



Akta Agrosia

Delivery of Arbuscular Mycorrhiza Fungus Spores via Seed Coating with Biodegradable Binders for Enhancement of the Spores Viability and Their Beneficial Properties in Maize

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ABSTRACT

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(11 pt) The development of microbial seed coating with the use of a biodegradable binder to enhance plant growth while having minimal impact on the environment has been receiving a lot of attentions. Tapioca starch mixed together with a synthetic biodegradable polymer such as PVA is considered the most promising candidates for developing the sustainable sticker. The objective of this study was to determine the most suitable PVA + TS blends as adhesives agent for AMF spores inoculation via seed coating which can enhance the spores viability and their beneficial properties in maize. The polythene bag experiment was performed in a screen house of the Department of Plant Protection Faculty of Agriculture, University of Bengkulu Indonesia in 2015. Six adhesive blends were employed: 100% PVA + 0% TS, 75% PVA + 25% TS, 50% PVA + 50% TS, 25% PVA + 75% TS, 0% PVA + 100% TS, and no coating. The six experimental treatments were laid out in a completely randomized design with three replications. The results show that root colonization, AMF spore population, and shoot dry weight in 75% PVA + 25% TS were equal to those in 100% PVA. Root colonization, AMF spore population, shoot P content, and shoot P concentration were greater for 50% PVA + 50% TS than 100% PVA, 100% TS, and no coating. A mixture of 50% PVA + 50% TS was considered the preferred sticker. Thus, the tapioca starch can be used to substitute 25 - 50% of the PVA used without reducing AMF inoculant adhering to seed.

INTRODUCTION

Globally, together with rice and wheat, corn provides approximately two-thirds of all energy in human diets, emphasizing the economic importance and nutritional value of this crop (Cassman *et al.*, 2003). Intensive farming practices that lead to high corn yield require

chemical fertilizers including P, which are not only expensive but may also create environmental problems (Schröder *et al.*, 2011). Consequently, there is an increasing interest in the use of plant beneficial microorganisms (PBMs) such as arbuscular mycorrhizal fungi (AMF) as alternatives to the synthetic fertilizers in agricultural production (O'Callaghan, 2016).

The role of AMF to promote plant fitness have been demonstrated by many researchers (Fedderman *et al.*, 2010; Njeru *et al.*, 2015; Oliveira *et al.*, 2017b; Holečková *et al.*, 2017; Thonar *et al.*, 2017). The application of AMF in the field is usually accomplished by broadcasting a lot of inoculum with zeolite as a carrier. This inoculation method was considered economically unfeasible due to high cost need per plant (Vosátka *et al.*, 2012; Oliveira *et al.*, 2016b). These drawbacks are encouraging the development of an efficient AMF inoculation technique in which active ingredients are applied at the lowest possible dose rate so as to have minimal impact on the environment. Seed coating has the potential to meet these economic and safety requirements and is here proposed as an inoculation mechanism for maize seeds (Colla *et al.*, 2015; Ehsanfar and Modarres-Sanavy, 2004; Oliveira *et al.*, 2016b).

Seed coating is the technique of enclosing the seed surface with foreign materials such as AMF spore inoculants or other materials combined with an adhesive and to some extent filler as a carrier to enhance seed performance and modify the physical properties of seed (Pedrini *et al.*, 2017). This inoculation method allows the use of minor amounts of inoculum in a precise application and provides an opportunity for reduced application rate per hectare by reducing the need to treat the seed furrow or bulk soil (Amirkhani *et al.*, 2014; Oliveira *et al.*, 2016a; Rouphael *et al.*, 2017; Accinelli *et al.*, 2018a; Accinelli *et al.*, 2018b; Rocha *et al.*, 2019a; Rocha *et al.*, 2019b).

The success of seed coating mostly relies on an accurate selection of the coating material such as adhesives, which may influence AMF spore viability. When the beneficial fungi are applied via seed coatings, the adhesive materials function as a binding agent between seeds with the carriers that carry AMF spores and the filler materials play a role as AMF inoculum carriers. With this technique, AMF can infect the roots directly on newly germinated seeds (Amirkhani *et al.*, 2014; Oliveira *et al.*, 2016a). Thus, the adhesives used should have good coating properties such as non-phytotoxic, readily biodegradable, environmentally safe, good moisture retention,

and inexpensive. With the proper type of a sticking agent and amount of inoculant, the number of AMF spores adhering to the seed substantially increased (Berruti *et al.*, 2016).

The AMF inoculation on different seeds through seed coating with various types of adhesives such as arabic gum and sodium alginate have been demonstrated by many researchers (Sari, 2009; Khodijah, 2009; Musfal, 2010). In their studies, the amount of AMF spores that were inoculated on the seeds was 50 spores per seed to ensure successful infection of germinating AMF spores on actively growing plant roots. As a binding agent, arabic gum is more tenacious and provides an excellent adhesion. However, it is awkward to use, not readily available and is too expensive for most farmers. The higher price is more apparent for sodium alginate than arabic gum. Also, it is also difficult to obtain on the market since it is originated from brown algae.

