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REVIEW

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Utilization of biopesticides as sustainable solutions for management of pests in legume crops: achievements and prospects

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Abstract

Grain legumes remain important to meet the projected targets relating to food and nutritional security worldwide. The complementation of cereal-based food with grain legumes is a vegetarian diet with high-quality protein. However, the performance of these crops is severely influenced by a number of biotic and abiotic stresses, of which pests and pathogens remain the crucial affecting plants at different growth stages. Chemical pesticides are mainly employed across the world for management of pests and pathogens. The risk associated with the environmental pollution and health hazards to man, plants, domestic animals, and wild life makes these pesticides ecologically unacceptable. Also, major damage caused by pests of grain legumes are systemic in nature, and their management through chemicals often yields unsatisfactory outcome. This has led to increasing shift in the attention of scientific community towards eco-friendly and safer technologies for pest management in legumes. Sustainable protection demands implementation of strategies that rely upon biological control agents (BCAs) and their formulations. In recent years, such formulations have been promoted to mitigate the pest problem and improving crop yield. This review presents an updated summary on BCAs including the present status of BCA application, mode of actions, and delivery systems under controlled and field conditions to address major pest problems on legume crops.

Keywords: Legumes, Pest management, Biological control agents, Bioformulations, Ecological impacts

Background

The legume family is represented by nearly 400 genera and 10,000 species which harbors various pulse crops including chickpea (*Cicer arietinum* L.), pigeon pea (*Cajanus cajan* L.), field pea (*Pisum sativum* L.), lentil (*Lens esculenta* L.), green gram (*Vigna radiata* L.), black gram (*Vigna mungo*L.), cowpea (*Vigna ungiculata* L.), faba bean (*Vicia faba*), lathyrus (*Lathyrus sativus*), and rajmash (*Phaseolus vulgaris* L.), which are grown for both seeds and grains in different parts of the world (http://www.botany.hawaii.edu/faculty/carr/fab.htm).

Capable of releasing nitrogen into the soil, these crops are cultivated to obtain dry seeds that are rich in proteins and important minerals and micronutrients (Bohra et al., 2015, Nene, 2006). Frequent attacks by pathogens, insect pests, and nematodes cause impairments to the plants at various stages of crop growth. The average annual loss in legumes due to pests and pathogens was estimated to be up to 20% (Dhaliwal et al., 2010). In other words, the efforts dedicated to protect the crop may witness substantial increment in food legume production. Up to 100% losses have been reported in various legume crops in Asia and Africa in case of the conditions that favor diseases and pests (Vijay et al., 2015). In India, a considerable extent of yield in pigeon pea and chickpea is lost due to pod borer [*Helicoverpa armigera* (Hubner)]. In pigeon pea, spotted pod borer [*Maruca vitrata* (Fabricius)] remains the second most important pest causing up to 84% reduction in crop yield, amounting to a loss of nearly US$30 million in monitory terms (Margam et al., 2011). Similarly, more than 40% damage to the pods in pigeon pea is caused by pod fly [*Melanagromyza obtusa* (Malloch)] (Singh et al.,

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2013). The other pathogens that plague legume production include wilt, dry root rot, *Phytophthora* blight, collar rot, stem/white rot, *Macrophomina* blight, and yellow vein mosaic virus (YVMV). Losses caused by wilt in legumes may vary from 0 to 100% depending on the crop stage (Pande et al., 2013).

Besides being an expensive affair, the application of hazardous pesticides has detrimental effects on the environment, negatively impacting upon soil fertility and soil microfauna. Also, extended use of chemical pesticides not only often causes to the development of resistance (to pesticides) in insect pests, pathogens, and nematodes but also leads to carcinogenic, teratogenic, and mutagenic effects in human and animals as well. A range of strategies aiming to contain various pests and pathogens of grain legumes are currently available, which includes development of resistant varieties, genetically engineered plants, and use of pesticides and cultural and physical methods.

This review offers an update on the current status of biological control applications especially microbial organisms and their formulations in crops with an emphasis on legume crops. Future prospects of sustainable use of biological control agents (BCAs) to improve performance of legume crops are also discussed.

