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Evaluation of AMF formulations on growth, root colonization and biophysical parameters of maize (*Zea mays* L.)

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Abstract

Mycorrhizal formulations are becoming increasingly popular in many countries and for many crops, but less studies on their effect on plant parameter and mycorrhizal studies. There for we have evaluated different AMF formulations under green house studies. In this study soil samples from the UAS, Dharwad experimental plots were collected, sterilized and used in a pot experiment. The maize Hybrid (GPMH-1101) was grown in the pots upto 45 days. The plant dry weight, root length, bio physical parameters, P concentrations in plant tissues was measured and the extent of root colonization by arbuscular mycorrhizal fungi (AMF) was determined. AMF formulations have shown significant influence on the growth and biophysical parameters of plants that could be related to differences in the infective propagules in formulations. The inoculum potential and efficacy of the AMF formulations can promote P uptake and benefit plant growth.

Keywords: Mycorrhizal formulations, talc, lignite, glycerol based, encapsulation and alginate based formulation.

Introduction

Maize (*Zea mays* L.), the main source of food for humans and animals in many parts of the world, has a unique root system that is highly efficient not only in anchoring the plant to the soil but also in the acquisition of nutrients and water from the soil. In addition, the root system of maize also possesses several morphological and metabolic traits that are essential for increased efficiency like adventitious roots, long and dense root hairs, basal-root shallowness, root etiolation and cortical aerenchyma (Hochholdinger & Tuberosa 2009). Several studies reported the mycorrhizal status and colonization patterns like Arum-, Paris- and intermediate type in maize (Muthukumar & Prakash 2009; Muthukumar & Tamilselvi 2010; Chandra Gandhi *et al.* 2017) [21, 20, 5].

Further, maize genotypes exhibit variation in their responsiveness to mycorrhizal colonization (Kaepler *et al.* 2000) [15]. Recently, Wang *et al.* (2017) [28] in a long term experiment showed that increasing P fertilization in spite of reducing root colonization and community structure of AM fungi can still contribute substantially to P nutrition of maize plants. It also augment water uptake, induces resistant against diseases, suppress parasitic weeds and boost the crop yield. AM fungi are now available in different formulations. Formulation is essentially a blend of microbial propagules with a range of carrier or adjuvants to produce a material that can be effectively delivered to the target application (Adholeya, 2003) [2]. The main objective of the present study was to find the influence of AMF formulation on plant growth, nutrient uptake, phosphatase enzyme activities and mycorrhizal load and colonization in maize under green house pot culture studies.

Materials and methods

Evaluation on the efficacy of AMF formulations in pot culture condition using maize as host

Sterilized soil was used to test the efficacy of the AMF formulations. The AMF formulations were screened for their

ability to promote growth of maize under pot culture studies (Plate 1 and 2). The sterilized soil was used to test the efficacy of AMF formulations.

Experimental details

Treatment details		
Talc with AMF Product	T ₁	1:1
	T ₂	1:2
	T ₃	1:3
Lignite with AMF Product	T ₄	1:1
	T ₅	1:2
	T ₆	1:3
Humic acid with AMF Product	T ₇	1:1
	T ₈	1:2
	T ₉	1:3
Glycerol with AMF Product	T ₁₀	1:1
Encapsulated AMF product	T ₁₁	0.5 g capsule ⁻¹
Sodium alginate bits with AMF product	T ₁₂	0.5 g bit ⁻¹
Uninoculated control	T ₁₃	--

