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Evaluation of Carrier Materials for the Development of *Actinoalloteichus cyanogriseus* Bio-formulation with better Shelf-life

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ABSTRACT: Unlike other diseases of crops, biocontrol agents could provide better control of soil borne diseases than chemicals. One of the constraints in employing the biocontrol agent is their shelf-life of the product. For the better shelf-life of bioformulations, best compatible carrier has to be identified and utilized for formulation development. In this study physico-chemical properties of four carrier materials and their effect on black gram seed germination, seedling vigour were analysed. In addition, the carrier materials *viz.*, rice husk ash, saw dust, rice husk and talc were used to study the shelf-life of the potential antagonistic actinobacterial strain *Actinoalloteichus cyanogriseus* at 30 days interval up to 150 days. Among all, rice husk ash (RHA) showed better physico-chemical properties and maintained higher colony count (95 × 10^7 CFU) even after 150 days. Also, the seeds treated with RHA showed the higher germination percentage (94% and 90%) and seedling vigour (2705 and 1823) in the roll towel and protray studies, respectively.

Keywords: Carrier material, seedling vigour, actinobacteria, shelf-life.

INTRODUCTION

The chemical method of plant disease management is widely adopted across the world, because it provided better results in short term. But due to the residual effect of chemicals in both soil and plant, it caused more ecological problem and health hazards to humans (Pandya *et al*., 2011). Soil borne pathogens have wide range of hosts and survive in soil for many years by resisting the adverse climatic conditions, fungicidal effect, etc. with the help of resting structures. Moreover, the chemical method achieved only certain degree of control against soil-borne pathogens, hence the biological method has been preferred now-a-days (Amin *et al*., 2010). Biological method of combating plant diseases using antagonistic organisms provided a powerful and eco-friendly alternative instead of using synthetic chemicals (Emmert and Handelsman 1999).

Actinobacteria considered as an important source of many known antibiotics, secondary metabolites and reported as potential agent for managing many phytopathogens under *in vitro* condition (Khanna *et al*., 2011) and most of them could not be effectively employed under field condition due to lacuna in bioformulation development. However, any good bioformulation must be easy for handling, transport, shelf life, storage and application to the target site (Gopalakrishnan *et al*., 2016). The efficacy and shelf-life of these antagonistic microorganism based bioformulations are largely depending on various factors such as carrier material, storage conditions etc. The selection of carrier material should focus on improving the shelf-life of biocontrol agent and retaining their antagonistic activity for longer time. They should provide suitable micro environment to prevent sudden decline of microbial population. Carrier materials normally used were talc, peat, farm yard manure, vermicompost, lignite, compost and bagasse (Kumar, 2014). One of the delivery methods of bioformulation to combat soil-borne pathogens is seed treatment, therefore the carrier material should support both the microorganism and seed germination.

MATERIALS AND METHODS

Biocontrol agent. Actinobacterial strain *Actinoalloteichus cyanogriseus* - M12 has been obtained from the Microbial Type Culture collection, Department of Plant Pathology, TNAU, Coimbatore and maintained in Starch Casein Agar (SCA) at 30 ± 2 °C.

Carrier materials. Carrier materials used *viz*., rice husk (RH), rice husk ash (RHA), saw dust (SR), talc (T) were procured from different sources. Physico-chemical characteristics of the carriers like pH, particle size, colour, organic matter, water holding capacity (WHC) were analysed (Jayasudha *et al*., 2017; Nelson *et al*., 1974; Hariz *et al*., 2015).

Effect of carrier material on seed germination. To analyse the effect of carrier material on seed germination, black gram seeds (VBN 8) were used. Seeds were evenly coated with carrier materials *viz*., rice husk (RH), rice husk ash (RHA), saw dust (SD), talc (T) @10 g per kg of seeds using the binding agent carboxy methyl cellulose (CMC @ 10g per kg of carrier material). Germination test was carried out using roll towel method (ISTA, 1993) and also in the protray (Desai *et al*., 2020).

Roll towel studies. Black gram seeds coated with different carrier materials was placed on the germination paper (25 seeds/ paper) with four replications. Rolled germination paper was kept in standing position with the seeds at topside in a beaker. Beaker was half-filled with water and covered by polythene sheet to avoid drying. After incubation for seven days at 28˚C germination paper was unrolled to calculate germination per cent and seedling vigour (Jambhulkar *et al*., 2013). The germination per cent and seedling vigour were calculated using the formula.

$$\text{Germination percentage} = \frac{\text{No. of seeds germinated}}{\text{Total no. of seeds tested}} \times 100$$

Seedling vigour = (shoot length + root length) × Germination %

Protray studies. Black gram seeds coated with different carrier materials were sown in sterilized soil at the rate of one seed/ well (4.5 cm × 4.5 cm × 4 cm) in a protray. For each treatment, four replications were maintained with 10 seeds/ replication in protrays at glass house condition. Germination per cent and seedling vigour were measured at 7th and 14th day after sowing (DAS) respectively using the above formula.