At present, coating material's degradability is an important focus of the research in this field because of the renewed attention towards environmental protection issues (Taylor *et al.*, 2001). Tapioca starch (TS) is of interest and considered as a promising candidate among the natural polymers as adhesives due to its complete biodegradability, low cost, abundant availability, and renewability. Unfortunately, the starch has disadvantages, i.e. no physico-mechanical characteristics. The products from starch are mostly water soluble and brittle (Majeed and Kamil, 2013).

Improving some properties of starch can be accomplished by mixing with synthetic polymers (Lu *et al.*, 2009) such as polyvinyl alcohol (PVA). The reason is that it is well known as a synthetic biodegradable polymer and non-phytotoxic hydrophilic polymer and possesses excellent mechanical properties (Song *et al.*, 2018). PVA is one of the best options to be blended with starch and valued for its solubility and biodegradability, which contributes to its minimal environmental impact (Majeed and Kamil, 2013). PVA is a kind of excellent membrane material for preparation of a hydrophilic layer that swells quickly and even dissolves in water. With its hydrophilic characteristic, PVA is suitable used

as an adhesive for seed coating because it prevents AMF spores from dehydrating in elevated soil temperature, thus maintaining their viability. The mixture of PVA + TS is widely used in packaging and agricultural applications. Despite numerous reports on the mechanical properties and biodegradability of PVA + TS blend films, surprisingly little data exist on their inherent properties as adhesives for inoculation of corn seed with AMF spores via seed coating. Therefore, further research is needed before the benefits of a full range of potential PVA + TS blends as an adhesive for seed coating with AMF can be captured for use in open agricultural fields. The present study was conducted with the objective was to determine the most suitable PVA + TS blends as an adhesives agent for AMF spores inoculation via seed coating which can enhance the spores viability and their beneficial properties in maize.

MATERIALS AND METHODS

Production of arbuscular mycorrhizal mother inoculum in polythene bags

The arbuscular mycorrhizal fungal species of *Glomus* sp. was obtained and multiplied in the Laboratory of Plant Protection Department of Plant Protection Faculty of Agriculture University of Bengkulu Bengkulu Indonesia. A commercial corn was utilized as a host plant to produce the arbuscular mycorrhizal fungi propagules. Before planted in 10 kg poly bags, corn seeds were surface sterilized for 10 minutes with a 10 % of household bleach. The 10 % of household bleach was prepared by mixing 1 volume of household bleach containing 5% sodium hypochlorite (NaOCl) plus nine volumes of water. Then, they were rinsed three times in running water and planted in a 10 kg polybag containing 1:1 sterile sand: soil mixture. The soil was collected from the uppermost 10-cm layer of research farm in the University of Bengkulu, sieved through a 4-mm mesh. The soil and sand mixture was autoclaved before its utilization at 121⁰C for 40 minutes. Soil type used the AMF spore multiplication method was Ultisol obtained from the field trial.

Corn seed was pre-germinated for 3-4 days at room temperature on tap water wetted tissue

paper by placing the seeds in rows and rolling up the tissue paper to encase the seeds. For AMF inoculation and plant growth, three holes were made in sand-soil mixture medium per each polythene bag. About 5 g of sterile zeolite containing 55 spores of *Glomus* sp. were put into each hole. Spores were covered with soil, and corn seedlings were placed into the hole. The plants were regularly watered to 12.5% weight of medium, and they were grown in the glasshouse. Along with watering all of the plants were fertilized with a nutrient solution spray of Hoagland's plant nutrient solution once in a week. The infection was allowed to develop in the host plants for at least three months before the inoculum was harvested.

Plants were harvested at 16 weeks and after a stressing period for stimulating spores of AMF. After the crop had been harvested, shoots and roots were separated. The certain weight of root and the soil were used as a source of AMF propagules, while the shoot was not used or was discarded. The 'mother inoculum' (containing a mixture of spores, hyphae and colonized root bits of *Glomus* sp.) was obtained by cutting all the root of the host plant to 1cm size and mixing it with the soil-sand mixture. The 'mother' inoculum containing AM infected roots, fungal hyphae, and spores was further used for these experiments.

Isolation of AMF spores from the 'mother' inoculum

Arbuscular mycorrhizal fungi spores were obtained from the mother inoculum by combining the wet sieving and decanting method with a sucrose solution centrifugation process (Brundrett *et al.*, 1996). Spores were collected by grinding infected corn roots in an Omni mixer, and the wet sieving the material through 355 μ m and 45 μ m (325 meshes) screens to remove debris. Spores were then concentrated with a sucrose gradient (20/40/60%). The number of spores was counted under a dissecting microscope with scale Petri dish. The AMF spores were maintained at 5⁰C until use and served as coating material.