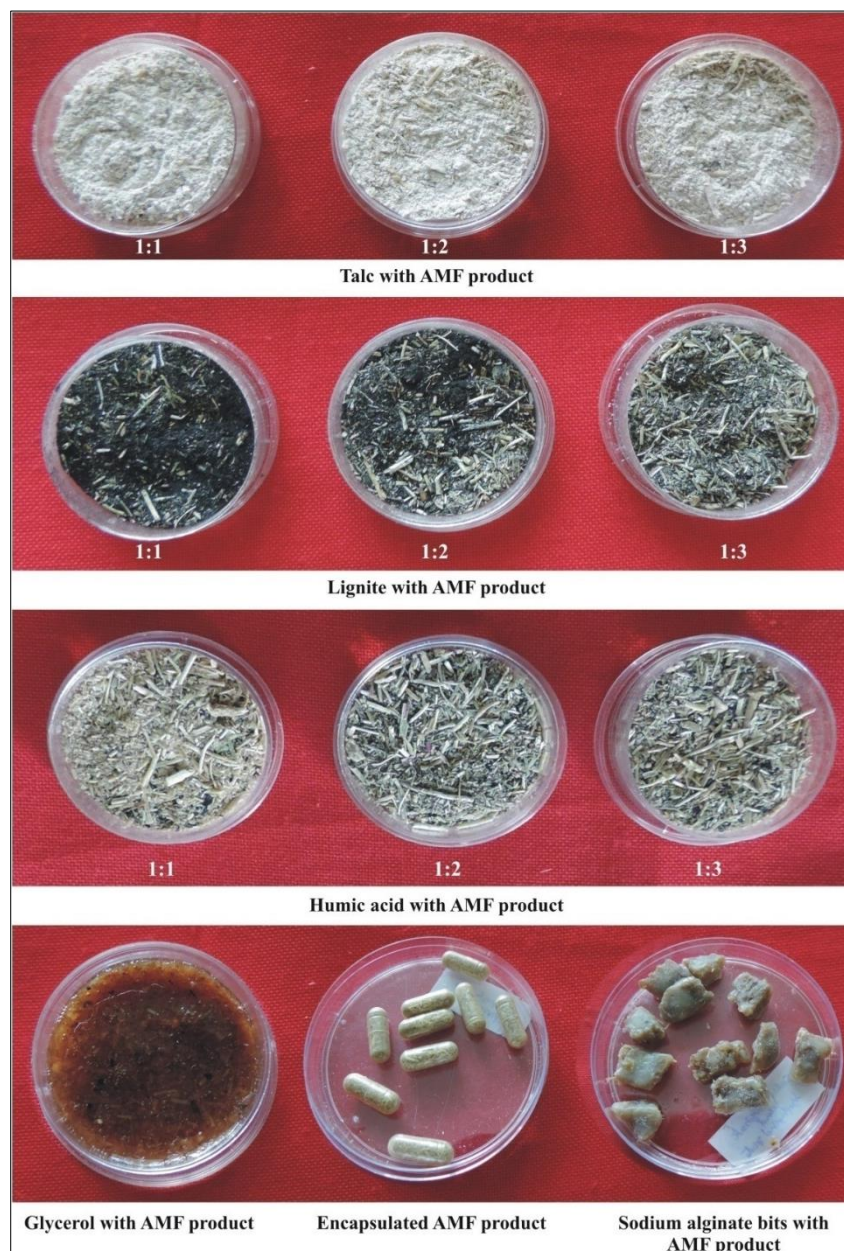


Plate 1: AMF formulations



Plate 2: General view of the pot experiment

Pot culture studies

The pots of 15 cm diameter was selected and soil was collected from top 10 cm in the field. The soil was air dried and sieved through a 2 mm sieve to remove stone and plant debris. Sterilized in autoclave for one hour at 15 lbs pressure and 121°C temperature and then cooled it at room temperature. After following this, pots was filled with 4 kg of sterilized soil. The pots were inoculated with different formulation @ 6 g per pot.

Maize seeds were obtained from seed unit (UAS, Dharwad). Thereafter, four seeds per pot was sown directly, later two seedlings were maintained per pot in the greenhouse. Plants were watered regularly.

Observations (Up to 45 DAS)

1. Plant parameters

During experiment, growth parameter was selected for recording the growth response in the inoculated and control plants. The details of the material used and the techniques adopted during the course of investigation are described below. The plant height was recorded from the base of the plant to the tip of the growing point. The stem girth was recorded on 45 DAS. The average diameter of shoot of each plant was calculated and expressed in centimetre. The dry weight of root and shoot was recorded. The maize plants were uprooted, root portion and shoot portion were separated and dried in oven at 70°C until a constant weight was obtained. The average weight of root and shoot of each plant was calculated and expressed in grams plant⁻¹.

2. Mycorrhizal parameters

a. Spore count

The chlamydospores in rhizosphere of sugarcane were determined by wet sieving and decantation method as outlined by Gerdemann and Nicholson (1963) [11]. AM spores were isolated using wet sieving and decantation method. One hundred gram of soil sample was taken in one liter beaker, made up to 1000 ml with water, stirred well, heavier soil particles were allowed to settle for a few seconds. Then the suspension was passed through a series of different size sieves (500 µm, 250µm, 106 µm, 75 µm, 45 µm, 37 µm) arranged in the descending order of their mesh size. Again water was added up to 1000 ml, stirred well and allowed for few seconds this was repeated for five to six times, till the suspension appeared clear. Sievates were collected from each sieve separately in beakers. The supernatant from each beaker was

separately filtered through Whatman No. 1 filter paper and the content of the filter papers were examined for spores under a stereo zoom microscope (Labomed).

