



Efficient application of *Trichoderma viride* on soybean [*Glycine max* (L.) Merrill] seed using thin layer polymer coating

P. Kuchlan*, M.K. Kuchlan and M.M. Ansari

ICAR-Indian Institute of Soybean Research,
Khandwa Road, Indore-452 001, Madhya Pradesh, India.

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ABSTRACT

Soybean crop is very sensitive to change in climate and suffers from vagaries of climate. The productivity thus gets affected in different years due to the influence of climatic condition, incidence of diseases and insects. *Trichoderma* sp. is considered as a potential biocontrol and growth promoting agents for many crop plants. *Trichoderma* spp. is generally being applied to the soil and seed as talcum powder form. Though powder formulations may be applied directly to seeds at the rate of 5g/kg seed, the effectiveness of *Trichoderma* seed treatment is lost due to poor adhesion on the smooth seed coat surface of soybean. Thus, it was targeted to achieve maximum benefit by *Trichoderma* seed treatment mediated through polymer coating. Two soybean cultivars were treated with *Trichoderma viride* with different formulation namely *Trichoderma* culture solution with polymer, *Trichoderma* culture talc with polymer, *Trichoderma* culture talc powder dry dressing. Endophytic growth of *Trichoderma viride* in root, stem and leaf was studied by agar plate method at $27\pm1^{\circ}\text{C}$ for seven days. The percentage of plants with *Trichoderma* endophytic growth was 97-100 per cent in plants from seeds treated with *Trichoderma* with polymer as compared to 37-45 per cent in plants from seeds treated with *Trichoderma* talc powder dry dressing. Control plants did not show any endophytic growth of *Trichoderma*. Significant increase in plant height was observed due to endophytic growth of *Trichoderma*. 24 per cent yield advantage was observed due to *Trichoderma* culture solution treatment with polymer. Seedling mortality due to collar rot (0.61%) and disease incidence of *Myrothecium* Leaf Spot, *Anthracnose* and *Rhizoctonia* Aerial Blight (0.34%) was significantly lower than control (9.8% and 9.169%). Proper application of *Trichoderma viride* on soybean seed through polymer coating has significant potential to reduce diseases and to improve plant growth and seed yield.

Key words: Polymer coating, Seed treatment, Soybean disease control, Soybean seed, *Trichoderma*.

INTRODUCTION

Soybean represents half of the global legume crop area, 68 per cent of global production and fixes nitrogen 16.4 TgN/year which is more than three fourth of the N fixed by crop legume (Herridge *et al.*, 2008). Within a short time of inception of commercial cultivation of soybean in India (in 1970's) it has occupied the first position among the oil seed crops in terms of acreage and production. Soybean is grown solely as rainfed crop without much involvement of costlier technologies. Soybean crop is very sensitive to change in climate and suffers from vagaries of climate. The productivity thus gets affected in different years due the influence of unfavorable climatic condition, incidence of diseases and insects. The world loss of more than seven million tonnes of soybean is due to diseases alone (Sinclair, 1988). Fungal diseases are main obstacle to obtain high yield of soybean during commercial cultivation and generally some chemical fungicides are applied to control these diseases. Application of chemical fungicide has been replaced by biocontrol agents because of emergence of fungicide resistant

strain and public concern regarding the health and environmental impact of the chemicals (John *et al.*, 2010). *Trichoderma* spp. are considered as a potential biocontrol and growth promoting agents for many crop plants (Verma *et al.*, 2007, Bai *et al.*, 2008 and Savazzini *et al.*, 2009). *Trichoderma* spp. is common in soil and root ecosystem as well known for their biocontrol potential (Woo *et al.*, 2006 and Qureshi *et al.*, 2012). The application of *Trichoderma* spp. combined with other microbial species may be a good strategy to increase growth, nutrient uptake and plant yield (Rudresh *et al.*, 2005). Biological control with endophytes offers an effective strategy for pest management (Afzal Saima *et al.*, 2013). Endophytic fungi are those fungi that live in plant tissues without doing substantial harm or gaining benefits other than residency (Kobayashi and Palumbo, 2000). Endophytes colonize an ecological niche similar to that of phytopathogens, which makes them suitable as biocontrol agents (Berg *et al.*, 2005). *Trichoderma viridae* is a well documented biocontrol and is reported to give systemic protection against many seed borne foliar diseases