Preparation of inoculant carrier, adhesive, and adhesion of inoculant carrier

The process of coating corn seed with AMF

spores included an initial step of preparing coating materials. The said coating materials consisted of AMF spores species *Glomus* sp., inoculant carrier in the form of a (1:1) v/v mixture of sterilized milled zeolite and peat soil, and adhesive polymeric materials consisting of a blend of PVA + TS in a different ratio. A (1:1) v/v mixture of sterilized milled zeolite and peat soil was selected as AMF spores carrier. The carrier was prepared by weighing 1 kg of each. The peat soil was obtained from Agriculture Faculty Research Farm, Bengkulu University. Then, zeolite and peat were separately oven-dried at 75°C for three consecutive days and finely powdered in a rotary mill. A set of sieves in series consisting of 42 mesh (355 µm), 100 mesh (150 µm), and 200 mesh (75 µm) and a collecting pan were stacked up and clamped to a sieve shaker. Milled zeolite and peat were separately put to the uppermost sieve, and the shaker was activated for 60 minutes. The fraction caught on the 200 mesh sieve was collected and used to make the inoculant carrier. The remainder was returned to the mill and ground again.

About 100 g of milled zeolite and 100 g of peat was then mixed thoroughly. The zeolite-peat mixture moisture content was made equal by mixing 10 g of the mixture with 5 ml water. Then, the mixture placed in an autoclavable polypropylene bag and sterilized for 60 minutes at 121°C. The sterilized zeolite-peat mixture was used as AMF spores carrier and served as a coating material.

Spores of AMF species *Glomus* sp. obtained from the 'mother inoculum' were suspended in water and then aseptically mixed into a small volume of the inoculant carrier. The certain amount of AMF spore suspension and carrier material was adjusted such that the solution nearly saturated the carrier material and allowed the AMF spores to be evenly distributed over the surface of the carrier particles.

About 2.5% w/v PVA and 5% w/v TS were selected as adhesive polymeric materials. The polymeric stickers were prepared by mixing PVA and tapioca starch at a five different weight blend ratio, i.e. 100% PVA + 0% TS, 75% PVA + 25% TS, 50% PVA + 50% TS,

25% PVA + 75% TS, and 0% PVA + 100% TS.

A 2.5% PVA sticker solution was aseptically prepared by dissolving in water at 80°C with a magnetic stirrer. A 5% tapioca starch adhesive solution was made by dissolving in water at boiling temperature while stirring with a glass rod. The two solutions were then separately cooled down to room temperature. After cool down, the two sticker solutions were used to prepare the five PVA + TS polymeric adhesives at a five different weight blend ratio. The powdered zeolite-peat blend based inoculant carrier containing AMF spores was inoculated onto corn seed via seed coating using various adhesive agents (Table 1).

The hybrid seed of corn cv BISI was obtained from a local market and subjected to viability test at the Agronomy Laboratory Bengkulu University to assess the initial quality of the un-coated seed. Its initial seed viability was determined by the standard germination test (SGT) (AOSA, 2014).

Table 1. Different adhesive agents containing PVA + TS blends used to attach inoculant carrier containing AMF spores on corn seed.

Treatment	Adhesive Blend Composition	
	PVA (%)	Tapioca Starch (%)
Coating 1 (C ₁)	100	0
Coating 2 (C ₂)	75	25
Coating 3 (C ₃)	50	50
Coating 4 (C ₄)	25	75
Coating 5 (C ₅)	0	100
Coating 6 (C ₆)	0	0

The corn seeds were surface sterilized in batches of sufficient seeds by 5 minutes dip in 3% hydrogen peroxide followed by five washes with deionized water. The seeds were then air dried overnight on a sterile filter paper in the transfer chamber. The corn seed was misted with deionized water and coated by gradually spraying with each corresponding binding agent separately, followed by adding inoculant carrier containing AMF spores in a modified pan granulator till uniform seed coating was achieved. The proportion of corn seed: adhesive: inoculant carrier was 10:1:1 by

weight. Coated corn seed had an average loading of at least 100 AMF spores per seed. Coated seeds were then air dried at 25⁰C for 72 hours. Noninoculated control seeds were prepared without coating. The coated seeds were then used for the polythene bag and field experiments.

Pot Experiment

The soil was analyzed for chemical properties before the start of the experiment. The soil properties were pH (H₂O) 5.6, soil organic matter content 3.43%, total N 0.32%, P -available 5.68 ppm, and K-exchangeable 0.26 me g⁻¹. Black polythene bags (30 cm X 40 cm) filled with 10 kg or air-dried soil consisting of a mixture of 7.5 kg of top soil and 2.5 kg of cow dung were used. The soil mixture was sterilized (autoclaved at 121⁰C for 15 minutes).