Bioformulation preparation. *A. cyanogriseus* M12 strain was grown in Starch Caesin Broth (SCB) for 14 days at 30±2˚ C. The broth containing more than 50 × 10^7 CFU/ ml was used for bioformulation preparation using different carriers. For 100 g of carrier material 40 ml of broth, 1g of CMC was added. It was packed in polythene bag with moisture content less than 20 % and stored at room temperature (28±2˚ C) for shelf-life studies (Vidhyasekaran and Muthamilan 1999).

Shelf-life estimation. Shelf-life was estimated using serial dilution and pour plate technique (Patil *et al*., 2021). One gram of bioformulation was added to 10 ml of sterilized distilled water (10^{-1} dilution), vigorously mixed and serially diluted up to 10^{-8} dilution. From this, 1 ml of suspension was added to Petri dish, 15 ml of SCA medium poured over. Replicated thrice and incubated at 30 ± 2 ˚C for 14 days. The number of colonies were counted and the procedure was repeated at 30 days interval up to 150 days. Fresh sample was drawn each time and analysed (Jayasudha *et al*., 2017).

RESULTS AND DISCUSSION

The potential antagonistic *Actinoalloteichus cyanogriseus* M12 strain used was isolated from mulberry plants, and found to be effective against pathogens like *Macrophomina phaseolina*, *Lasiodiplodia theobromae*, *Athelia rolfsii*, *Fusarium* sp and *Rhizoctonia solani* (Saratha *et al.*, 2022).

Agricultural by-products are available in huge quantities at low cost and now, these were utilized for promoting soil and plant health. The physico-chemical characters analysed (Table 1) for different carrier materials showed that SD and RHA had higher water holding capacity. These agro industry wastes have good organic matter content than conventionally used inert talc carrier material. Sarin and Riddech (2018) also studied the effect of various agricultural residues *viz.,* rice husk ash, rice straw, cane leaves, coconut fibre as carriers for bio-fertilizer production and tomato growth promotion. Among the seeds treated with different carrier materials in the roll towel and protray studies, rice husk ash (RHA) showed higher germination (94 % and 90 %) on par with saw dust (92 % and 90 %) (Table 2 & 3).

Table 1: Physico-chemical characteristics of carrier material.

Sr. No.	Carriers	Particle size	pH	Colour	Water Holding Capacity (WHC) (%)	Organic matter (%)
1.	Rice husk	7 mm	7.9	Light brown husk	58	40
2.	Saw dust	267 µm	5.21	Brown dust	424	76
3.	Rice husk ash	145 µm	9.5	Black dust	400	5
4.	Talc	20 µm	9.03	White powder	52	0

Table 2: Effect of carrier materials on seed germination using roll towel method.

Sr. No.	Carriers	Germination (%)	Seedling biometrics (7DAS)		Seedling vigour
			Shoot length (cm)	Root length (cm)	
1.	Rice husk	91^{ab}	14.94^{b}	12.27^{b}	2476^{c}
2.	Sawdust	92^{a}	16.01^{a}	12.30^{b}	2605^{b}
3.	Rice husk ash	94^{a}	16.02^{a}	12.76^{a}	2705^{a}
4.	Talc	88^{bc}	15.71^{a}	12.75^{a}	2504^{c}
5.	CMC	88^{bc}	15.86^{a}	11.38^{c}	2398^{d}
6.	Control	86^{c}	16.05^{a}	11.72^{c}	2389^{d}
	SEd	1.734	0.203	0.189	36.047
	CD (.05)	3.695	0.433	0.402	76.834

DAS – Days After Sowing

Table 3: Effect of carrier material on seed germination grown in portray.

S. No.	Carriers	Germination (%)	Seedling height (cm)		Seedling vigour	
			7 DAS	14 DAS	7 DAS	14 DAS
1.	Rice husk	85[b]	6.28[e]	16.30[c]	533[d]	1386[c]
2.	Sawdust	90[a]	9.70[a]	19.90[ab]	873[a]	1791[a]
3.	Rice husk ash	90[a]	9.08[b]	20.25[a]	816[b]	1823[a]
4.	Talc	85[b]	8.55[c]	19.63[b]	726[c]	1668[b]
5.	CMC	70[c]	7.18[d]	16.03[c]	502[e]	1122[d]
6.	Control	60[d]	5.80[f]	8.45[d]	348[f]	507[e]
	SEd	0.901	0.121	0.243	9.920	20.043
	CD (.05)	1.920	0.257	0.518	21.144	42.722