Status of biological control agent (BCA) research and development

An increase in selection pressure consequent to indiscriminate application of chemical pesticides leads to emergence of pesticide resistance. In such conditions, alternate options of pest/disease management are much sought. During the past two decades, an urgent need was realized for management strategies that are safe vis-a-vis the environment and the land. Farmers are shifting towards eco-friendly technology for the management of pests, i.e., BCAs or BCA-based formulations, referred to as biopesticides. Examples include *Trichoderma* spp., *Pseudomonas* spp., *Bacillus* spp., *Agrobacterium radiobacter*, nonpathogenic *Fusarium spp.*, *Coniothyrium spp.* and *Aspergillus niger*, *Bacillus thuringiensis* (Bt), *Metarhizium* spp., *Beauveria bassiana*, and nuclear polyhedrosis virus (NPVs), which are popularly used in plant protection (Keswani et al. 2015; Mishra et al., 2015). According to a recent report (NAAS, 2013), nearly 1400 BCA products were sold and 175 biopesticide active ingredients and 700 products were registered worldwide for their commercialization. In India, only 15 biopesticides have been registered so far under the Insecticides Act 1968 (Table 1). A growing body of research articles report on the identification and efficacy of different BCAs against a number of pests and pathogens; however, their slow embrace is evident from the fact that only 2% biopesticides are currently used for crop protection worldwide. On the positive side, the usage of BCAs has witnessed an increasing trend (Ranga Rao et al., 2007; Singh et al., 2012). The rise is notably high at international level. Though literature on BCAs is growing worldwide (Fig. 1), the filing of patents on BCA technology is not in sync with the number of publications.

Table 1 Biopesticides registered in India (Anonymous, 2014)

S. no.	Name of the biopesticide	Use for
1.	*Bacillus thuringiensis* var. *israelensis*	Diamondback moths
2.	*Bacillus thuringiensis* var. *kurstaki*	Diamondback moths
3.	*Bacillus thuringiensis* var. *galleriae*	*Helicoverpa armigera*
4.	*Bacillus sphaericus*	Diamondback moths
5.	*B. firmus*	Diamondback moths
6.	*Trichoderma viride*	Root rots and wilts
7.	*Trichoderma harzianum*	Root rots and wilts
8.	*Pseudomonas fluorescens*	Bacterial and fungal pathogen
9.	*Beauveria bassiana*	Mango hoppers and mealy bugs and coffee pod borer
10.	NPV of *Helicoverpa armigera*	*Helicoverpa* on chickpea
11.	NPV of *Spodoptera litura*	*Spodoptera litura*
12.	Neem based biopesticides	Insect white fly
13.	Cymbopogon	Insect
14.	*H. bacteriophora*	Borers
15.	*Trichogramma* parasitoid	Sugarcane borers

Development of a stable and economically viable bioformulation remains central to biological control. Of the various carrier-based formulations available worldwide, alginate pellet- and talc-based formulations of BCAs have emerged as the most important carrier for the application in the management of crop diseases (Lewis et al., 1985). During initial phase of biological control development, it was viewed as an interaction of a single BCA with a pathogen in the rhizosphere (Wilson and Backman, 1999). Still, the majority of the reports on BCA document one target disease. This, however, has yielded inconsistent performance given that a single agent might not remain active in all soil conditions. Integrating microbes in a biological control formulation may serve as more effective management strategy in longer term (Duffy and Weller, 1995). Few biological control formulations based on combination of two or more BCAs are available in the market. Notwithstanding their significant role in sustainable agriculture, consortia-based formulations have not received adequate attention. Production of BCAs for sustainable agriculture relies on cost-effectiveness of the procedure and viability of a potential strain. Further, enabling mass production with high level of microbial count and viability also assumes greater significance.

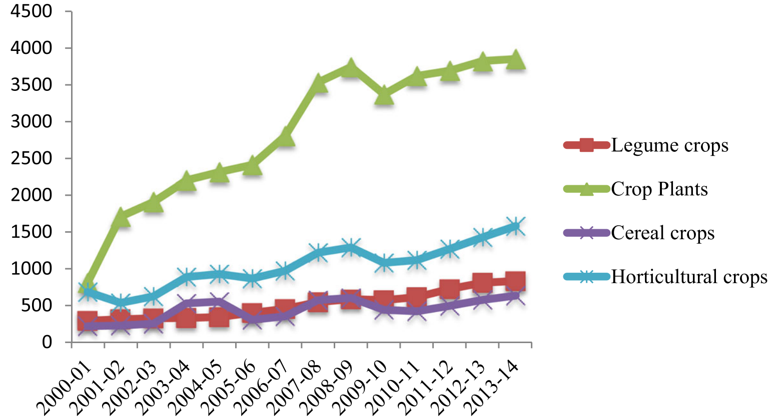

Fig. 1 Increasing use of BCAs in different crop plants as reflected in the form of patents and publications (from year 2000 to 2014, a rising trend is seen in the number of patents and publications pertaining to BCAs in plants. The data were obtained by searching the Google Scholar with biological control agents + formulations + diseases + insect + management + crop plants/legume crops as key words)

Mechanism of BCAs

BCAs interact with a variety of factors to control diseases and insect pests of crop plants. Therefore, an improved understanding of the BCA mechanisms can facilitate optimization of control in addition to permitting search for more efficient strains. Also, knowledge about the mechanisms underlying BCA-driven pest management may allow us to select and construct the BCAs with greater effectiveness. Studied by different researchers (Adams, 1990; Lo et al., 1998), these mechanisms encompass (i) antibiosis, (ii) competition, (iii) mycoparasitism/hyperparasitism, and (iv) induced resistance.