A mixture of PVA + TS in a different ratio (Table 1) was tested with a control (no coating) for their effectiveness as adhesive agents for AMF spores via seed coating. The six experimental treatments were laid out in a completely randomized design having three replications and making provision for three sampling times. Two coated corn seeds having the same initial AMF spore density were planted and thinned to one plant per polythene bag during the seedling stage. The maize was grown for 60 days. The plants were fertilized (except liming) for optimum corn growth. Each polythene bag received basal fertilizers of 7.5 mg N, 7.5 mg P₂O₅, and 7.5 mg K₂O kg⁻¹ soil. The cow dung contains 4.5 mg P₂O₅ kg⁻¹. The addition of fertilizer and manure made the initial content of P₂O₅ in the soil was 27.5 mg P₂O₅ kg⁻¹.

Plant harvest, sampling and measurements

Plant harvest was done at 60 days after planting (silking stage). At harvest, shoot dry weight, % AMF root colonization, AMF spore population, shoot phosphorus (P) content, and shoot P concentration were measured. At this stage, whole maize plants were cut at the soil surface, placed in labeled paper bags, and oven dried at 70 °C for 72 hours. Shoot dry mass was determined after oven-drying. Dry shoots were ground into a powder using a blender. Ground shoots were digested with HNO₃-HClO₄-H₂SO₄ solution. Phosphorus content

(mg plant⁻¹) and concentration (%) in the digested solution was determined calorimetrically with the vanadomolybdate-yellow assay (Olsen and Sommers, 1982).

Assessment of mycorrhizal root colonization

Immediately after removing plant shoots, the roots were gently removed from the soil, washed with tap water and dried on paper towels. A 1 to 1.5 g sample of the finest roots was removed using scissors. Percent root colonization was quantified in 1 cm long root segments obtained from a 1.0 to 1.5 g fresh weight root sample free of adhering soil. The root segments were cleaned using 10% KOH, bleached in ammonium and hydrogen peroxide solution, neutralized in 1% HCl and stained with 0.05% trypan blue in acid glycerol according to method described by Brundrett *et al.* (1996) and Giovannetti and Mosse (1980). The stained root samples were then mounted on a microscopic glass slide and observed under the light microscope (10 x magnification) for either the presence or absence of mycorrhizal structures (hyphae, spore, vesicles and arbuscules). The percent root colonization by AM fungi was calculated using the gridline intersect method (Brundrett *et al.* 1996; Giovannetti and Mosse 1980) and expressed as percentage of colonization.

Estimation of AMF spore population in soil after harvesting

Soil rhizosphere under each coating treatment was also assessed for AMF spore population. After harvesting, 50 g of thoroughly mixed soil without roots from each polythene bag was weighed and used for spore extraction by combination of wet-sieving and decanting method with sucrose-centrifugation techniques (Brundrett *et al.*, 1996; Daniels and Skipper, 1982). The spores were examined and counted under a stereomicroscope. Obviously empty, broken or flattened spores were ignored.

Data Analysis

The data obtained was subjected to analysis of variance. The comparison of the mean values was made using the Least Significant Difference Test at a level of 5% probability (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Adhering ability of sticker agents

Data from the screen house study demonstrated that adhesive agents significantly affected AMF spores inoculum adhering to seed (Table 2).

Table 2. Adhesion of powdered inoculant to corn seeds as affected by adhesive agent.

Adhesive agent PVA (%)	Tapio- ca Starch (%)	Inoculant adhering to seed (mg)		Attaching spores per seed
		Initial	2 hours drying	
100	0	1017.8 a	815.3 a	105.2 a
75	25	1010.2 a	809.8 a	102.8 a
50	50	1001.0 a	800.2 a	102.6 a
25	75	658.7 b	525.0 b	82.6 b
0	100	620.0 b	496.3 b	80.2 b

Note: Average followed by the same letters within each column were not significantly different each other according to LSD test.

The function of adhesive agents (“stickers”) for seed coating was mainly to bind AM fungal inoculum to the seed, thus maintaining the required intimate contact between germinating AMF spore and the newly emerged roots. Three adhesive agents (100% PVA + 0% TS, 75% PVA + 25% TS, and 50% PVA + 50% TS blends) apparently performed this function properly. They bound over 1000 mg of the inoculant per seed, demonstrating their excellent potential as adhesive agents for AMF inoculum delivery system. Their binding ability as adhesive agents increased along with PVA content due to its excellent mechanical properties (Han *et al.*, 2009). The other adhesive agents (25% PVA + 75% TS and 0% PVA + 100% TS blends) bound similar amounts of inoculant, which was approximately 55% less inoculant to the seed than that bound by the three previous adhesive agents. The two adhesive agents were classified as poor stickers. The increasing of starch content in the blends probably reduced their binding properties. The reason was because tapioca starch has disadvantages, i.e. no physic-mechanical characteristics and the products from starch are mostly brittle (Majeed and Kamil, 2013).

