Compatibility of colored commercialpolymers used by the seed industryfor maize biological seed coating treatments

Abstract:

Introduction: An efficient biological seed coating begins with the choice of a suitable polymer. Experiments were conducted at the Department of Seed Science and Technology, Professor Jayashankar Telangana State Agricultural University, Hyderabad, to determine if commercial polymers and the biocontrol agents were compatible.Methodology: Five polymers commercially used by the seed industry were procured from seed companies.Three bioformulations were purchased from the commercial biofertilizer units and thepure cultures of*Trichoderma viride,Pseudomonas fluorescens,* and *Bacillus subtilis* were isolated from the bioformulations through serial dilutions, culturing and subculturing on microbial media. The compatibility ofcommercially available coloured polymers has been tested with biocontrol agents using poisoned food technique (for fungal bioagents) and the inhibition zone technique (for bacterial bioagents). Observations on the radial growth and reduction in radial growth of the fungal bioagent and zone of inhibition of bacterial bioagents was recorded in mm.Results: The results revealed that all the fivecoloured polymers commerciallyused by the seed industry were tested to show 96–100% compatibility with *Trichoderma viride*, 100% compatibility with *Pseudomonas fluorescens,* and 95–100% compatibility with *Bacillus subtilis*. Interpretation: These findings indicate that there is a greater compatibility of all the coloured polymers used in the seed industry with the bioagents which can also be used for effective seed coating with bioagents economically without incurring any additional inventory from the part of the industry.

Key words:*Bacillus subtilis,* compatibility, *Pseudomonas fluorescens,*polymers, *seed coating, Trichoderma viride.*

Comment [A1]: Highlight the need of the word "coloured" …… Is there other polymers which are colourless? Please clear it.

Introduction

Maize (*Zea mays* L.) is one of the most important cereal crops cultivated throughout the globe. It is the third most important cereal crop in India after rice and wheat in terms of area and production.Post- flowering stalk rots (PFSR) are the world's most destructive diseases of corn**.** The incidence of PFSR complex (Charcoal rot, Fusarium stalk rot, late wilt) varying from 5 to 40% in different parts of the country. As the pathogen is seed and soil borne, chemical method alone can't offer the protection throughout the crop period even though various disease management methods have been implemented to reduce and eradicate pathogenic fungi.

One of the new-generation seed treatment methods for enhancing seed quality and for comprehensive crop protection is coating with beneficial microorganisms.Seed coating combines beneficial materials with a binder and applies it to the seed [1].Seed coating with plant beneficial microbes (PBMs) allows a precise application of minor amounts of inocula at the seed-soil interface [2], ensuring that the PBMs are readily accessible at germination and early development plant stages, stimulating healthy and rapid establishment, and consequently maximizing crop production [3]. PGPM is proposed as an eco-friendly and cost-effective alternative to conventional seed treatment methods [4].The bioinoculants offers a solution to the challenges arising from excessive use of chemical fertilizers and pesticides in agriculture [5].

It is an ecological plant disease management approach and a potential alternative to chemical control through the use of selected bioagents against the soil and seed borne pathogens.Seed coating with fungi or bacteria increased the soluble protein and antioxidant enzyme activity of seed, the bioactive chemicals, nutrients, and useful microbes can be added to seeds through coating and pelleting technologies [1]. Large scale delivery of beneficial microbial inoculants occurs through seed coating [6]. *P. fluorescens* and *T.*

Comment [A2]: Need to properly address the research gap. As the seed companies are already using the polymers for seed coating what new things can be addressed with the paper.

Comment [A3]: Cite suitable reference.

harzianum moderately modified the negative effects of drought stress and improved the growth parameters of cumin seed[1].*Bacillus spp* has the ability to produce auxin, siderophores, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase [7].The bioagents are ecofriendly and cost effective than chemicals for the control of pests and diseases and there are no/ meagre studies to encourage the use of bioagents after seed processing and before packaging.