Antibiosis

The production of biochemicals by one microorganism that inhibits the growth of other organisms is known as antibiosis. Antibiotics generated by BCAs serve in different ways in order to minimize pests and pathogens. Production of multiple antibiotics probably helps suppress diverse microbial competitors, some of which are likely to be plant pathogens. More recently, *Pseudomonas putida* WCS358r strain was genetically engineered to produce phenazine, and 2,4-diacetylphloroglucinol (DAPG) had greater capacity to control plant diseases in field-grown wheat (Glandorf et al., 2001). Antibiotics thus produced generally are nonpolar/volatile, polar/nonvolatile, and water soluble. Among these, the greater effectiveness of volatile antibiotics can be accounted to their ability to serve at the sites away from the site of production. Table 2 enlists antibiotics produced by the BCAs that suppress the activity of plant pathogens. Delivered through sprays or expressed in plant, *Bacillus thuringiensis* (Bt) crystalline proteins (Cry toxins) manifest insecticidal activities against *H. armigera* (Dhillon and Sharma, 2010). Avermectins, milbemycins, and spinosyns, collectively forming macrocyclic lactones, are derived from culture broths of actinomycetes. Similarly, spinosyns are obtained through fermentation of two species of *Saccharopolyspora* (Herbert, 2010). According to Deshmukh et al. (2010), spinosyns and avermectins, ingredients in insecticides spinosad (45 SC at 0.009%) and emamectin benzoate (5 SC at 0.0015%), respectively, remain highly effective against *H. armigera* population and pod damage in chickpea. Likewise, active rhizosphere colonizers *Trichoderma harzianum* and *Trichoderma virens* produce some cell wall-degrading enzymes (Larito et al., 1976), antibiotics such as gliotoxin and viridin (Tronsmo and Harman, 1992), and also certain biologically active heat-stable metabolites such as ethyl acetate, which inhibit various pathogens present in the soil. Studies have shown that *T. harzianum*, *Trichoderma hamatum*, and *Pseudomonas fluorescence* could be effective BCAs for management of lentil wilt caused by *Fusarium oxysporum* f. sp. *lentis*. Apart from producing antifungal enzymes, it negatively impacts wilt pathogen by posing competition for key nutrients and/or ecological niches (El-Hassan et al., 2013). Researchers have found chitinolytic activity in *Bacillus cereus* 28-9 (Huang et al., 2005) and *Alcaligenes xylosoxidans* (Vaidya et al., 2001). Likewise, chaetominis compound produced by *Chaetomium globosum* had significant role in antibiosis (Di Pietro et al., 1992).

Competition

Soils and plant surfaces constitute nutrient limited environment, thereby putting pressure on a microbe to compete for the available nutrients (Pal and Gardener, 2006). Both BCAs and the pests compete with each another for nutrients and space to establish in the environment. This process involves an indirect interaction between BCAs and pathogens, eventually resulting in pathogen exclusion by means of diminishing food base and physical occupation of site (Lorito et al., 1994). Also, BCAs

Table 2 Antibiotics produced by BCAs for different target pathogens

Source	Antibiotic	Target pathogens	Diseases	References
Pseudomonas fluorescens F113	2,4-diacetyphloroglucinol	*Pythium* spp.	Damping off	Shanahan et al. (1992)
Agrobacterium radiobacter	Agrocin 84	*Agrobacterium tumefaciens*	Crown gall	Kerr (1980)
Bacillus subtilis AU195	Bacillomycin D	*Aspergillus flavus*	Aflatoxin contamination	Moyne et al. (2001)
Bacillus amyloliquefaciens FZB42	Bacillomycin, fengycin	*Fusarium oxysporum*	Wilt	Koumoutsi et al. (2004)
Lysobacter sp. strain SB-K88	Xanthobaccin A	*Aphanomyces cochlioides*	Damping off	Islam et al. (2005)
Trichoderma virens	Gliotoxin	*Rhizoctonia solani*	Root rots	Wilhite et al. (2001)
Pantoea Agglomerans C9-1	Herbicolin	*Erwinia amylovora*	Fire blight	Sandra et al. (2001)
B. subtilis QST713	Iturin A	*Botrytis cinerea* *R. solani*	Damping off	Paulitz and Belanger (2001), Kloepper et al. (2004)
B. subtilis BBG100	Mycosubtilin	*Pythium aphanidermatum*	Damping off	Leclere et al. (2005)
P. fluorescens 2–79 and 30–84	Phenazines	*Gaeumannomyces graminis* var. *tritici*	Take-all	Thomashow et al. (1990)
P. fluorescens Pf-5	Pyoluteorin, Pyrrolnitrin	*Pythium ultimum* *R. solani*	Damping off	Howell and Stipanovic (1980)
Burkholderia cepacia	Pyrrolnitrin, Pseudane	*R. solani* *Pyricularia oryzae*	Damping off and rice blast	Homma et al. (1989)
Bacillus cereus UW85	Zwittermicin A	*Phytophthora medicaginis* *P. aphanidermatum*	Damping off	Smith et al. (1993)
Bacillus thuringiensis	Endotoxin	*Helicoverpa armigera*	Pod borer	Van Rie et al., (1990)