The two hours initial drying in desiccator was intended to simulate the air-drying required before newly inoculated seeds were bagged. Approximately 20% of the initially adhering inoculant was lost during this two-hours drying period. In general, those adhesive agents which bound the highest initial amount of inoculant (100% PVA + 0% TS, 75% PVA + 25% TS, and 50% PVA + 50% TS blends) retained the greatest amount of spores attaching to seed (Table 2). The reason was because their mechanical properties such as binding ability as adhesive agents increased along with increasing PVA content (Han *et al.*, 2009). Taylor *et al.* (2001) stated that with the proper type of a sticking agent and amount of inoculant, the number of AMF spores adhering to the seed substantially increased.

Mycorrhizal root colonization and spore population

Root colonization is a prerequisite for the functioning of AM symbiosis and for obtaining the plant benefits that are derived from this plant–fungus association (Smith and Read, 2008). Therefore, the primary objective of application of AM fungal inoculum to plants is to achieve root colonization. The test for the performance of an adhesive agent to bind AM fungal inoculum to the seed was the assessment of root colonization. Data from the present study showed that adhesive agents significantly affected root colonization (Table 3). Mycorrhizal colonization was observed in all inoculated plants due to presence of arbuscules in the infected roots. Meanwhile, no root colonization was noted in an uninoculated control plant (Table 3). The highest root colonization was achieved when 50% PVA + 50% TS blend was used as an adhesive agent followed by 75% PVA + 25% TS and 100% PVA + 0% TS blend, respectively. The 75% PVA + 25% TS blend was as effective adhesive agent as the 100% PVA + 0% TS blend in generating root colonization. The other adhesive agents (25% PVA + 75% TS and 0% PVA + 100% TS blends) generated similar percentage of root colonization, which was approximately 45% less root colonization than the 50% PVA + 50% TS blend. They were classified as poor stickers in promoting root

colonization. Good adhesive agents must bind the inoculant to the seed, protect the seed from desiccation, and some may provide nourishment to the germinating AMF spores. The 50% PVA + 50% TS blend apparently performed all three of these functions simultaneously. PVA is suitable used as an adhesive for seed coating because of its excellent mechanical properties (Han *et al.*, 2009) and its hydrophilic characteristic. With its hydrophilic characteristic, PVA prevents AMF spores from dehydrating in elevated soil temperature, thus maintaining their viability. While tapioca starch provided nourishment to the germinating AMF spores.

Table 3. Root colonization (%) and estimation of AMF spore population in soil after harvesting at silking stage

Adhesive agent		Root coloni- zation (%)	Spore pop- ulation (spore)
PVA (%)	Tapioca Starch (%)		
100	0	38.50 b	35.42 b
75	25	39.00 b	37.57 b
50	50	49.52 a	58.24 a
25	75	27.50 c	24.30 c
0	100	27.30 c	23.30 c
Non- coating		0.00 d	10.00 d

Notes: Means followed by the same letters within each column did not differ significantly each other ($P < 0.05$) by LSD test

Spores and fungi in root fragments are the main sources of infection at the beginning of corn growth. Data from the present study indicated that there was a significant variation among the treatment in the number of inoculated spores at harvest (Table 3), indicating that spore population from soil after harvesting was significantly influenced by adhesive agents. All the treatments recorded higher number of inoculated spores except for no coating treatment. A study conducted by Kartika (2004) also showed that the number of spores in flax plants inoculated with AMF inoculum was 62.2% greater than non-inoculated treatment. According to Abdullah and Musa (2005) the number of AMF inoculum formed in corn rhizosphere was influenced by the adaptation of AMF that

developed in rhizosphere host plants. As an adhesive agent, 50% PVA + 50% TS blend recorded the highest number of AMF spore population which was approximately 1.5 times greater than 100% PVA + 0% TS or 75% PVA + 25% TS and 2.5 times greater than 25% PVA + 75% TS or 0% PVA + 100% TS (Table 3). According to Minister of Agriculture Regulation No. 70 / Permentan / SR.140 / 10/2011 on Organic Fertilizer, Soil Fertilizer and Soil Enhancer, the standard quality for total AMF propagules is ≤ 50 spores / g dry weight of sample.

Shoot P uptake, shoot P concentration, and shoot dry weight

The results of this experiment showed that there was a significant variation among the treatment in shoot P uptake and concentration, indicating that plant P concentration and content (i.e. total P uptake per plant) significantly increased with AMF spore inoculation (Table 4). The result of this study agreed with the study reported by Valentine *et al.* (2001) for cucumber plants.

Table 4. Shoot P uptake, shoot P concentration, and shoot dry weight after harvesting at silking stage.