*Corresponding author's e-mail: punam124@rediffmail.com

(Hanson, 2000). *Trichoderma* sp. in general is being applied to the soil and seed as talcum powder form. A large volume of *Trichoderma* powder was applied to the crop and soil. Powder formulations recommended at the rate of 5g/kg seed is poorly adhered to soybean seed due to very smooth seed coat surface. Thus the efficiency of this biocontrol agent is reduced. Biological control of damping off in cucumber and radish caused by *P. ultimum* and *Rhizoctonia solani*, was obtained using *Trichoderma* spp. applied to seed as an industrial film coating (Cliquet and Scheffer, 1996). Therefore it will be more beneficial to utilize the maximum benefit of this beneficial biocontrol agent by treating the seeds with this biocontrol agent mediated through polymer coating.

Therefore, present work was undertaken to find out the efficiency of *Trichoderma viride* to control soybean diseases applying through thin layer polymer coating of different formulations.

MATERIALS AND METHODS

Source and maintenance of *Trichoderma viride* culture: *Trichoderma viride* isolates originally from ICAR-Indian Institute of Soybean Research (formerly Directorate of Soybean Research) field and maintained in the plant pathology laboratory was used for the seed treatment. *Trichoderma viride* culture for application to seed was multiplied at 3.9 percent Potato Dextrose Agar (PDA) medium.

Preparation of *Trichoderma* powder for seed treatment: *Trichoderma* powder was prepared by mixing one Petri plate *Trichoderma* culture in 10 ml sterile water and homogenized on a rotary shaker at 150 rpm. Spore suspension was counted in a haemocytometer and diluted to give a concentration of approximately $1.5\text{--}1.7 \times 10^7$ conidia ml^{-1} . 10ml of this spore suspension was mixed with 10g talcum powder. Mixture was air dried and powder form of *Trichoderma* culture was stored in a plastic bottle for use under ambient condition.

Application of *Trichoderma* powder to seeds with polymer coating: 3g polymer and 4g *Trichoderma* powder and 3.5 ml distilled water were mixed together and applied to 1 kg soybean seed. After seed treatment with this solution seeds were surface dried and stored for 7 days at ambient condition in polythene packets. Treated seeds were then sown in the field.

Application of *Trichoderma* spore suspension with polymer to the seed: 10 ml suspension medium of *Trichoderma* sp. was prepared by thoroughly mixing one plate *Trichoderma* culture in 10 ml sterilized distilled water. Spore suspension was counted in a haemocytometer and diluted to give a concentration of approximately $1.5\text{--}1.7 \times 10^7$ conidia ml^{-1} and homogenized on a rotary shaker at 150 rpm. This spore suspension was then used for seed treatment. For seed treatment with *Trichoderma* spore 3g

polymer, 3 ml spore suspension of *Trichoderma* sp., and 3.5 ml of distilled water were mixed thoroughly and applied to the seed.

Another set of seeds was dry dressed with *Trichoderma* powder at the rate of 4g per kg seed. Survival of *Trichoderma* sp. on treated seed was observed in Potato Dextrose Agar medium incubated at $27 \pm 1^\circ\text{C}$ for 7 days.

Studies on efficacy of biocontrol agent applied to the seed through polymer coating: Soybean seeds treated with *Trichoderma* sp. (powder and polymer, culture solution and polymer and Trichoderma Powder dry dressing) and untreated control were sown in the field in randomized block design in the ICAR- Indian Institute of Soybean Research Farm during kharif 2012 to 2015. After 20 and 40 days of sowing 20 plants were selected and leaf and stem samples were collected randomly from each plots. Leaf and stem samples were surface sterilized with sodium hypochlorite (1%). The leaf and stem samples were incubated in the agar medium at $27 \pm 1^\circ\text{C}$. After 3-4 days of incubation plates were observed for presence or absence of *Trichoderma* in the plant parts.