Microbes found in the solid or liquid bioformulations will be employed for coating, coupled with a suitable adjuvant, to coat seeds. Adjuvant is absolutely essential for the microbiome's survival and shelf life as well as for the solid adherence of the organism to the seed surface.Polymer is the substance applied to the seed that does not obscure its shape. The plasticizer polymer forms a flexible film that adheres and protects the fungicides and insecticides during handling. The film being water soluble reduce imbibition damageand do not impede the germination of film coated seed but improve germination and seedling emergence and can be stored for longer duration without loss of seed viability. Polymer coating acts as a temperature switch, regulating intake of water by seed coat, the stress imposed by accelerated ageing, which includes fungal invasion and improves the seedling emergence at changing soil moisture regime especially in the sub-optimal range [8].The presence of additives can enhance polymer properties and protect microorganisms[9].

The appropriate polymer for biological coating should be compatible with the bioagent, maintain the microbial population on the seed surface, cause little to no dust-off issues, be phytotoxic-free, sticky, moisture-retentive, nutrient-releasing, porous, temperature-stable, biodegradable, serve as a source of nourishment for the microbes during storage, improve seed quality, germination, and growth of seedlings, and in turn, improve the yield. It also needs to allow for the exchange of gases and water for respiration. The polymer needs to provide

controlled release and be friendly to bioactive chemicals. It must be easy to use, reasonably priced, environmentally friendly, and in compliance with all applicable laws. The selection of polymer depends on the crop requirement, the environment, and the objectives of the seed coating.

Different seed companies use different types of polymers for general seed treatments with agrochemicals. But the selection of compatible polymers is the first step to be considered for effective biological seed coating. It is very important for the seed company to know whether the polymer existing with them can be used for biological coating or not. Little or no research has been done on the feasibility of using commercial polymers available in the seed industry for seed coating with biologicals. Hence, the present study has been taken up to study the compatibility of coloured commercial polymers and seed coating agrochemicals with biological agents so that the seed company can use the existing polymers for coating biologicals as well.

Material and methodology

The present investigation was aimed to study the compatibility of bio control agents with different commercially available colored polymers. The experiments were carried out during *Kharif*, 2019 and *Rabi*, 2019-20 at the Department of Seed Science and Technology, Seed Research and Technology Centre and Department of Agricultural Microbiology and Bio-energy, College of Agriculture, PJTSAU, Rajendranagar, Hyderabad, Telangana. The liquid bioformulations of biological agents *Trichoderma viride, Pseudomonas fluorescens* and *Bacillus subtilis* were obtained from the Department of Agricultural Microbiology and Bio-energy, College of Agriculture, PJTSAU, Rajendranagar, Hyderabad, Telangana.The commercially used

and available coloured polymers i.e.,P1, P2, P3, P4 and P5 were collected from location stores, Hyderabad.

The compatibility testing of fungal biocontrol agent *Trichodermaviride* with different commercially available colored polymers using poisoned food technique:

The compatibility of fungal biocontrol agent *Trichodermaviride* with5 commercially available coloured polymers (P1, P2, P3, P4 and P5) were tested using poisoned food technique[10]. In this test, 60 ml of PDA (20 ml per replicate) was taken in 100 ml of sterilized conical flask and at lukewarm temperature a specified quantity of the colored polymer was added, mixed thoroughly and were poured into the sterilized petriplates aseptically and allowed to solidify. Mycelial discs (5 mm diameter) of one week old pure culture of *Trichoderma viride* was transferred to the centre of poisoned medium in each of the petriplate. Suitable controls were maintained by placing discs of *Trichoderma viride* in petriplates containing untreated medium (i.e. without chemical). Three replicate plates were maintained for every treatment. All the inoculated petriplates were incubated at 25 ± 2^0C in a BOD incubator. The colony diameter of the *Trichoderma viride* was measured in treatment plates when the colony growth in the control plate was full.

Per cent inhibition (I) of the bio control agent over the control was calculated by using the following formula

$$I = \frac{C - T}{C} X 100$$

Where,

I = Per cent inhibition

C = Colony diameter of biocontrol agent in control

T = Colony diameter of biocontrol agent in treatment

Treatments:

Table 1: Compatibility of *Trichoderma viride*with commercially available colored polymers under in-vitro conditions using poisoned food technique:

T1	*Trichoderma viride*+ Polymer 1 (Red Colourant + transparent polymer)
T2	*Trichoderma viride*+ Polymer 2 (Red Polymer)
T3	*Trichoderma viride*+ Polymer 3(Red Polymer)
T4	*Trichoderma viride*+ Polymer 4 (Red Polymer)
T5	*Trichoderma viride*+ Polymer 5 (Pink Polymer)
T6	*Trichoderma viride* (control)

The compatibility of bacterial biocontrol agents *Pseudomonas fluorescens* and *Bacillus subtilis* with different commercially available colored polymers through the Inhibition zone technique.