Adhesive agent		Shoot P content (mg plant ⁻¹)	Shoot P con- centra- tion (%)	Shoot dry weight (g plant ⁻¹)
PVA (%)	Tapio- ca Starch (%)			
100	0	20.4 b	0.14 b	24.25 a
75	25	29.7 a	0.20 a	22.40 a
50	50	34.5 a	0.21 a	25.05 a
25	75	32.9 a	0.20 a	23.65 a
0	100	19.2 b	0.15 b	24.00 a
No coating		12.4 c	0.07 c	12.15 b

Note: Means followed by the same letters within each column were not significantly different ($P < 0.05$) each other according to LSD test.

The use of different adhesive for coating of corn seed with AMF spores improved P uptake and concentration as indicated by higher shoot P content and concentration of inoculated plants than that of non-inoculated plants (no coating). Three adhesive agents (75% PVA +

25% TS, 50% PVA + 50% TS, and 25% PVA + 75% blends) were the most effective stickers for increasing P uptake and concentration. With the three adhesive agents the highest P uptake and concentration were achieved. Phosphorus uptake by inoculated plants with the three adhesive agents was about 2.5 times higher than non-inoculated plant (no coating), and P concentration was about 3.5 times higher. Meanwhile, non-inoculated plants had the lowest shoot P uptake and concentration. Other adhesive agents (100% PVA + 0% TS and 0% PVA + 100% TS blends) were better than no coating treatment in improving P uptake and concentration, but less effective than the three best stickers.

It is well established that AMF promote the growth of many plants by enhancing P uptake (Smith and Read, 2008; Oliveira *et al.*, 2016). They further stated that enhanced plant P uptake and concentration was due to the elongation of the extra radical hyphae of AMF into soil. The hyphae ultimately increased the surface area for the uptake of P, which was often depleted in rhizosphere soil solution.

The use of adhesive agents for coating of corn seed with AMF spores significantly affected shoot dry weight (Table 4). Irrespective of the adhesive agents used, shoot dry weight in AMF inoculated plants were higher than that in non-inoculated plant even though between AMF inoculated plants itself did not showed a difference in their shoot dry weight. The similar result was reported by Oliveira *et al.* (2016a) who reported that mycorrhizal inoculation via seed coating significantly increased the dry weight of shoot of wheat. This finding demonstrated that delivery of AMF spores via seed coating had the opportunity of applying a lower number of infective propagules per plant. The observed growth enhancement of inoculated corn plant was probably attributed to increased uptake of soil P nutrients assisted by mycorrhiza (Oliveira *et al.*, 2016a).

CONCLUSIONS

Three adhesive agents (100% PVA + 0% TS, 75% PVA + 25% TS, and 50% PVA + 50% TS

blends) bound over 1000 mg of the inoculant per seed and retained the greatest amount of spores attaching to seed, demonstrating their excellent potential as adhesive agents for AMF inoculum delivery system via seed coating. Three adhesive agents (75% PVA + 25% TS, 50% PVA + 50% TS, and 25% PVA + 75% TS blends) produced similar shoot P uptake, shoot P concentration, and shoot dry weight. From the three prospective adhesive agents, 50% PVA/50% TS blend was considered the most effective sticker as supported by the highest root colonization, the highest number of AMF spore population, higher shoot P uptake, shoot P concentration, and shoot dry weight followed by 75% PVA + 25% TS and 100% PVA + 0% TS blend, respectively.

REFERENCES

- Abdullah, S.Y. and F.H. Musa. 2005. Perbanyak cendawan mikoriza arbuskula (CMA) pada berbagai varietas jagung (*Zea mays* L.) dan pemanfaatannya pada dua varietas tebu (*Saccharum officinarum* L.). *J. Sains & Teknologi*. 5 (1): 12 – 20.
- Accinelli, C., H.K. Abbas, N.S. Little, J.K. Kotowicz, and W.T. Shier. 2018a. Biological control of aflatoxin production in corn using non-aflatoxigenic *Aspergillus flavus* administered as a bioplastic-based seed coating. *Crop Prot.* 107: 87–92. doi: 10.1016/j.cropro.2018.02.004.
- Accinelli, C., H.K. Abbas, and W.T. Shier. 2018b. A bioplastic-based seed coating improves seedling growth and reduces production of coated seed dust. *J. Crop Improv.* 32: 318–330. doi: 10.1080/15427528.2018.1425792.
- Amirkhani, M., M. Mortaz, A. Netravali, and A.G. Taylor. 2014. Seed Coating Technologies Employing a Plant-based Green Binder. doi: 10.13140/2.1.2744.0000.
- AOSA. 2014. Rules for testing seeds: Principles and procedures. *Assn. Off. Seed Anal.* 1: 6–25.
- Berruti, A., E. Lumini, R. Balestrini, and V. Bianciotto. 2016. Arbuscular mycorrhizal fungi as natural biofertilizers: Let's benefit from ast successes. *Front Microbiol.* 6:1559. doi:10.3389/fmicb.2015.01559.
- Brundrett, M., N. Bougher, B. Dell, T. Grove

