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RESEARCH ARTICLE Agricultural Science Digest

Seed Coating with Biodegradable Stickers for Enhancement of Inoculant Viability and Their Beneficial Properties on Seed Germination of Blackgram [*Vigna mungo* (L.) Hepper.]

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ABSTRACT

Background: This study assessed the effect of different sticking agents *viz*., gum arabic, guar gum and xanthan gum for coating *Rhizobium* sp. and Arbuscular Mycorrhizal Fungi (AMF) on seed germination and vigour of blackgram seeds.

Methods: The surface sterilised seeds were first coated with different sticking agents as per the following treatments, T_0 - Dry seed, T_1 - Water, T_2 - Gum arabic 20% w/v, T_3 - Guar gum 0.5% w/v, T_4 - Xanthan gum 0.3% w/v followed by coating with *Rhizobium* sp. and AMF liquid cultures.

Result: Among the different sticking agents, gum arabic (20%) when used as a sticker for coating *Rhizobium* sp. and AMF, increased the seed germination (95%) and vigour index (21.85) of blackgram compared to the control. The efficacy of inoculation depended on the survival of inoculants on seeds. On using sticking agents, the survival of microbes could be extended up to 48 h of treatment and 87 percent AMF colonization with gum arabic (20%) as deduced from the viability studies.

Key words: Blackgram, Germination, Gum arabic, Microbial survival, Seed coating, Sticking agents.

INTRODUCTION

Leguminosae family pulses are the main source of protein in the vegetarian diet. Among the pulses, black gram contains 25-26% of protein (Amuthaselvi *et al*., 2019). India is the world's largest producer and consumer of blackgram, with a 41.4 lakh ha area. In 2020-2021, production and productivity are estimated to be 22.3 lakh tonnes and 538 kg/ha, respectively (Indiastat, 2022). The low productivity in pulses was due to the reason that they were grown in poor unfertile lands with low soil moisture and uncertain rainfall patterns. Instead of utilising chemical fertilisers to increase production, using microbial inoculants offers a solution to the afore mentioned issues as well as the advantage of benefitting the environment since it is an environmentally sustainable approach.

The legume-rhizobium seed inoculation has been known long back due to its role in symbiotic nitrogen fixation in the root nodules and Arbuscular mycorrhizal fungi (AMF) forms a symbiotic relationship with plant roots and has long been recognised as having an impact on several plant characteristics, phosphorous uptake and mobilization, improves soil structure and reduces the negative effects of various biotic and abiotic stresses (Vessey, 2003; Manoharachary *et al*., 2009; Riaz *et al*., 2023). The positive influence of microbes on co-inoculation of *Rhizobium* sp. - AMF have been reported in common bean (Tajini *et al*., 2011) and blackgram (Choudhury and Azad, 2004).

The selective assemblage of the microbial community in the root zone of legumes can be achieved through seed enhancement techniques (Annadurai *et al*., 2021). Standardising seed enhancement techniques will take care of the initial germination process which is the crucial stage as it is the beginning of the life cycle of crop plants and the benefits can extend well beyond seedling establishment. Seed coating is one of the methods of microbial inoculation in which a thin inseparable even coating of microbes with a binder material is coated onto the seeds.

Microbes that have been inoculated in soil faces harsh environment and competition from native microorganisms. To secure the greatest benefits of seed coating, inoculated seeds must maintain a high number of viable cells. One of the methods to protect the coated microbes from the adverse environment and to ensure a threshold level of microbes on the seed is adding sticking agents. The development of microbial seed coating with the use of biodegradable stickers

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How to cite this article: Monisha, S., Renganayaki, P.R., Sundareswaran, S., Nakkeeran, S. and Varanavasiappan, S. (2023). Seed Coating with Biodegradable Stickers for Enhancement of Inoculant Viability and Their Beneficial Properties on Seed Germination of Blackgram [*Vigna mungo* (L.) Hepper.]. Agricultural Science Digest. doi: 10.18805/ag.D-5724.