Compatibility of bacterial biocontrol agents were determined using inhibition zone technique[10]. In this technique, 60 ml of the specific medium (King's B medium for *Pseudomonas fluorescens*, and Pikovskaya's agar for *Bacillus subtilis*) was poured in the sterilized petriplates over which 15 µl of bacterial sample is spread uniformly by sterilized spreaders. Four discs of sterilized Whatman No.1 filter paper of about 10 mm diameter dipped in the commercially available coloured polymers; air dried and placed over bacteria seeded agar plates. Plates along with a control (discs dipped in sterilized water) were incubated at 28 ± 2^0C for 1-2 days.

The inhibition zone (mm) around the discs was measured and per cent inhibition of each antagonistic bacteria was calculated by using the following formula.

$$\text{Percent inhibition of growth (mm) of antagonistic microbe} = \text{Growth in control (mm)} - \text{Growth in treatment (mm)}$$

Growth in control (mm)

Table 2: Compatibility of *Pseudomonas Fluorescens* with commercially available colored polymers under *in vitro* conditions using Inhibition zone technique:

T1	*Pseudomonas fluorescens* + Polymer 1 (Red Colourant + transparent polymer)
T2	*Pseudomonas fluorescens* + Polymer 2 (Red polymer)
T3	*Pseudomonas fluorescens* + Polymer 3 (Red polymer)
T4	*Pseudomonas fluorescens* + Polymer 4 (Red polymer)
T5	*Pseudomonas fluorescens* + Polymer 5 (Pink polymer)
T6	*Pseudomonas fluorescens* (control)

Table 3: Compatibility of *Bacillus subtilis* with commercially available colored polymers under *in vitro* conditions using Inhibition zone technique:

T1	*Bacillus subtilis* + Polymer 1 (Colourant + Transparent polymer)
T2	*Bacillus subtilis* + Polymer 2 (Red polymer)
T3	*Bacillus subtilis* + Polymer 3 (Red polymer)
T4	*Bacillus subtilis* + Polymer 4 (Red polymer)
T5	*Bacillus subtilis* + Polymer 5 (Pink polymer)
T6	*Bacillus subtilis* (control)

Statistical analysis:

The data recorded were analyzed statistically by adopting Completely Randomized Design (CRD), as described by Panse and Sukhatma[11] and the standard error of difference was calculated at 5% probability level to compare the mean difference among the treatments. The data recorded as percentage were transformed to the respective angular (arc sin) values before subjecting them to statistical analysis.

Results and discussion:

The compatibility of bioagent *Trichodermaviride* with different commercially available coloured polymers using poisoned food technique:

All coloured polymers under testing recorded high compatibility (> 95%) at 6000 ppm with *Trichoderma viride*by recording low reduction in mycelial growth compared to control. Among the5 polymers, T5 and T3 at 6000 ppm showed high compatibility (100 and 99.3 %, respectively) followed by T2(98 %), T4 (96.55 %) and T1(96.45 %) (Plate 1). This indicates that the commercially available coloured polymers are compatible with no antagonistic effects on the radial growth of *T. viride*. The research finding of compatibility of bio friendly polymer with *T. viride*was in conformity with the previous finding stating thatthe high compatibility (100%) of *T. viride* with biofriendly polymer which was used in biological seed coating[12, 13].

Similarly,in another finding it isreported that biological seed coating with bio friendly polymer showed more viability and long shelf life (CFU) of *T. viride*[14].*Trichoderma* have not shown any inhibition with film forming ingredients [15]. Highest compatibility of biopolymerswith *T. asperellum* reported in chilli seeds [16].