compete for the essential micronutrients such as iron (Fe) and manganese (Mn) especially in highly oxidized and aerated soils. However, the competition for micronutrients exists due to the BCAs having more efficient uptake system for the substances than the pests. While competing with pathogen for physical occupation of site, BCAs reduce or delay the root colonization by the pathogen. Owing to a rapid colonization of root surface of plants by the BCAs, the failure of pests and pathogens to establish on host helps lessening the severity of diseases that affect roots of plants.

Parasitism

Fungi that are parasitic on other fungi are usually referred to as mycoparasites (Baker and Cook, 1974). Parasitism requires the host fungus to be recognized by the mycoparasite followed by production of hydrolytic enzymes and antibiotics. Direct parasitism or lysis of the mycelium of a fungal pathogen by mycoparasitic fungus is known as hyperparasitism. Weindling (1932) observed *Trichoderma lignorum* parasitizing the hyphae of *Rhizoctonia solani*. The mycoparasitism of *Trichoderma* species enabled by appressoria-like structures to penetrate the target fungus cell wall (Chet, 1987) to contributes towards the development of biological control strategies (Harman et al., 2004). A range of pests with different kinds of parasitisms were observed viz., simple-, super-, multiple-, hyper-, auto-, and cleptoparasitisms. Larval parasitoid, *Campoletis chlorideae*, shows simple parasitism on *H. armigera* (Pillai et al., 2016). Similarly, *Maruca obtusa* is parasitized by *Euderus lividus* at larval-pupal stages (Moudgal et al., 2005).

Induced resistance

Induced resistance varying from local to systemic in nature is the most indirect form of antagonism. With regard to systemic acquired resistance, salicylic acid (SA) and non-expresser of pathogenesis-related genes 1 (NPR1) are key players. *Trichoderma harzianum* when inoculated into the root system or on leaves of crops to manage the *Botrytis cinerea* on leaves spatially separated from the site of application of the BCA (Desmukh et al., 2006). While inducing resistance in plants, different kinds of compounds are released by *Trichoderma* spp. into the zone where the interaction occurs. Proteins with enzymatic or other activity constitute the foremost class in this regard. For instance, fungal proteins such as xylanase, cellulases, and swollenins are secreted by *Trichoderma* spp. (Martinez et al., 2001). Similarly, *Trichoderma* endochitinase can also enhance defense, probably through induction of plant defense-related proteins.

Physical thickening of cell walls in plants caused by BCAs contributes to induced resistance via diverse mechanisms that involve lignification, callose deposition and accumulation of antimicrobial low-molecular-weight substances (e.g., phytoalexins), and synthesis of chitinases, glucanases, peroxidases, and other pathogenesis-related (PR) proteins. Several endophytes such as *Bacillus* and *Paenibacillus* have been reported to inhibit soil-borne phytopathogens of pulse crops by production of siderophore, hydrolytic enzymes, antibiotics, and hydrogen cyanide (Senthilkumar et al., 2009). Florescent pseudomonad bacterium produces phenazine (Toohay et al., 1965), phloroglucinol (Howell and Stipanovic, 1980), siderophores (Sakthivel et al., 1986), and pyrrolnitrin (Burkhead and Geoghegan, 1994).

