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Development of Pusa 5SD for seed dressing and Pusa Biopellet 10G for soil application formulations of *Trichoderma harzianum* and their evaluation for integrated management of dry root rot of mungbean (*Vigna radiata*)

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ABSTRACT

Various seed dressing and soil application formulations were developed from *Trichoderma viride*, *T. virens* and *T. harzianum* to increase the shelf life of bio-formulations used to manage dry root rot (*Rhizoctonia bataticola*) of mungbean (*Vigna radiata*), a major yield limiting factor in mungbean production. The shelf life of the formulations developed in the present study was monitored by counting colony forming units (cfu) up to 25 months of storage at room temperature (26 ± 8 °C). A newly developed seed dressing formulation, Pusa 5SD based on peat powder (47.5%), Sabudana powder (*Manihot esculenta*) (47.5%) and carboxymethyl cellulose (5%) and a newly developed soil application formulation, Pusa Biopellet (PBP) based on sodium alginate, aluminium silicate, Sabudana powder and tap water (1:5:5:100 w/w/w/v) exhibited longer shelf life. Another formulation Pusa Biogranule (PBG) based on wheat and pulse brans varied in cfu counts during different periods of storage. Pusa 5SD could be used up to 25 months of storage while PBP 10G and PBG 5 could be used up to 15 months of storage (>10^5 cfu). The efficacy of the formulations was evaluated in pot experiments against the disease. In these experiments, *T. harzianum* based PBP 10G and PBG 5 for soil application, and Pusa 5SD for seed treatment were found to be superior to others in reducing the dry root rot incidence, and increasing the seed germination and shoot and root lengths. However, a combination of soil application of PBP 10G (*T. harzianum*) and seed treatment with *T. harzianum* based Pusa 5SD + carboxin was found superior to the use of any of these formulations alone in reducing the dry root rot incidence (87.2%) and increasing the seed germination (43.0%), shoot length (40.3%), root length (37.0%) and grain yield (54.6%) of mungbean crop over those of untreated control under sick field conditions.

1. Introduction

Rhizoctonia bataticola (Taub) Butler, a soil inhabiting fungus, is a destructive root pathogen which causes dry root rot in a large number of plant species (Wyllie, 1989). Mungbean (*Vigna radiata* (L.) Wilczek), is an important pulse crop which is prone to many diseases. Amongst them, dry root rot caused by *R. bataticola* is one of the most widespread diseases in all mungbean growing areas of the world and causes up to 60% losses (Deshkar et al., 1974). The crop is grown under rainfed conditions and moisture stress predisposes the crop to dry root rot infection. The pathogen is both soil and seed borne.

Control of soil borne diseases through antagonistic microorganisms is often effective (Cook and Baker, 1983). The filamentous fungus, *Trichoderma* has attracted the attention because of its effectiveness against various plant pathogens (Harman et al., 2004). *Trichoderma viride* Pers. ex Gray and *Trichoderma harzianum* Rifai were found to be most effective in reducing the mycelial growth and sclerotial formation of *R. bataticola* (Khan and Gupta, 1998). Other workers (Kumar and Khare, 1990; Praveen and Ghaffar, 1991; Mathur, 2006) have also reported *T. harzianum* as a potential biocontrol agent of *R. bataticola*. The development of a stable, cost effective and easily applicable biocontrol formulation is critical in biocontrol (Lisansky, 1985). The application of alginate pellet and talc based formulations of bioagents is an important approach for the management of plant diseases. It has been recognised that in the formulation of alginate gels with *Trichoderma* species, a food base such as groundnut or wheat bran is preferred to inert clay as a carrier (Lewis and Papavizas, 1985, 1987; Lewis et al., 1985). The viability of conidia of *Trichoderma* species gradually decreased in alginate formulation and remained less than 10% after 24 weeks of storage at 5 or 25 °C (Lewis and Papavizas, 1985). Similarly, seed dressing formulations also showed drastic reduction in their viability after 6 months of storage at room temperature (Jayarajan et al., 1994; Tewari and Mukhopadhyay, 2001).