The compatibility of *Trichoderma* with commercially available coloured polymers might be due to the presence of some nutrients and guarding factors in addition to adhesive factors as reported by Accinelli *et al.*,[17]. The biodegradable polymers can serve both as a nutrient source for a biocontrol agent.Binders/fillers can be used to extend microbial survival[6].

The compatibility of bioagent *P.fluorescens* with different commercially available coloured polymers through the inhibition zone technique:

Pseudomonas fluorescens showed highest compatibility of 100% with all the commerciallyavailable coloured polymers (Plate 2). The treatments T1, T2, T3, T4 and T5 used

in the experiment at 6000 ppm by recording no zone of inhibition.

This research finding of compatibility of biofriendly polymer with *P. fluorescens* was in conformity with the previous finding who have reported that the compatibility of *P. fluorescens* with biofriendly polymer[12,13] and also in conformity with Chin *et al.*,[16] who stated that all the biopolymers were compatible with *P. fluorescens*. The pre-inoculated seed treatment with polymer coating has not affected the microbial population in the seed[18].Cts-PEG film containing with *Trichoderma* increased their populationwhen applied in the soil, by the degradation of hydrolytic enzymes of chitosan film served as the nutrient source for *Trichoderma*[19].

The compatibility of bioagent *Bacillus subtilis*with different commercially available coloured polymers through inhibition zone technique:

B. subtilis showed less / no zone of inhibition the treatmentsT5 and T2.The treatments T1showed **2.4 %**and T4with 3.4% zone of inhibition followed T3with 5% zone of inhibition (Plate 3). And this inhibition is negligible which might not show a drastic reduction in the colony counts after coating.

This finding is in conformity with the previous findings[12, 13, 20]who has reported compatibility of biofriendly polymer with *Bacillus*and seed coating materials.

Conclusion: All the bioagents under study have shown more than 95% compatibility with the commercially available colouredpolymers used by the seed companies and have not shown any negative effect on the radial growth of bioagents *Trichoderma viride and no inhibition zone withPseudomonas fluorescens* and *Bacillus subtilis.* The coating of the seed with these bioagents as single or in consortia can be effectively utilized for biological seed coating in controlling the seed and soil borne diseases. As there is an increase in global concern with the use of chemical

pesticides and their dust-off on the environment, these bioagents can be used effectively as a coating with thin film layer of polymer whereby the seed shape is not altered and theinocula of these bioagents can be maintained without any dust-off. The seeds can be coated with the bioagents on farm before sowing or immediately before packing and storage and it doesn't have any serious effect on human and animal health. Though the use of bioagents is limited, the promising effects of the bioagents in combinations or consortia or as single is gaining interest and opening new perspectives of the seed industry.

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Table 4: Compatibility of *Trichoderma viride* with commercially available colored polymers under *in vitro* conditions (Poisoned food technique)

Treatments	Details of the treatments	Radial growth of *T. viride* (mm) after 7 days*	Reduction in Radial growth of *T. viride* (mm)	Compatibility (%)
T1	*Trichoderma viride*+ Polymer 1	86.80	3.55	96.45
T2	*Trichoderma viride*+ Polymer 2	88.20*ab	2.00	98.00*
T3	*Trichoderma viride*+ Polymer 3	89.33*ab	0.67	99.30*
T4	*Trichoderma viride*+ Polymer 4	86.90	3.40	96.55
T5	*Trichoderma viride*+ Polymer 5	90.00*a	0.00	100.00*
T6	*Trichoderma viride* (control)	90.00a	0.00	Control
	Mean	88.54		
	C.D (0.05)	2.427		
	SE (m)	0.779		
	SE (d)	1.102		
	C.V %	1.524		

When $p < 0.05$ ANOVA Tukey statistical test (95% confidence interval) was performed. Means within a column followed by different letter are significant at 5% level of significance and those following by the same letter do not differ significantly at 5% level of significance

Table 5. Compatibility of *Pseudomonas fluorescen*s with commercially available coloured polymers under *invitro* conditions (Inhibition zone technique)

Treatments	**Details of the treatments**	**Zone of inhibition (mm) after 72 h***	**Growth reduction over control (mm)**	**Compatibility (%)**