BCAs and their formulations for pest management in legumes

Considerable efforts have been made to manage important pests of legumes by incorporating BCA-colonized natural substrates into the rhizosphere. Bacterial endophytes like *Bacillus*, *Paenibacillus*, and *Pseudomonas* show antifungal activity against major pathogens like *Rhizoctonia solani* (Kuhn.), *Rhizoctonia bataticola* (Taub.) Butler, *Fusarium udum* Butler., *F. oxysporum* f. sp. *cireri* (Padwick) Snyd. & Hans., and *Sclerotium rolfsii* Sacc. infecting pulse crops (Senthilkumar et al., 2009). Among the most pronounced antagonistic fungi, *Trichoderma* species have been extensively investigated as potential BCAs in pulse-based ecosystem. These are demonstrated to be effective against wilt and root rot (root, collar, and stem) diseases conditioned by different *Fusarium* spp., *R. solani*, *R. bataticola*, *Sclerotium rolfsii*, *Sclerotinia sclerotiorum* (Lib.) de Bary, *Phytophthora drechsleri* f. sp. *cajani* Tucker, and *Pythium* spp. in different pulses and other field crops (Chaudhary et al., 2004). The fungi belonging tohyphomycetes such as *Metarhizium anisopliae*, *Beauvaria bassiana*, *Nomuraea*, *Verticillium*, and *Paecilomyces* have been employed as biopesticides in legume crops. In soybean, the application of *Paecilomyces lilacinus*, *Pochonia chlamydosporia*, *Aspergillus nidulans var. dentatus*, and *T. harzianum* at 2 g/kg soil can effectively reduce nematode (*Rotylenchus reniform*) population along with promoting plant growth (Gurjar et al., 2012; Singh and Prasad, 2014). Apart from controlling diseases in pulses, BCAs are also reported to enhance nodulation.

Several isolates of *Trichoderma* spp. have been characterized and evaluated against different fungal pathogens of pulse crops (Dubey et al., 2006; 2007; 2009; 2011; 2012; 2013; Jamali et al., 2004 Chaudhary et al., 2004; Mishra et al., 2015). A list of BCAs that are employed to manage different pests of pulses is given in Table 3. Besides, *B. thuringiensis* is one of the most promising biopesticides used worldwide for managing many lepidopterous pests. According to Roh et al. (2007), more than 100 Bt-based biopesticide formulations have been developed. Nuclear polyhedrosis viruses (NPVs) are specific biopesticides widely used in cotton, chickpea, pigeon pea, maize, groundnut, tomato, sorghum, sunflower, vegetables, and other crops (Pawar and Thombre, 1992).

Researchers at Indian Institute of Pulses Research (IIPR), India, has identified several native potential strains of *Trichoderma* spp. (*T. harzianum*, *T. asperellum*, *T. longibrachiatum*, and *T. reesei*) and plant growth-promoting rhizobacteria (PGPRs) isolated from rhizospheres in major pulse-growing areas in India and evaluated these for their antagonistic potential against a variety of pathogens (Fig. 2a, b). Accordingly, mass production technology has been developed and popularized among the pulse-growing farmers in different agro-ecosystems (Chaudhary et al., 2004; Mishra et al., 2015, 2016).

Types of BCA formulations

BCAs are formulated by several means including dry formulations such as dusts, granules, and microgranules; seed dressing formulations such as powder for seed dressing; dry formulations for dilution in water including dispersible granules and wettable powders; and liquid formulations for dilution in water such as emulsions and suspension concentrates (Knowles, 2005, 2006). Globally, biopesticides are currently available in the market as wettable powder, liquid, and granular formulations (Singh et al., 2012, 2014). In pulses, researchers at IIPR employed 2% wettable powder formulations for seed treatment and soil application (Chaudhary et al., 2004).

Wettable powders and liquids

Generally, the BCAs such as fungal and bacterial species like *Pseudomonas*, *Bacillus*, and *Trichoderma* are applied as seed treatment and seedlings/root dip at the time of sowing (Tronsmo and Dennis, 1983). In pulses, 2% talc-based formulation was found to be effective against wilt and root rot pathogens (Purushottam et al., 2014).

Granular formulations

As reported by Jones et al. (1984), lignite- and vermiculite-based granular formulations were used for management of soil-borne pathogens in different crops. Examples include alginate-based granular formulations of *T. harzianum* that are used to control *R. solani* in various crops.

Delivery system of biological control formulations

Biological control formulations and their consortia are delivered through several means relying primarily on survival nature and mode of infection of the pathogen. These diverse modes of application include seed treatment, soil/foliar application, or through workable combination of different methods. A brief description about these methods is provided here.

Seed treatment

This forms the most effective mode of applying BCAs, particularly to counter soil-borne phytopathogens. The hydration level of seed is controlled through techniques like seed priming which in turn allows the pre-germinative metabolic activities while avoiding the emergence of the radical. Treating pigeon pea and