- and N. Malajczuk. 1996. Working with mycorrhizas in forestry and agriculture. ACIAR Monograph 32. 374 p.
- Cassman, K.G., A. Dobermann, D. T. Walters, and H. Yang. 2003. Meeting cereal demand while protecting natural resources and improving environmental quality. *Annu. Rev. Environ. Resour.* 28:315–58 doi: 10.1146/annurev.energy.28.040202.122858.
- Colla, G., Y. Roupael, P. Bonini, and M. Cardarelli. 2015. Coating seeds with endophytic fungi enhances growth, nutrient uptake, yield and grain quality of winter wheat. *Int. J. Plant Prod.* 9:171-190. doi:[10.22069/ijpp.2015.2042](https://doi.org/10.22069/ijpp.2015.2042).
- Daniels, B.A. and H.A. Skipper. 1982. Methods for the recovery and quantitative estimation of propagules from soil. *In*: Schenck, N.C.(ed) *Methods and Principles of Mycorrhizal Research*, American Phytopathological Society, St. Paul, 29-35.
- Ehsanfar, S. and S.A. Modarres-Sanavy. 2004. Crop protection by seed coating. *Commun. Agric. Appl. Biol. Sci.* 70:225–229.
- Fedderman, N., R. Finlay, T. Boller, and M. Elfstrand. 2010. [Functional diversity in arbuscular mycorrhiza - The role of gene expression, phosphorus nutrition and symbiotic efficiency](https://doi.org/10.1016/j.funeco.2009.07.003). *Fungal Ecology* 3:1-8. doi: 10.1016/j.funeco.2009.07.003.
- Giovannetti, M. and B. Mosse. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist.* 84: 489-500. doi:10.1111/j.1469-8137.1980.tb04556.x
- Gomez, K.A. and A. A. Gomez. 1984. *Statistical Procedures for Agricultural Research*. 2nd Edition, New York: John Wiley and Sons Inc.
- Han, X., S. Shen, and X. Hu. 2009. Controlled-release fertilizer encapsulated by starch/polyvinyl alcohol coating. *Desalination.* 240:21-26.
- Holečková, Z., M. Kulhánek, and J. Balík. 2017. Use of active microorganisms in crop production—A review. *J. Food Process. Technol.* 8:10. doi: 10.4172/2157-7110.1000696.
- Kartika, R. 2004. Pengaruh Inokulasi Cendawan Mikoriza Arbuskula dan Dosis Pupuk P terhadap Serapan Hara P, Pertumbuhan dan Hasil Serat Rami (*Boehmeria nivea* L. Gaud). Skripsi. Departemen Agronomi dan Hortikultura. Fakultas Pertanian IPB. Bogor.
- Khodijah, S. 2009. Evaluasi Efektivitas Bahan Perekat dan Pelapis untuk Pelapisan Benih Kedelai (*Glycine max* Merr.) dengan Cendawan Mikoriza Arbuskula. Skripsi. Departemen Agronomi dan Hortikultura. Fakultas Pertanian IPB. Bogor.
- Lu, D.R., C. M. Xiao, and S. J. Xu. 2009. Starch-based completely biodegradable polymer materials. *Polymer Letters.* 3 (6): 366–375. doi: 10.3144/expresspolymlett.2009.46.
- Majeed, K.J. and M.I. Kamil. 2013. Effect of formaldehyde Content and nanoparticles on biodegradability of PVA/Corn Starch blend films. *IJAIEEM.* 10 (10):78-85.
- Musfal. 2010. Potensi Cendawan Mikoriza Arbuskula untuk Meningkatkan Hasil Tanaman Jagung. Balai Penelitian Teknologi Pertanian Sumatera Utara. *Jurnal Litbang Pertanian*, 29(4):154-158.
- Njeru E, L. Avio, G. Bocci, C. Sbrana, A. Turrini, P. Barberi, and F. Oehl. 2015. Contrasting effects of cover crops on “hot spot” arbuscular mycorrhizal fungal communities in organic tomato. *Biol. Fertil. Soils.* 51:151–166. doi: 10.1007/s00374-014-0958-z.
- O’Callaghan, M. 2016. Microbial inoculation of seed for improved crop performance: issues and opportunities. *Appl. Microbiol. Biotechnol.* 100, 5729–5746. doi: 10.1007/s00253-016-7590-9.
- Oliveira, R. S., I. Rocha, Y. Ma, M. Vosátka, and H. Freitas. 2016a. Seed coating with arbuscular mycorrhizal fungi as an ecotechnological approach for sustainable agricultural production of common wheat (*Triticum aestivum* L.). *J. Toxicol. Environ. Health A.* 79: 329–337. doi: 10.1080/15287394.2016.1153448.
- Oliveira, R. S., Y. Ma, I. Rocha, M.F. Carvalho, M. Vosátka, and H. Freitas. 2016b. Arbuscular mycorrhizal fungi are an alternative to the application of chemical fertilizer in the production of the medicinal and aromatic plant *Coriandrum sativum* L. *J. Toxicol. Environ. Health A* 79: 320–328. doi: 10.1080/15287394.2016.1153447.
- Oliveira, R.S., P. Carvalho, G. Marques, L. Ferreira, M. Nunes, I. Rocha, Y. Ma, M.F. Carvalho, M. Vosátka, and H. Freitas. 2017b. Increased protein content of chickpea (*Cicer arietinum* L.) inoculated