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In the present study, alginate pellets and seed dressing formulations with a new food base have been developed from the isolates of *T. viride* (IARI P-1; MTCC No. 5369), *T. virens* (Miller, Giddens & Foster) Arx (IARI P-3; MTCC No. 5370) and *T. harzianum* (IARI P-4; MTCC No. 5371) to increase the shelf life of the product and its effectiveness against plant diseases. Therefore, the formulations were evaluated alone and in combinations in different modes of application in pot and field conditions for integrated management of dry root rot and to increase the grain yield of mungbean.

2. Materials and methods

2.1. Development of seed dressing formulation

The isolates of *T. viride* (IARI P-1 = MTCC No. 5369), *T. virens* (IARI P-3 = MTCC No. 5370) and *T. harzianum* (IARI P-4 = MTCC No. 5371) were used for the development of seed dressing bio-formulations. The formulations were prepared by mixing their ingredients (Table 1). The products were named after Pusa, the popular name of Indian Agricultural Research Institute, New Delhi, India (Pusa is being used as a trade name for the products/crop varieties developed by the Institute). Before adding carboxymethyl cellulose (CMC), the fine mixture (200 mesh) of peat and Sabudana (*Manihot esculenta* Crantz) powders were dried 12 h at 60 °C in a hot air oven. The mixture of substrates was inoculated with the inoculum of *Trichoderma* species multiplied either on solid state (sorghum grains) or liquid state (potato dextrose broth) medium. Fifteen days after incubation (25 ± 1 °C), the mycelial growth along with conidia were filtered from potato dextrose broth and dried at room temperature before making fine powder. Sorghum grains (*Sorghum vulgare* Pers.) were soaked in tap water for 12 h, strained and filled into 500 ml conical flasks (250 g flask^{-1}). The flasks containing sorghum grains were autoclaved for two subsequent days at 1.1 kg/cm^2 for 30 min and inoculated with 7-day-old culture of *Trichoderma* spp. The flasks were incubated at 25 ± 1 °C for 15 days. Well-colonized sorghum grains were taken out from the flasks and dried at room temperature and finally made into a fine powder. Colony forming units (cfu) per gram of powdered product were determined by serial dilution method on potato dextrose agar medium (PDA). The required quantity of the inoculum was added into the substrate separately to make final cfu at 10^{10} g^{-1} of the product. A similar method was also followed for the preparation of Pusa 8, Pusa 17 and Pusa 26 as per their ingredients (Table 1) for evaluation under pot conditions. The colony forming units of formulation was counted by serial dilution method on PDA medium immediately after preparation of the formulations and at regular intervals up to 25 months of storage at room temperature (26 ± 8 °C).

2.2. Development of soil application formulation

Earlier mentioned three isolates of *Trichoderma* species were used for the development of granules and pellets suitable for soil application.

2.2.1. Granules

Two carriers, namely, wheat (*Triticum aestivum* L.) and pulse brans were used as basic substrates for the development of different granules (Table 1) which were designated as Pusa Biogranule (PBG). All three *Trichoderma* species were grown separately in

Table 1
Bio-formulations developed and used in the present study.