Table 3 Management of major diseases of legumes by biological control agents

Crops	Diseases	Pathogens	Effective biological control agents
Pigeon pea	Wilt	*Fusarium udum*	*Trichoderma harzianum*, *T. hamatum*, *T. viride*, *T. koningii*, *B. subtilus*
	Phytophthora stem blight	*Phytophthora drechsleri* f. sp. *cajani*	*T. harzianum*, *T. hamatum*, *Glomus mosseae*, *Pseudomonas fluorescens*, *Bacillus subtilis*
	Seed-borne diseases	*Pseudomonas campestris* pv. *vinae*	*T. viride*, *T. harzianum*
Chickpea	Wilt	*Fusarium oxysporum* f. sp. *ciceri*	*Trichoderma harzianum*, *T. viride*, *T. virens*, *B. subtilus*, *A. niger* AN 27
	Root rot	*Rhizoctonia bataticola*	*Trichoderma harzianum*, *T. viride*
	Collar rot	*Sclerotium rolfsii*	*T. viride*, *T. harzianum*, *P. fluorescens*
	Gray mold	*Botrytis cinerea*	*Trichoderma* spp.
	Stem rot	*Sclerotinia sclerotiorum*	*T. harzianum*
Cowpea	Wilt	*Fusarium oxysporum*	*T. harzianum*
	Charcoal rot	*M. phaseolina*, *F. oxysporum f. sp. tracheiphilum*	*T. viride*, *T. harzianum*, *T. koningii*, *T. pseudokoningii*
Lentil	Wilt	*Fusarium oxysporum* f. sp. *lentis*	*T. viride*, *T. harzianum*, *G. virens*, *Pseudomonas fluorescens*
	Root rot	*Macrophomina phaseolina*	*T. viride*, *T. harzianum*, *G. virens*, *P. fluorescens*
Mung bean	Root rot	*M. phaseolina*	*T. harzianum*, *T. viride*
Soybean	Dry root rot	*M. phaseolina*	*T. harzianum*, *T. viride*
Field pea	Rust	*Uromyces fabae*	*Pseudomonas fluorescens*, *P. aeruginosa*, *Bacillus subtilis*
	Powdery mildew	*Erysiphe polygoni* DC	*T. harzianum*, *T. koningii*, *T. longibrachiatum*, *P. fluorescens*, *Bacillus subtilis*
	Root rot	*Rhizoctonia solani*	*T. harzianum*, *T. longibrachiatum*, *P. fluorescens*, *B. subtilis*

chickpea seeds with talc-based formulation of *T. harzianum*, *Trichoderma viride*, *T. hamatum*, *T. virens*, *Bacillus subtilis*, and *Pseudomonas fluorescens* facilitates management of *Fusarium* wilt in both crops (Chet and Baker, 1981; Chand et al., 1991; Kaur and Mukhopadhayay, 1992; Vidhyasekaran et al., 1997; El-Hassan and Gowen, 2006; Khan et al., 2012). On-farm demonstrations have evidenced that the seed treatment with 2% talc-based formulation of *Trichoderma harzianum* (IPT-31) led up to 32.1 and 14.3% decrease in root rot incidence in chickpea and lentil, respectively, thus correspondingly improving crop yield by 16.6 and 12.6% (Purushottam et al., 2014).

Seed priming

Priming of seeds with BCAs is a promising approach to protect seeds from various seed- and soil-borne pathogens. This technique is able to incite changes in plant characteristics apart from facilitating uniform seed germination (Bisen et al., 2015). Likewise, PGPRs also improve germination and seedling establishment. Callan et al. (1990) reported a 10-fold increase in the antagonist population load on the seeds as a result of seed bio-priming using bacterial antagonists, thus protecting rhizosphere from phytopathogen invasion. Priming of field pea seeds with *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, and *Bacillus subtilis* causes nearly 20% decrease in incidence of *Uromyces fabae* under field conditions (Mishra and Pandey, 2010). Raguchander et al. (1998) reported that seed pelting with *B. subtilis* effectively controlled root rot (caused by *Macrophomina phaseolina*) in soybean.

Soil application

Trichoderma powder formulation can be applied into the soil prior to sowing or drenched at initial stages of crop growth. According to Vidhyasekaran and Muthamilan (1995), soil application of peat-based formulation with *P. fluorescens* (Pf1) at 2.5 kg of formulation mixed with 25 kg of well-decomposed farm yard manure improved management of chickpea wilt. Combining *P. fluorescens* with safer fungicides reduced the wilt complex in pigeon pea (Siddiqui et al., 1998). Likewise, seed treatment with wheat husk-based formulation of *T. harzianum* in chickpea reduced dry root rot incidence up to 28% as compared to 70% in untreated control (Parakhia and Vaishnow, 1986).