Plant height (cm): Randomly selected 20 plants of each plot were observed for plant height (cm) after 14 and 40 days of sowing.

Seedling mortality: Seedling mortality rate was calculated by number of seedling died due to collar rot out of total plant population multiplied by 100.

Different foliar disease incidence on the soybean field and treated plots performance was calculated with the help of following formula.

$$\text{Disease incidence (I)} = \frac{\text{Number of infected plant units} \times 100}{\text{Total number (healthy and infected)}}$$

$$\text{Disease intensity (\%)} = \frac{\text{Total read score} \times 100}{\text{Total number of reading} \times \text{highest}}$$

The experimental data were analyzed using MSTAT C programme for analysis of CD at 5%. The percentage values were converted to Arc sine values before analysis.

RESULTS AND DISCUSSION

The *Trichoderma* seed treatment revealed endophytic growth of *Trichoderma* in the plant system (Fig 1 and Fig 2). The efficacy of different treatment varied significantly. 97.67 to 100 per cent tested plants from seeds treated with *Trichoderma* culture through thin layer polymer coating showed endophytic growth of *Trichoderma* whereas 37 to 45 per cent plants when applied through conventional way of dry dressing of seeds with *Trichoderma* talc powder (Table 1). In control plants no *Trichoderma* growth in plant system was observed. Thus it was found that efficacy of *Trichoderma* treatment was maximum when it was applied

through thin layer polymer coating. This technique had been reported to be very effective for proper application of seed treating chemicals by adherence of the chemicals for longer times to seeds during sowing and seedling emergence. Soybean seed germination is epigeal. Therefore, the seed comes out of the soil and new leaves come out of the cotyledons. The polymer coating had been found to remain intact on the surface of the seed coat even after field emergence and the biocontrol agents got more time to germinate and penetrate into the seed and further into the plant system. Otherwise, in dry dressing the *Trichoderma* powder coating on seeds got shed from seed to soil during sowing and seedling emergence. This phenomenon caused accumulation of *Trichoderma* in soil and reduced the efficiency of *Trichoderma* to penetrate into the seeds and further growth in the plant system. McQuilkent *et al.*, (1997) reported that the application of biological control agents by commercial seed coating procedure is more economical method of delivery. With this result it was also proved that *Trichoderma* culture remain viable inside polymer film coating. It was found that seedling mortality percentage due to collar rot was significantly controlled in seed treatment with thin layer polymer coating of *Trichoderma* spore solution and talc powder (Table 1) in both the cultivar as compared to control. Biological control induced by seed treatment of *Trichoderma viride* by polymer against pathogenic species of *Sclerotium rofsi* could protect soybean plants resulting lesser seedling mortality rate. Kashem *et al.*, (2016) reported that *Trichoderma harzianum* controlled 64.07-99.4 per cent root rot of lentil due to *Sclerotium rofsi*. *Trichoderma* isolate have been reported to successfully control the damage caused by soil borne pathogens in green houses and under field conditions (Papviza, 1985). John *et al.*, (2010) also reported that *Trichoderma* treatment controlled the severity of pathogen infection and improved plant growth.

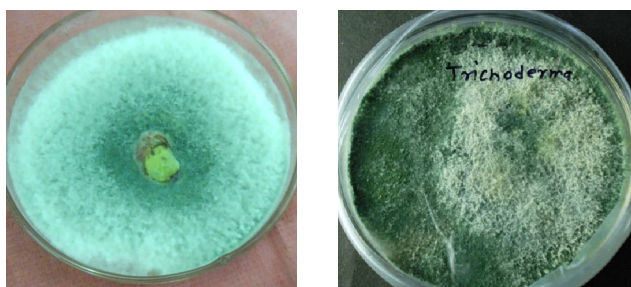


Fig 1: Growth of *Trichoderma* from seeds coated with *Trichoderma* and polymer.