Submitted: 03-01-2023 **Accepted:** 14-02-2023 **Online:**

to enhance plant growth while having minimal impact on the environment has been receiving a lot of attention. Sticking agents include alginate, gum arabic, carboxymethyl cellulose, sucrose solutions, vegetable oils, agar, α- and k-carrageenan, gellan gum, guar gum, bean gum, starch, xanthan, pectin, chitosan, waxes and lignin can also be used. In this view, the objectives of this study were to analyse the effectiveness of different sticking agents in aiding the inoculation of *Rhizobium* sp. and AMF. The response to inoculation was determined by measuring the germination, seedling growth and *Rhizobium* sp. survival after different hours of inoculation root colonization ability of AMF on blackgram seeds.

MATERIALS AND METHODS

Materials

The laboratory experiment was carried out at the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore during the year 2021-2022. Freshly harvested black gram seeds of the VBN 8 variety were obtained from Krishi Vigyan Kendra, Vamban, Tamil Nadu, South India. The bio-inoculants *viz., Rhizobium* sp. BMBS, used in the experiment was obtained from the Department of Microbiology, Tamil Nadu Agricultural University, Coimbatore. *Rhizobium* sp. was cultured in YEMA broth and was incubated for 48 h to get a maximum cell population of 10^8 CFU ml^{-1}. The cells were harvested by centrifugation at 8,000 rpm for 20 minutes. The cell pellet was resuspended in sterile phosphate buffer and adjusted to get an OD value of 1.0 at 600 nm and Arbuscular Mycorrhizal Fungi (AMF) liquid inoculant composed of *Glomus* sp. and *Acaulospora* sp. was procured from Uyir Organic Farmers Market, Coimbatore with a spore count of 1×10^3 spores ml^{-1}.

Sticking agent preparation

The sticking agents such as gum arabic, guar gum and xanthan gum were dissolved by heating. The concentration and source of the sticking agents used in the study were mentioned in Table 1. The treatment details were as follows, T_0 - Dry seed, T_1 - Water, T_2 - Gum arabic 20% w/v, T_3 - Guar gum 0.5% w/v, T_4 - Xanthan gum 0.3% w/v.

Seed coating procedure

The seeds were surface sterilized with 70% ethanol for 1 min followed by sodium hypochlorite solution (0.5%) for 3 min and then rinsed in sterile distilled water five times. The seeds were first coated with respective sticking agents according to the treatment in a separate inflated polythene bag at a rate of 15 ml kg^{-1} of seeds followed by coating with a microbial consortium of *Rhizobium* sp. containing 1×10^8 cells and AMF with 1×10^3 spores ml^{-1} mixed in equal ratios. Seed coating was performed by vigorous shaking and after that seeds were subjected to shade drying at room temperature.

Seed physiological parameters

The coated black gram seeds were tested for different physiological parameters *viz*., germination percentage (ISTA, 2015), root length, shoot length, dry matter production and vigour index values along with control. The experiment was conducted by adopting a Completely Randomized Block Design (CRD) in five replications. Root length was the mean of ten normal seedling lengths measured from the collar region to the tip of the primary root in cm while shoot length was measured from the growing tip to the collar region. After measurements, the seedling was shade dried for 24 h and subjected to hot air oven drying at 80°C for 24 h. The weight after drying was the dry matter production of seedlings. The seedling vigour index was calculated as per Abdul-Baki and Anderson (1973) and the mean was expressed as a whole number.

$$\text{Seedling vigour index} = \text{Germination (\%)} \times \text{Dry matter production.}$$

Microbial survival of *Rhizobium sp.*

The survival of *Rhizobium* sp. was determined by the plate count method on Yeast Extract Mannitol Agar (YEMA) plates at regular intervals of every 12 h by transferring 1 g of coated seeds to 100 ml sterile water followed by 5 min agitation and the suspension was plated by serial dilutions on Congo-red YEM agar. Colony counts of *Rhizobium* sp. were made after incubation at 30°C for 2 days. The experiment was terminated when the seeds were cross-contaminated by other organism at varying intervals depending on the treatment. Data were the mean values of four independent counts and the number of rhizobial cells was expressed as the log number of cells $gram^{-1}$ of seeds.

