects the viability of cfu's. Biopriming *T. atroviride* Cs2-1(T.a9) in combination with bran, enhanced the activity of glutathione reductase to 157% as compared to control.

Future research needs

Biological control agents as seed treatments are being actively developed by a number of companies across many countries. These products may provide a good solution for growers, who are working toward the organic

status of their crop. Therefore, there is the need for identification of efficient and economical priming agent for particular seed and bio-agent. The protocols for bio-priming of different seeds, for the control of seed and soil-borne diseases, should be developed. Researches should be carried out at optimum temperature and humidity required for the storage of primed seed and competent strains of bioagents for bio-priming should be identified.

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