Significant increase in plant height was observed due to endophytic growth of *Trichoderma*. It was found that maximum height of 23.07 cm after 14 days and 62.16 cm after 40 days of sowing was obtained with thin layer polymer coating of *Trichoderma* culture solution as compared to 19.46 and 50.95 cm in control plants respectively (Table 2). The result clearly showed the significant improvement in the plant height when *Trichoderma* culture powder or *Trichoderma* culture solution were applied to the seed mediated with in polymer as compared to dry dressing. John *et al.*, (2010) also reported that *Trichoderma viride* showed growth promoting action on the soybean plant. Vinale *et al.* (2008) reported that the secondary metabolite such as auxin like compounds or auxin substances by *Trichoderma* plant interaction might be reason for the improved growth. The impact of plant growth and reduction in disease incidence was reflected as increased seed yield in the treated plots as compared to control and dry dressing treatment. In NRC 7 the yield was increased from 1.52 to 1.87 kg/plot for polymer coating of *Trichoderma* culture solution which was 23.02 per cent higher over control. Similarly, in JS 335 the increase was 24 per cent over control. Maximum increase was observed in case of polymer coating of *Trichoderma* spore



Fig 2: Endophytic growth of *Trichoderma* sp. in plant parts after 40 days of sowing.

Table 1: Frequency of entophytic growth of *Trichoderma* by different mode of application onto the soybean seeds and impact on seedling mortality in varieties NRC 7 and JS 335 due to collar rot.

Treatment name	Plants with endophytic growth of <i>Trichoderma</i>			Seedling mortality (%)	
	Leaf	Stem	Root	NRC 7	JS 335
Polymer coating of <i>Trichoderma</i> spore solution	99.67 (88.09)	99.67 (88.09)	100 (90.00)	0.61	1.1
Polymer coating of <i>Trichoderma</i> talc	96.67 (79.65)	97.67 (81.53)	99.00 (84.43)	0.71	1.3
Dry dressing of <i>Trichoderma</i> talc	36.67 (37.25)	38.67 (38.44)	45.67 (42.50)	3.75	3.6
Untreated control	0	0	0	9.8	8.5
LSD value at alpha= 0.050	6.499	7.063	5.579	0.8663	0.5825

Table 2: Effect of *Trichoderma viride* on plant height and seed yield applied with polymer technique on NRC 7 and JS 335.

Seed Treatment	Plant height at 14 DAS (cm)		Plant height at 40 DAS (cm)		Seed Yield (Kg/plot)	
	NRC 7	JS 335	NRC 7	JS 335	NRC 7	JS 335
Polymer coating of <i>Trichoderma</i> spore solution	23.07	19.13	58.16	52.40	1.87	1.76
Polymer coating of <i>Trichoderma</i> talc	21.3	19.00	57.76	51.4	1.79	1.73
Dry dressing of <i>Trichoderma</i> talc	19.46	16.95	50.95	46	1.68	1.60
Untreated control	17.8	16.35	47.3	43	1.52	1.41
LSD value at alpha= 0.050	2.629	2.455	7.59	6.933	0.2278	0.2278

solution but effect of polymer coating of *Trichoderma* talc powder was at par. The yield of soybean was improved with *Trichoderma* seed treatment. It was also found that *Trichoderma* treated plants remain healthy throughout the crop stage whereas in the untreated control plot the seedling mortality rate was highest in both the cultivar. It may be also one of the reasons to get higher yield in the treatment plot as compared to untreated control. According to Rudresh *et al.*, (2005) the application of *Trichoderma* sp combined with microbial species may be a good strategy to increase growth, nutrient uptake and plant yield.