Root colonization of AMF

The root colonization efficiency by AMF was assessed by trypan blue staining of roots taken from the seven days old seedlings. Root bits of 1 cm were cleared in 10% KOH at 80°C for 30 min. After incubation, 2 % hydrochloric acid (HCl) was added and then root bits were washed in tap water followed by staining in 0.008% trypan blue (Phillips and Hayman, 1970). 50 segments of each replicate were observed. The stained roots were observed in stereomicroscope at 5 X magnification for the presence of arbuscules, vesicles, or both, mycelia and spores. the root colonization percent by AMF was calculated with the formula,

$$\text{Root colonization (\%)} = \frac{\text{Number of root segments infected}}{\text{Total number of root segments investigated}} \times 100$$

Statistical analysis

The data obtained were subjected to analysis in AGRESS software. The comparison of the mean values was made using the least significant difference test at a level of 5% probability (Panse and Sukhatme, 1954).

RESULTS AND DISCUSSION

In terms of germination and seedling vigour, coating of *Rhizobium* sp. and AMF to blackgram seeds performed

better than the control seeds. A highly significant difference was found in gum arabic 20% (T_2) followed by guar gum 0.5% (T_3) when used to coat *Rhizobium* sp. and AMF to seeds than the control in terms of germination (95%, 93% and 89%), root length (19.53 cm, 18.45 cm, 17.60 cm), dry matter production (0.230, 0.199, 0.106 g/10 seedling) and vigour index (21.85, 18.51, 9.45) respectively (Table 2 and Fig 1). Similar results were found by Priya *et al*. (2019), who registered that the *Rhizobium* strain increased the germination and vigour index of groundnut on seed treatment. Mia *et al*. (2012) opined that inoculation of *Rhizobium* strains promoted seedling emergence and vigour by the production of phytohormones. Significant increase in root length was a growth response that might be attributed due to the production of IAA by *Rhizobium* sp. (Mohite, 2013). Microbes through phytohormone and hydrolysing enzyme production interacted with the seedlings and facilitated the nutrient mobilization from the endosperm to the embryo, which could have reflected in the dry matter production. Through enhanced nutrient uptake by AMF, might have increased dry matter production (Clark *et al*., 1999).

The effect of the sticking agent on the survival of *Rhizobium* sp. on coated seeds was evaluated by the plate count method. Among the different sticking agents, Gum arabic 20% recorded a higher initial cell concentration of Log 6.2 CFU g^{-1} than all other treatments. Up to 60 h after treatment, it showed a rhizobial cell concentration of Log 0.3 CFU g^{-1} (Fig 2). Followed by gum arabic 20%, guar gum 0.5% recorded higher survival at various hours after treatment. With the proper type of sticking agent and amount of inoculant, the number of microbes adhering to the seed substantially increased (Berruti *et al*., 2016). When water was used, less than 100 rhizobia g^{-1} of seed was reached within 12 h of coating whereas, when gum arabic was used at least 100 rhizobia g^{-1} was found till 48 h after coating (Fig 2). Researchers had suggested that at least 100 rhizobia/seed should be applied, when planting under ideal conditions (Waggoner *et al*., 1979).

Per cent mycorrhizal colonization in the roots of blackgram was significantly influenced by sticking agent. The seedling roots were observed after seven days for colonisation (Fig 3). Selvaraj and Thangavel (2022) documented the colonization of blackgram roots by AMF within 10 days of inoculation. For the sticking agents analysed, the spore count per seedlings were 0, 5, 35, 30 and 17 for control seed, water, gum arabic, guar gum and xanthan gum treatments respectively.

The root colonization percent for different sticking agents were assessed. The maximum AMF colonization was 87% recorded with gum arabic 20% (T_2), followed by guar gum 0.5% (64%). When water was used only 20% root colonization was detected (Table 3). The use of sticking agent which were in sticky consistency might enhance the microbial spore adherence to the seed surface and immobilised it. AMF spore abundance on the seed surface is directly proportional to the percent root mycorrhizal colonization (Selvaraj and Thangavel, 2022).