Formulations	Short name	Ingredients
Pusa 2 seed dressing	Pusa 2SD	Talc powder – 47.5% + Sabudana powder (*Manihot esculenta*) – 47.5% + carboxymethyl cellulose (CMC) – 5%
Pusa 4 seed dressing	Pusa 4SD	Peat powder – 95% + CMC – 5%
Pusa 5 seed dressing	Pusa 5SD	Peat powder – 47.5% + Sabudana powder –47.5% + CMC – 5%
Pusa 8 seed dressing	Pusa 8SD	Multani soil – 47.5% + Sabudana powder –47.5% + CMC – 5%
Pusa 17 seed dressing	Pusa 17SD	Karanj cake (*Pongamia glabra*) powder – 47.5% + sabudana powder – 47.5% + CMC – 5%
Pusa 26 seed dressing	Pusa 26SD	Pulse bran powder – 47.5% + Sabudana powder – 47.5% + CMC – 5%
Pusa Biopellet 2C	PBP 2C	Sodium alginate + Pioneer™ + water (1:10:100 w/w/v) for *T. viride*
Pusa Biopellet 2G	PBP 2G	Sodium alginate + Pioneer + water (1:10:100 w/w/v) for *T. viride*
Pusa Biopellet 3G	PBP 3G	Sodium alginate + Pioneer + rice + water (1:5:5:100 w/w/w/v) for *T. viride*
Pusa Biopellet 4C	PBP 4C	Sodium alginate + Pioneer + Sabudana + water (1:5:5:100 w/w/w/v) for *T. viride*
Pusa Biopellet 4G	PBP 4G	Sodium alginate + Pioneer + Sabudana + water (1:5:5:100 w/w/w/v) for *T. viride*
Pusa Biopellet 5G	PBP 5G	Sodium alginate + Pioneer + wheat bran + water (1:5:5:100 w/w/w/v) for *T. viride*
Pusa Biopellet 8C	PBP 8C	Sodium alginate + Pioneer + water (1:10:100 w/w/v) for *T. harzianum*
Pusa Biopellet 8G	PBP 8G	Sodium alginate + Pioneer + water (1:10:100 w/w/v) for *T. harzianum*
Pusa Biopellet 9G	PBP 9G	Sodium alginate + Pioneer + rice + water (1:5:5:100 w/w/w/v) for *T. harzianum*
Pusa Biopellet 10C	PBP 10C	Sodium alginate + Pioneer + Sabudana + water (1:5:5:100 w/w/w/v) for *T. harzianum*
Pusa Biopellet 10G	PBP 10G	Sodium alginate + Pioneer + Sabudana + water (1:5:5:100 w/w/w/v) for *T. harzianum*
Pusa Biopellet 11G	PBP 11G	Sodium alginate + Pioneer + wheat bran + water (1:5:5:100 w/w/w/v) for *T. harzianum*
Pusa Biopellet 14C	PBP 14C	Sodium alginate + Pioneer + water (1:10:100 w/w/v) for *T. virens*
Pusa Biopellet 14G	PBP 14G	Sodium alginate + Pioneer + water (1:10:100 w/w/v) for *T. virens*
Pusa Biopellet 15G	PBP 15G	Sodium alginate + Pioneer + rice + water (1:5:5:100 w/w/w/v) for *T. virens*
Pusa Biopellet 16C	PBP 16C	Sodium alginate + Pioneer + Sabudana + water (1:5:5:100 w/w/w/v) for *T. virens*
Pusa Biopellet 16G	PBP 16G	Sodium alginate + Pioneer + Sabudana + water (1:5:5:100 w/w/w/v) for *T. virens*
Pusa Biopellet 17G	PBP 17G	Sodium alginate + Pioneer + wheat bran + water (1:5:5:100 w/w/w/v) for *T. virens*
Pusa Biogranule 1	PBG 1	Wheat bran (400 g), kaolin powder (100 g) and acacia (*Acacia nilotica*) gum powder (50 g) for *T. viride*
Pusa Biogranule 2	PBG 2	Wheat bran (400 g), kaolin powder (100 g) and acacia gum powder (50 g) for *T. harzianum*
Pusa Biogranule 3	PBG 3	Wheat bran (400 g), kaolin powder (100 g) and acacia gum powder (50 g) for *T. virens*
Pusa Biogranule 4	PBG 4	Pulse bran (400 g), kaolin powder (100 g) and acacia gum powder (50 g) for *T. viride*
Pusa Biogranule 5	PBG 5	Pulse bran (400 g), kaolin powder (100 g) and acacia gum powder (50 g) for *T. harzianum*
Pusa Biogranule 6	PBG 6	Pulse bran (400 g), kaolin powder (100 g) and acacia gum powder (50 g) for *T. virens*

SD = Seed dressing, PBP = Pusa Biopellet, Pioneer™ = aluminium silicate.
Seed dressing formulations were prepared by mixing their respective ingredients and adding the inoculum of 15-day-old *Trichoderma* species.
Pusa Biopellets were prepared by mixing their respective ingredients in a blender. The mixture was autoclaved. 15-day-old conidia of *Trichoderma* (10^8 conidia ml^{-1}) were added. The product was pipetted into solution of calcium chloride (C) and calcium gluconate (G).
Pusa Biogranules were prepared by mixing their respective ingredients (sterilized at 72 °C for 3 days) in a grinder with 15-day-old inoculum of *Trichoderma* multiplied on potato dextrose broth (50 ml).