DAS – Days After Sowing

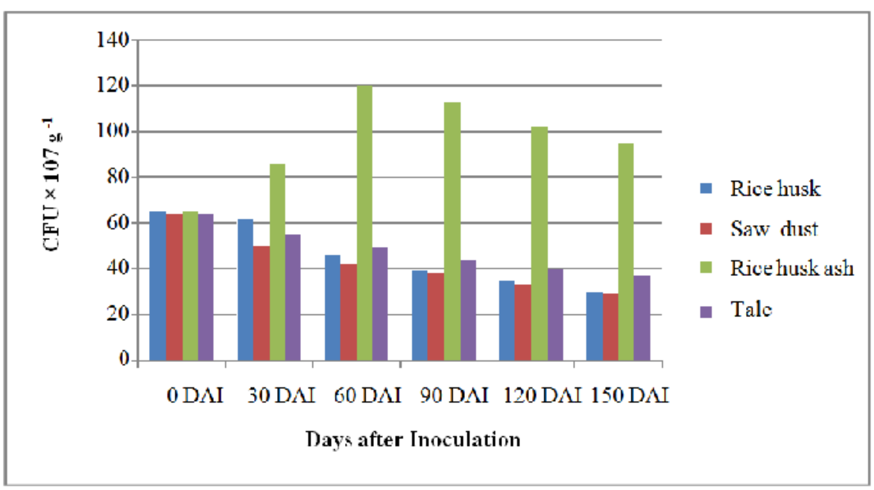
However, RHA showed significantly higher seedling vigour (2705 and 1823) in both tests. Comparatively, control seeds showed lower germination of 86 per cent in roll towel and 60 per cent in protray. Similarly, in case of seedling vigour, control showed lowest value in both roll towel (2389) and protray (507). The above results showed that RHA treated seeds had increased germination per cent and seedling vigour of black gram seeds by 9.3% and 48.4% respectively was corroborated with previous report by Lu *et al.* (2015). They reported that tomato seeds treated with nano silica powder prepared from RHA showed 22 per cent increase in germination and 92 per cent vigour index over control.

Presetya *et al.* (2018) reported that higher silica content (90%) in RHA helped in maintaining moisture in the seeds which could enable higher germination of black gram seeds. Similar results were reported by Tsakaldimi (2006) that addition of RHA with peat to the growth medium, *Pinus halepensis* showed higher shoot length and dry weight than control growth medium. Rice husk ash increased resistance to paddy against *Xanthomonas oryzae* pv. *oryzae* and showed significant difference in plant height, improved photosynthesis. Due to presence of silica in RHA, it alleviated water stress, salinity stress, nutrient deficiency and improved erectness of leaves, there by increased rice yield and mitigated abiotic stress (Medrano *et al*., 2021). Rice husk ash application improved the yield of wheat about 24 % in mean grain yield.

Table 4: Shelf-life study of *Actinoalloteichus cyanogriseus* in different bioformulations.

Sr. No.	Carriers	Number of colonies in different bioformulations of *Actinoalloteichus cyanogriseus* (CFU × 10^7 g^{-1})					
		0 DAI	30 DAI	60 DAI	90 DAI	120 DAI	150 DAI
1.	Rice husk	65	62[b]	46[b]	39[c]	35[c]	30[c]
2.	Saw dust	64	50[d]	42[c]	38[c]	33[c]	29[c]
3.	Rice husk ash	65	86[a]	120[a]	113[a]	102[a]	95[a]
4.	Talc	64	55[c]	49[b]	44[b]	40[b]	37[b]
	SEd		1.799	1.673	1.342	1.456	1.619
	CD (.05)	NS	3.920	3.646	2.923	3.172	3.527

DAI – Days after Inoculation



DAI – Days After Inoculation

Fig. 1. Shelf-life of *Actinoalloteichus cyanogriseus* in different bioformulations.

The shelf-life studies showed M12 strain found to have longer survival ability (Table 4) in almost all carriers used. However, among four carrier materials tested, RHA had significantly better results for shelf-life and maintained higher colony count (95×10^7 CFU g^{-1}) even after 5 months (Table 4). RHA found to have suitable properties for growth of antagonistic M12 strain like alkaline pH, with minerals and nutrients (Table 1). Previously, Saratha *et al.* (2022) found that M12 strain could survive at wide temperature and pH range. Durham and Simonton (1985) reported pH of RHA was about 8.4 and had high silica content, supported the better survival of actinobacteria.

Similar results were reported by Sarin and Riddech (2018) that RHA as best carrier, which showed the highest growth and survival of rhizobacteria at wide temperature regions (30- 50˚C), maximum number of rhizobacteria colonies was recorded (10^9 –10^{10} CFU g^{-1}) on 15th day of incubation at 30˚C. They also mentioned RHA as suitable for microbial immobilization and tomato growth promotion. Silica content in rice husk ash was used by actinobacteria for their growth (Mezan *et al.*, 2020). Ratna *et al.* (1996) reported that RHA increased the soil microbial activity.

CONCLUSION

Among the carrier materials tested rice husk ash enhanced the biometric parameters of black gram seedlings and the shelf-life of antagonistic actinobacterial strain. It had better physico-chemical properties especially WHC and the seeds treated with rice husk ash in the roll towel method and protray studies showed the higher germination percentage and seedling vigour. RHA maintained higher population of M12 strain even after 150 days and found as suitable carrier.

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Conflict of interest. Authors have declared that no conflict of interest exist.

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