T1	*P. fluorescens* + Polymer 1	0.00	Nil	100
T2	*P. fluorescens* + Polymer 2	0.00	Nil	100
T3	*P. fluorescens* + Polymer 3	0.00	Nil	100
T4	*P. fluorescens* + Polymer 4	0.00	Nil	100
T5	*P. fluorescens* + Polymer 5	0.00	Nil	100
T6	*P. fluorescensfluorescens*(control)	0.00	Control	
	Mean	0.00		
	C.D (0.05)	0.00		
	SE (m)	0.00		
	SE (d)	0.00		
	C.V %.	0.00		

When $p < 0.05$ ANOVA Tukey statistical test (95% confidence interval) was performed. Means within a column followed by different letter are significant at 5% level of significance and those following by the same letter do not differ significantly at 5% level of significance

Table 6. Compatibility of *Bacillus subtilis* with commercially available coloured polymers under *in vitro* conditions (Inhibition zone technique)

Treatments	Details of the treatments	Zone of inhibition (mm) after 72 hrs *	Growth reduction over control (mm)	Compatibility (%)
T1	*Bacillus subtilis*+ Polymer 1	2.17	2.41	97.59
T2	*Bacillus subtilis*+ Polymer 2	1.33ab	1.48	98.52*
T3	*Bacillus subtilis*+ Polymer 3	4.50	5.00	95.00
T4	*Bacillus subtilis*+ Polymer 4	3.08	3.42	96.58
T5	*Bacillus subtilis*+ Polymer 5	0.00a	Nil	100.00*
T6	*Bacillus subtilis* (control)	0.00a	Control	
	Mean	1.85		
	C.D (0.05)	1.50		
	SE (m)	0.48		
	SE (d)	0.68		
	C.V %	45.23		

When $p < 0.05$ ANOVA Tukey statistical test (95% confidence interval) was performed. Means within a column followed by different letter are significant at 5% level of significance and those following by the same letter do not differ significantly at 5% level of significance

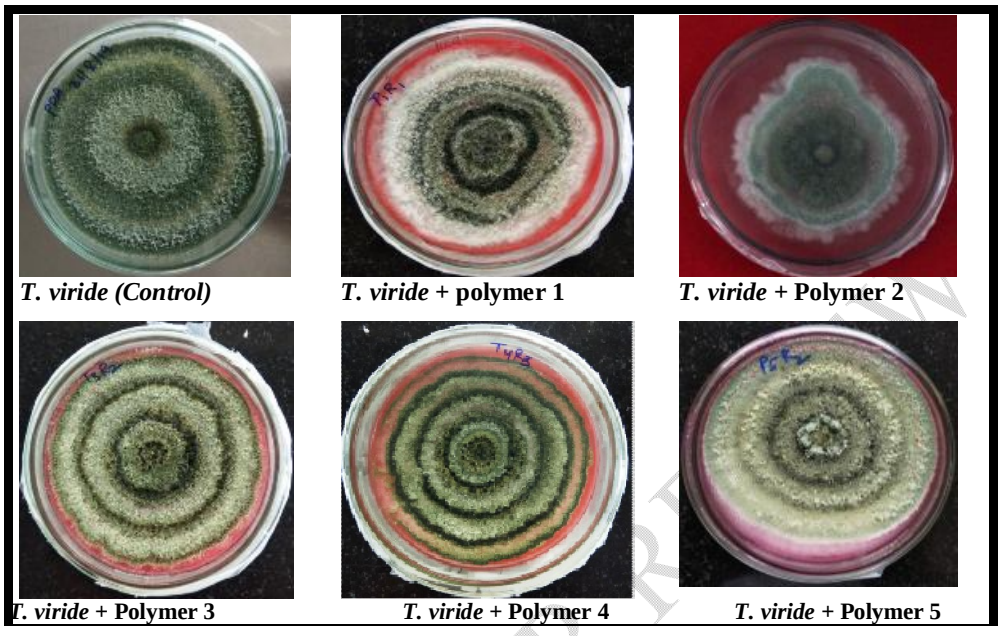

Plate 1: Compatibility of *T. viride* (Radial growth of *Trichoderma viride*) with commercially available coloured polymers