Foliar spray

Application of *Trichoderma* spp., *Pseudomonas* spp., and *B. subtilis* on leaves was reported to reduce the incidence of rust (*Uromyces phaseoli*) in beans (Baker et al., 1985). In a similar manner, seed treatment and foliar application of *P. fluorescens* (Pf1) reduced the severity of *Puccinia arachidis* of groundnut under field conditions (Meena et al., 2002). Under controlled environment,

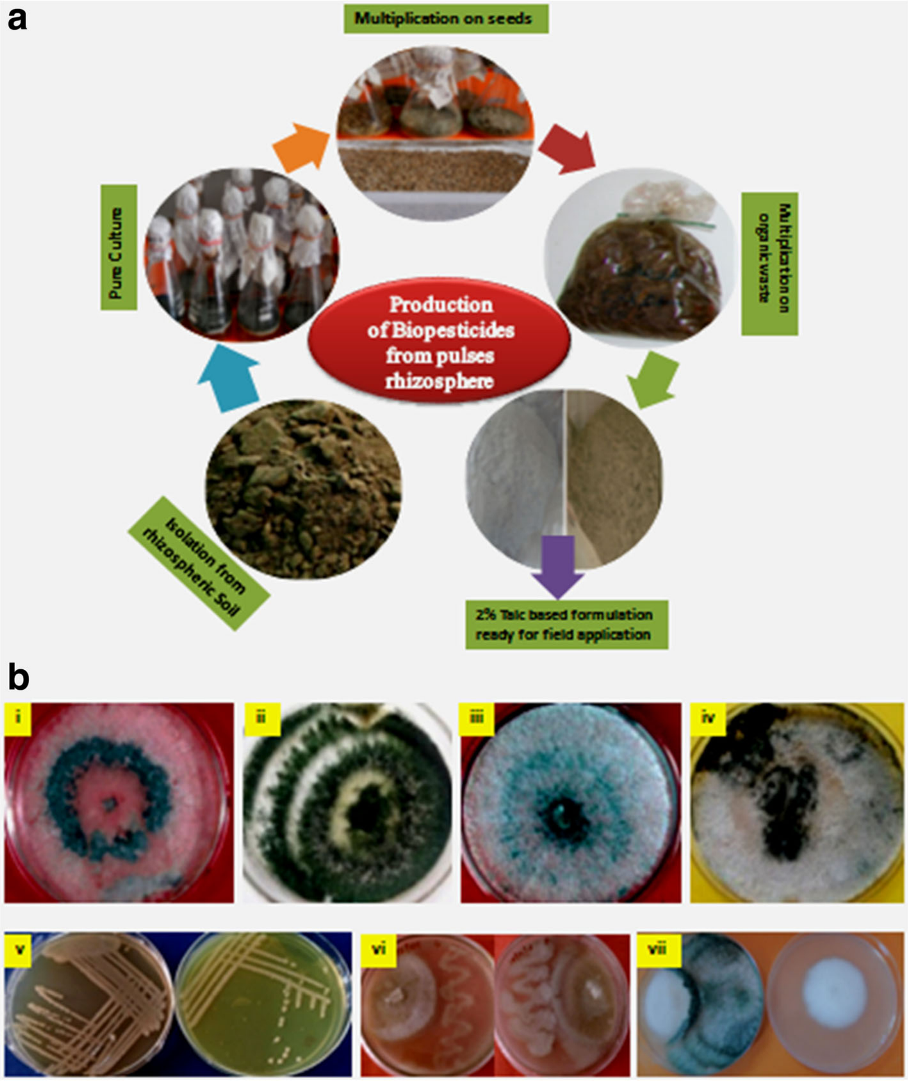

Fig. 2 **a** Procedure for formulation development. **b** Potential isolates of *Trichoderma* spp. from pulse rhizosphere in India: (i) *Trichoderma harzianum*, (ii) *T. asperellum*, (iii) *T. longibrachiatum*, (iv) *T. reesei*, (v) *PGPRs*, and antagonistic activity of (vi) *PGPRs* and (vii) *Trichoderma* spp. against *F. udum*

antagonistic activity of *T. harzianum* was demonstrated against botrytis gray mold (BGM) on chickpea foliage (Mukherjee and Haware, 1993). Ability of entomopathogenic PGPR strains to colonize phylloplane in a constant mode was notable with regard to foliar pest management in crop plants (Otsu et al., 2004).

Commercialization of biological control products in legumes

A series of experimentation and concerted efforts has led to commercialization of biological control products. The implementation of biological control products in managing plant diseases and insect pests is gaining ground. However, these still represent only 1% of the total plant protection products in comparison to fungicides accounting for 15% of total chemicals used in agriculture (Fravel, 2005). On the plus side, recent entry of several small- and large-scale entrepreneurs into commercial production of BCAs can be viewed as expanding scope of these in the world market.