Similar root colonization by seed coating of AM fungi was documented by Oliveira *et al*. (2016) and Rocha *et al*. (2019). Successful AMF inoculation can be confirmed by root colonization efficiency. Colonization is necessary for the AMF symbiosis to work and for plants to benefit from the interaction between fungi and plants (Calvo *et al*., 2014). No colonization was seen in the uninoculated control.

Sticking agents must bind the inoculant to the seed and protect them from desiccation and some might provide

Table 1: Concentrations and sources of the sticking agents used.

Treatment	Sticking agent	Concentration	Source
T_1	Water	15ml/kg	Distilled water
T_2	Gum arabic	20% w/v	HiMedia, RM682
T_3	Guar gum	0.5% w/v	HiMedia, GRM 1233
T_4	Xanthan gum	0.3% w/v	Loba chemie, 06494

Table 2: Effect of sticking agents on seed germination and seedling growth of blackgram VBN 8.

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	Dry matter production (g/10 seedlings)	Vigour index
T_0	89 (70.63)	17.60	20.06	0.106	9.45
T_1	92 (73.57)	17.70	20.16	0.123	11.32
T_2	95 (77.08)	19.53	21.56	0.230	21.85
T_3	93 (74.66)	18.45	21.22	0.199	18.51
T_4	92 (73.57)	18.18	20.40	0.154	14.17
Mean	92 (73.57)	18.29	20.68	0.162	15.06
SEd	1.32	0.20	0.42	0.003	0.22
CD	2.76	0.41	0.87	0.006	0.46

(Figures in parenthesis indicate arcsine transformed values) T_0- Dry seed; T_1- Water; T_2- Gum Arabic 20%; T_3- Guar gum 0.5%; T_4- Xanthan gum at 0.3%.

Table 3: Effect of sticking agents on the spore load and percent AM colonization in blackgram roots.

Treatments	Number of spores/ seedling	Per cent AM colonisation
T_0	0	0
T_1	5	20
T_2	35	87
T_3	30	64
T_4	17	45
SEd	0.40	0.99
CD (P=0.05)	0.82	2.04

T_0 - Dry seed; T_1 - Water; T_2 - Gum Arabic 20%; T_3 - Guar gum 0.5%; T_4 - Xanthan gum at 0.3%

nourishment and energy source for the inoculants. Gum arabic performed all three of these functions (Vincent, 1958; Date, 1970; Elegba and Rennie, 1984). Among the stickers investigated, gum arabic 20% was best in increasing the affinity between the seed coat and coating material and possessed qualities such as strength and plasticity that prevented the deterioration of the coated material.

Gum arabic is a complex carbohydrate extracted from Acacia plants, which protects the inoculants against desiccation and results in better survival on seed (Vincent *et al*., 1962). Among the stickers, lower viable cell value was found when water was used as a sticking agent. One of the most popular sticking agents, water, initially produced good adherence but does not nourish or shield microorganisms