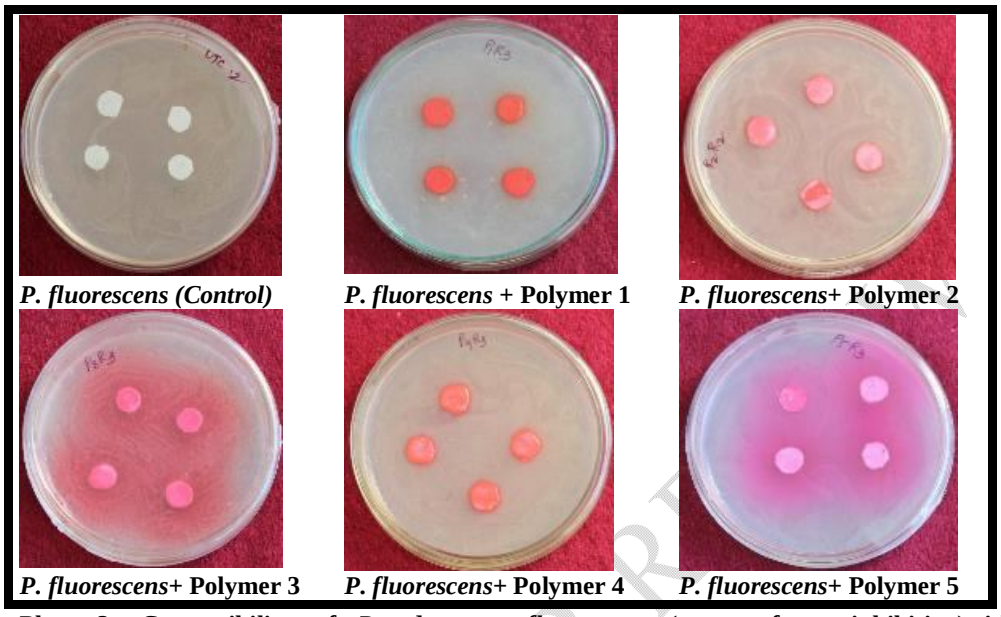

Plate 2: Compatibility of *Pseudomonas fluorescens* (zones of no inhibition)with commercially available coloured polymers

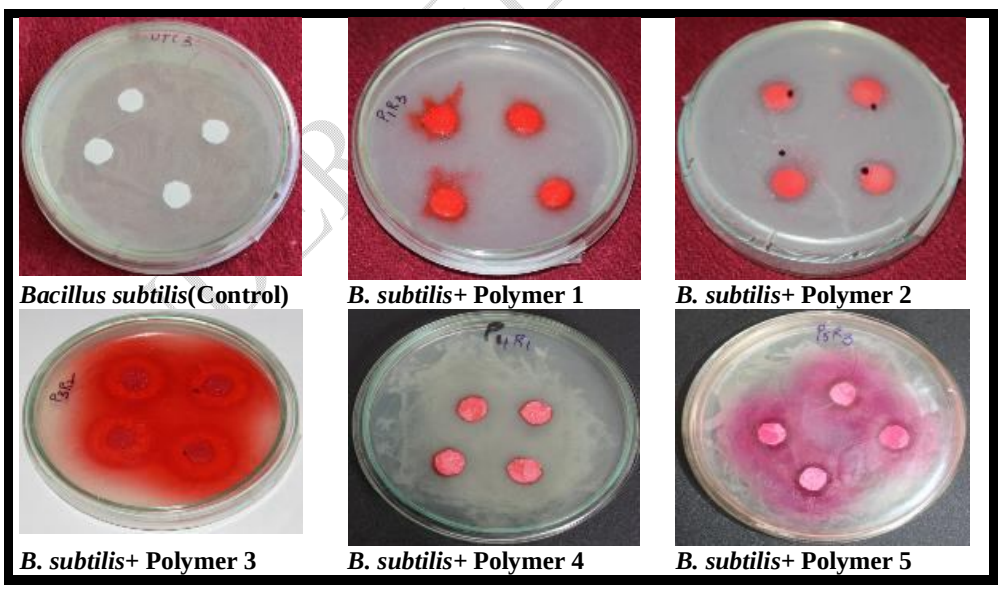

Plate 3: Compatibility of *Bacillus subtilis* (zones of inhibition) with commercially availablecoloured polymers