As shown in Table 4, several formulations have been registered and available in the market for managing the crop pests. Unfortunately, no effective and potential host and region-specific stable formulation as well as consortia of biological product neither registered nor commercially available for pulses so far. Some potential biological control agents have been identified for management of *Fusarium* wilt and root rot diseases. In India, *T. viride*, *Streptomyces gougeroti*, and several bacterial species were shown to be functional against lentil wilt (Mehrotra and Claudius 1972). Similarly, *T. harzianum* and *Trichoderma koningii* showed antibiosis and

Table 4 Commercial formulations registered in Indian market

Biopesticides	Registered formulations
Trichoderma viride	5% WP
T. harzianum	5%WP
T. harzianum + *T. viride* + *T. virens*	5% WP
Bt var. *kurstaki* serotype H3a, H3b (HD-1)	5% WP, 2.5% AS, 0.5% WP,
Bt var. *kurstaki* serotype H2a, 3b (NRD-12)	3.5% AS, WG
Bt var. *galleriae*	1.3% FC
Bt var. *israeliensis* serotype H14	Liquid and WP formulations, 5% AS, 12% AS
Bt *sphaericus* serotype H5a H5b	1.3% FC
B. *sphaericus* serotype B101	
Verticilium lecanii	1.15% WP
Beauvaria bassiana	1.15% WP, 1.0% WP,1.15%SC
Metarhizium anisopliae var. acridum	1.15% WP
HaNPV	0.43% AS or 2.0% AS
Consortia of *T. harzianum* and *B. thuringiensis*	2%WP
T. harzianum (IIPRTh-1)	2%WP

mycoparasitism (Prasad and Rangeshwaran, 1999; Chaudhary et al., 2004; Mishra et al., 2015). Role of *Bacillus* and *Pseudomonas* as biological control of bacterial wilt and root rot, respectively, in bean has been examined (Neeraj, 2011; Martins et al., 2013). Apprehensions that introducing foreign microbes could predispose existing microflora to disturbance warrant an elaborated examination of interactions involving foreign and native microbes, and this will also permit assessment of the negative impacts that such introductions exert on pulse rhizosphere.

Challenges and prospects of BCAs in legume-based cropping system

Pulses are grown mostly by the resource poor and small-scale farmers. Cultivation of these crops mostly in harsh and unpredictable environments renders these prone to attack by a wide range of pests and pathogens at various stages of the crops. The use of chemical pesticides as a control measure is not deemed environmentally safe, and at the same time, affordability of these chemicals by the marginal farmers also remains limited. Therefore, employing BCAs in pulses is safer in view of the fact that these crops are consumed directly as seeds.

In current agricultural scenario, the use of BCAs and their formulations is of utmost important in grain legumes for biotic stress management. However, their full potential remains to be seen because of the limited attention these have received so far in terms of commercial scale production of BCAs and formulations. In addition, commercially available formulations have not been accessible by the pulse growers. The major stumbling block impeding the progress in this regard is inadequate awareness about its application and advantages. This underlines the scope of making these BCA-based formulations and techniques popular at farmers' field.

The quality of BCAs and their different formulations and efficiency of the antagonist strain are utmost important. It is measured in terms of inoculum potential (propagules/unit weight and the aggressiveness), shelf life, and ease of application and purity of the formulation. Generally, the biological control formulations available in the market are of very poor quality with meager shelf life (Singh et al., 2012), which demand strict regulation through quality control agencies. Also, biological control agents despite of performing well under controlled environments may fail to yield the same results in the field conditions. Further, storage and marketing of microbial biopesticides is another issue that greatly limits the extensive use of BCAs in today's agriculture. It is a high time to educate the dealers and users (cultivators) on importance of appropriate storage conditions, shelf life, and mode of action of BCAs.

The pathogenic variability reported in soil-borne phytopathogens (especially in *Fusarium* spp. causing wilt) in many pulse crops poses a serious challenge aiming to develop potential BCAs. Further, before selecting BCA for developing its formulations, it should be thoroughly screened against a large number of phytopathogens or a large number of isolates/strains/races of phytopathogens.

Increasing development and deployment of such cost-efficient and environment-friendly techniques are the methods-of-choice to check the rising pest and pathogen problem and to sustain production of pulse crops in the face of growing agricultural adversities.

Conclusions

Grain legumes remain important crops not only from nutritional security perspective, but also for their contribution to the health and fertility of the soil. Cultivation of these crops faces a number of biotic and abiotic stresses, reflected in the form of deteriorated yield and quality. The use of chemicals, as a control measure, is not commercially viable. More importantly, it has considerable damaging consequences on the environment, soil and microorganisms. In this respect, BCAs have shown immense potential, thus increasingly playing a significant role in agricultural production systems. Management of pests and pathogens of legumes with BCAs offers several benefits over the traditional protection measures. We advocate application of BCAs in grain legumes crops in view of their potential to deliver sustained gains in crop productivity.

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Authors' contributions
RKM with AB and NPS conceived the idea of the manuscript. RKM, AB, NK and KK participated in preparing first draft of the manuscript. KG, SGK, PRS SNSJ, MM conducted literature surveys. BKS, DK and DKS provided inputs on BCA delivery systems. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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