- with arbuscular mycorrhizal fungi and nitrogen-fixing bacteria under water deficit conditions. *J. Sci. Food Agric.* 97:4379-4385. doi:10.1002/jsfa.8201
- Olsen, S.R. and L.E. Sommers. 1982. Phosphorus. In: Page AL (ed) *Methods of Soil Analysis Part 2 Chemical and Microbiological Properties*. Am. Soc. Agro., Madison, WI, pp. 403–430
- Pedrini, S., D.J. Merritt, J. Stevens, and K. Dixon. 2017. Seed coating: science or marketing spin? *Trends Plant Sci.* 22:106–116. doi: 10.1016/j.tplants.2016.11.002
- Rocha, I., M. Ying, M. F. Carvalho, C. Magalhães, M. Janoušková, M. Vosátka, H. Freitas, and R. S. Oliveira. 2019a. Seed coating with inocula of arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria for nutritional enhancement of maize under different fertilization regimes. *Arch. Agron. Soil Sci.* 65:31–43. doi: 10.1080/03650340.2018.1479061
- Rocha, I., Y. Ma, M. Vosátka, H. Freitas, and R.S. Oliveira. 2019b. Growth and nutrition of cowpea (*Vigna unguiculata*) under water deficit as influenced by microbial inoculation *via* seed coating. *J. Agron. Crop Sci.* 205: 447–459. doi: 10.1111/jac.12335
- Rouphael Y., G. Colla, G. Graziani, A. Ritieni, M. Cardarelli, S. De Pascale. 2017. Phenolic composition, antioxidant activity and mineral profile in two seed-propagated artichoke cultivars as affected by microbial inoculants and planting time. *Chem.* 234: 10–19. doi: 10.1016/j.foodchem. 2017, 04.175
- Sari, P. E. 2009. Pengaruh Kombinasi Bahan Pelapis dan *Methylobacterium* spp. terhadap Daya Simpan Benih dan Vigor Bibit Kacang Panjang (*Vigna sinensis* L.). Skripsi. Departemen Agronomi dan Hortikultura. Fakultas Pertanian IPB. Bogor.
- Schröder, J.J., A.L. Smit, D. Cordell, and A. Rosemarin. 2011. Improved phosphorus use efficiency in agriculture: A key requirement for its sustainable use. *Chemosphere* 84: 822-831. doi:10.1016/j.chemosphere.2011.01.065.
- Smith, S.E. and D.J. Read. 2008. *Mycorrhizal Symbiosis*, 3rd edn. Academic, London.
- Song, R., M. Murphy, C. Li, K. Ting, C. Soo, and Z. Zheng. 2018. Current development of biodegradable polymeric materials for biomedical applications. *Drug Des. Devel. Ther.* 12:3117–3145. doi:10.2147/DDDT.S165440.
- Taylor, A. G., C.J. Eckenrode, and R.W. Straub. 2001. Seed coating technologies and treatments for onion: Challenges and progress. *HortScience* 36: 199–205. doi: 10.21273/HORTSCI.36.2.199.
- Thonar, C., J.D.S.nLekfeldt, V. Cozzolino, M. Kulhánek, C. Mosimann, G. Neumann, A. Piccolo, M. Rex, S. Symanczik, F. Walder, M. Weinmann, A. de Neergaard, and P. Mäder. 2017. Potential of three microbial bio-effectors to promote maize growth and nutrient acquisition from alternative phosphorous fertilizers in contrasting soils. *Chem. Biol. Technol. Agric.* 4: 7. doi: 10.1186/s40538-017-0088-6.
- Valentine, A.J., B.A. Osborne, and D.T. Mitchell. 2001. Interactions between phosphorus supply and total nutrient availability on mycorrhizal colonization, growth and photosynthesis of cucumber. *Sci. Horti.* 88: 177-189.
- Vosátka, M., A. Látr, S. Gianinazzi, and J. Albrechtová. 2012. Development of arbuscular mycorrhizal biotechnology and industry: current achievements and bottlenecks. *Symbiosis* 58: 29–37. doi: 10.1007/s13199-012-0208-9.