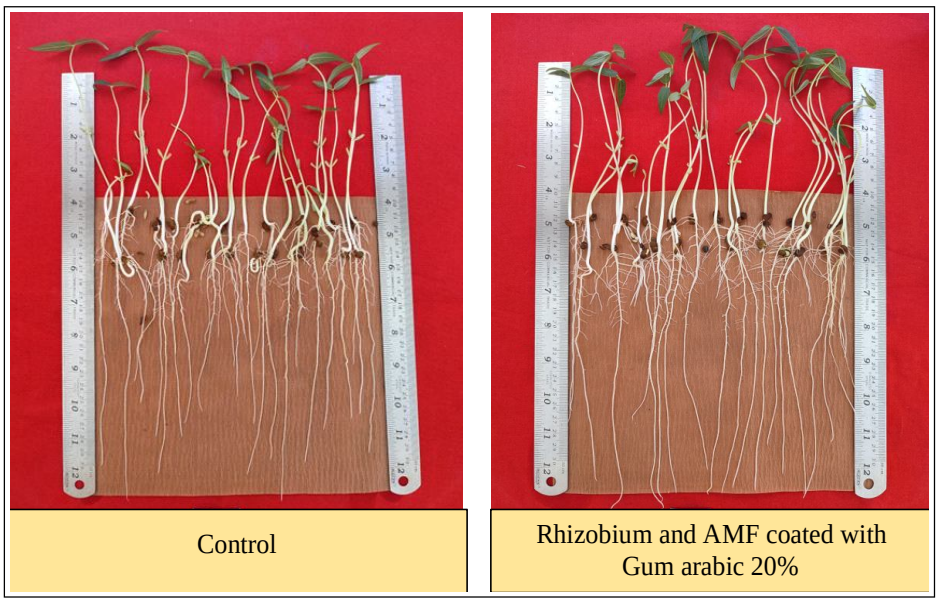

Fig 1: Effect of sticking agents on seed germination of blackgram.

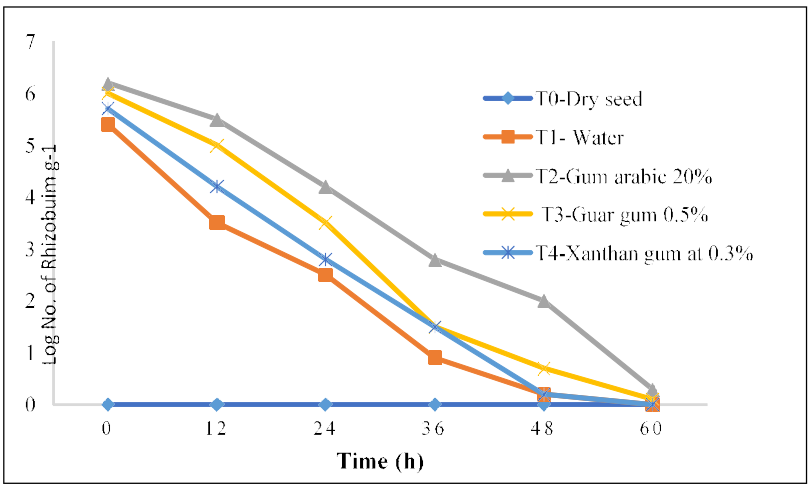

Fig 2: Survival of *Rhizobium* sp. coated with different sticking agents.

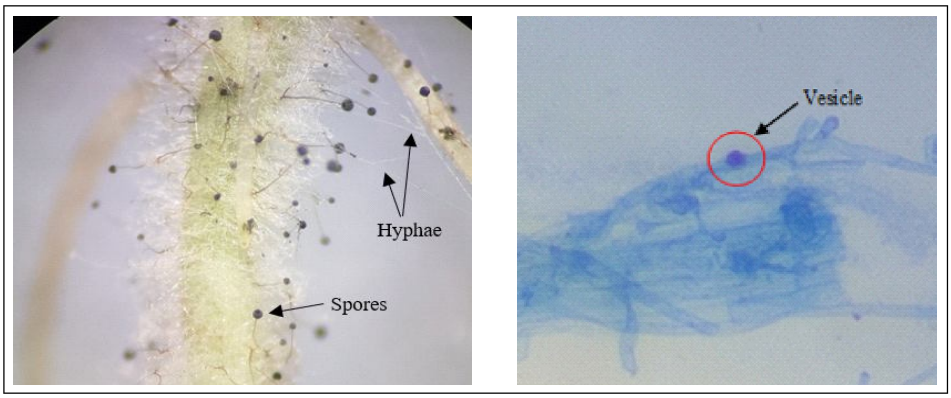

Fig 3: Microscopic view of AMF colonization in blackgram roots.

from desiccation (Hoben *et al*., 1991). Hence water as an adhesive agent cannot be recommended.

CONCLUSION

The present study concluded that the rhizobium and AMF inoculation showed increased seed germination and seedling vigour of blackgram. After screening with different sticking agents, gum arabic was selected for seed coating based on the survival study. Gum arabic (20%) coated seeds showed a higher cell population of *Rhizobium* sp. and higher root colonisation percent by AMF after treatment.

Conflict of interest

The authors declare that there is no competing interest.

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