Three foliar diseases namely Myrothecium leaf spot, Anthracnose and Rhizoctonia arial blight were observed with high intensity in untreated control plots (Table 3 and 4). Treatment 1 and Treatment 2 were highly effective to control these foliar diseases in the field. Only Myrothecium leaf spot was observed with lower intensity and significantly lesser number of plants were infected. Lorito *et al.*, (1996) reported that fungal pathogens are killed by the release of toxic compounds i.e. antibiotics gliotoxin, gliovirin and peptabols and a battery of lytic enzymes, mainly chitinases, glucanases and proteases produced by species of *Trichoderma*. These enzymes facilitate penetration into the host and utilization of host nutrients. Antibiotic production, mycoparasitism, the production of cell wall degrading

enzymes and competition for nutrient or space are considered as the action involved in biocontrol of pathogens during mycoparasitic interaction between *Trichoderma* and fungal pathogens (Zeilinger and Omann, 2007 and Vinale *et al.*, 2008). Similarly, Benhamou and Chet (1993) illustrated many interactions of *Trichoderma* with pathogens Rhizoctonia and Pythium.

This study has provided evidence for the value of the approach of polymer film coating *Trichoderma* sp. onto the seed using polymer coating technique. Endophytic growth of *Trichoderma* was traced in root, stem, leaf as well as pods. The technique was found successful to improve plant stand by controlling seedling mortality due to Collar rot, to improve plant health and seed yield by controlling foliar diseases - *Sclerotium rolfsi*, Rhizoctonia arial blight, Myrothecium leaf spot diseases. The study reveals that seeds treatment with *Trichoderma* sp. have the potential to highly reduce the disease on soybean.

The economic feasibility of the techniques can be determined by the cost of application and the benefit obtained in terms of increase in yield and reduction in cost of plant protection measures. The technique focused on the reduction in seedling mortality. Thus, it gives a scope to reduce seed rate and cost incurred on seed. The technique reduced the disease incidence and improved plant growth and increase

Table 3: Disease incidence in the NRC7 in different treatment plot.

Treatments	Myrothecium Leaf Spot		Anthracnose		Rhizoctonia Arial Blight		Total diseased plant / plot (%)
	Plant infected (%)	Intensity / plant	Plant infected (%)	Intensity / plant	Plant infected (%)	Intensity / plant	
Polymer coating of <i>Trichoderma</i> spore solution	0.34	3	-	-	-	-	0.34
Polymer coating of <i>Trichoderma</i> talc	0.41	3	-	-	-	-	0.41
Dry dressing of <i>Trichoderma</i> talc	2.024	3	2.30	3	1.316	-	5.64
Untreated control	3.114	5	3.925	5	2.123	5	9.169

Table 4: Disease incidence in JS 335 in different treatment plot.

Treatments	Myrothecium Leaf Spot		Anthracnose		Rhizoctonia Arial Blight		Total diseased plant / plot (%)
	Plant infected (%)	Intensity / plant	Plant infected (%)	Intensity / plant	Plant infected (%)	Intensity / plant	
Polymer coating of <i>Trichoderma</i> spore solution	0.27	3	-	-	-	-	0.27
Polymer coating of <i>Trichoderma</i> talc	0.56	3	-	-	-	-	0.56
Dry dressing of <i>Trichoderma</i> talc	1.95	3	1.86	3	1.22	3	5.03
Untreated control	3.594	5	4.57	5	2.123	5	10.287

yield by around 24 per cent. Concurrently, the cost of plant protection measures for costly agrochemical is reduced. Above all application of *Trichoderma* on seed through thin layer polymer coating is environment friendly green technology to reduce the dependence on toxic and polluting agrochemicals. The average cost of polymer in Indian market presently is approximately five hundred rupees per kilogram.

At the rate of two gram polymer per kg seed incur an seventy rupees per hectare for polymer in addition to recommended cost of *Trichoderma* and the application cost. The application of biocontrol agent replaces the recommended 3g carbendazime per kg seed which cost more than the cost of polymer and *Trichoderma*. The benefit of this technique is manifold in terms of economic return and environmental safety.

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