b. Per cent root colonization

Mycorrhizal root colonization was determined as per the procedure proposed by Philips and Hayman (1970) [22]. Fresh root samples were cut into 1 cm pieces and placed in screw cap vials. The clearing of the roots was achieved by treating them with 10 per cent KOH and leaving them overnight. The KOH solution was poured off and the roots were rinsed with tap water. Later, the roots were treated with 10 per cent HCl for 10 minutes to neutralize the residual effect of alkali and create an acidic environment required for further staining. The root bits were stained with 0.05 per cent trypan blue in lactoglycerol (lactic acid, glycerol and water in the ratio (40:20:20 respectively) by boiling them at 90° C for 30 minutes. Excess stain was decanted and the root samples were immersed in lactoglycerol for de staining. The stained root bits were placed on a clean glass slide and observed under microscope for colonization. The percentage of roots colonized by mycorrhizae was calculated by the formula

$$\text{Per cent root colonization} = \frac{\text{Root bits positive for colonization}}{\text{Total number of root bits}} \times 100$$

3. Soil enzymes

a. Estimation of dehydrogenase activity

Dehydrogenase activity in the soil samples were determined by following the procedure as described by Casida *et al.* (1964) [3].

Ten grams of soil and 0.2 g CaCO₃ were thoroughly mixed and dispensed in test tubes. To each tube, one ml of 3 per cent aqueous solution of 2, 3, 5- Triphenyl tetrazolium chloride (TTC), one ml of one per cent glucose solution and eight ml of distilled water were added. This was sufficient to leave a thin film of water above the soil layer. The tubes were stoppered with rubber cork and incubated at 30° C for 24 h. At the end of incubation, the contents of the tube were rinsed down into a small beaker and slurry was made by adding 10 ml methanol. The slurry was filtered through Whatman No. 50 filter paper. Repeated rinsing of soil with one ml methanol was continued till the filtrate ran free of red color. The filtrate was pooled and made up to 50 ml with methanol in a volumetric flask. The intensity of red color was measured at 485 nm against methanol as blank using UV- vis spectrophotometer. (Thermo Scientific, USA). The concentration of formazan in soil samples were determined by referring to a standard curve prepared using graded concentration of formazan. The results were expressed as µg of triphenyl formazan (TPF) formed g⁻¹ soil per day.

b. Estimation of phosphatase activity

The chemical analysis was done by using shoot and leaf samples of maize plants.

Phosphatase activity of soil samples were determined by following the procedure of Evazi and Tabatabai (1979) [8].

One gram of soil sample was placed in 50 ml Erlenmeyer flask to which 0.2 ml toluene followed by four ml of modified universal buffer (pH 7.5) was added. One ml of para-nitrophenol phosphate solution made in modified universal buffer was added to the flasks and contents of the flasks were mixed by swirling for 2 minutes. The flasks were stoppered and incubated at 37°C for one hour. After incubation, one ml

of 0.5M CaCl_2 and four ml of 0.5 M NaOH were added to the flask, swirled and filtered through Whatman No. 42 filter paper. The intensity of yellow colour developed was measured at 420 nm against the reagent blank using spectrophotometer. Controls were maintained for each soil sample and were analyzed by following the same procedure described above except that the para nitrophenol phosphate solution was added after the addition of 0.5 M CaCl_2 and 0.5 M NaOH and just before filtration. The phosphatase activity in the soil samples was expressed as μg para nitrophenol formed per gram soil per hour with reference to the standard curve prepared by using graded concentrations of p-nitrophenol phosphate.

4. The influence of AMF formulation on relative chlorophyll content (SPAD) and biophysical parameters of maize plants (IRGA)

a. Chlorophyll content (SPAD readings)

The single photoelectric analyzing diode (SPAD) meter (SPAD-502 KONICA-Japan) was used for recording the chlorophyll reading. The reading was taken between 10.00 and 12.00 hours of the day.

b. Biophysical parameters of maize plants (IRGA)

Measurement of photosynthetic rate, stomatal conductance, rate of transpiration and leaf temperature were made on the top fully expanded leaf at different locations by using a portable photosynthesis system (LI-6400 LICOR, Nebraska, Lincoln USA,). These measurements were made between 10.00 am to 12.00 noon on all the sampling dates.

c. Chemical analysis of Maize plants at 45 DAS

The chemical analysis was done by using shoot and leaf samples of maize plants. P was estimated by vanadomolybdate phosphoric yellow colour method (Jackson, 1973) [14].

Statistical analysis

The data were subjected to analysis following Completely Randomised Design (CRD) as defined by Gomez and Gomez (1984).

Results

1. Effect of AMF formulations on plant growth parameters in maize (15, 30 and 45 DAS)

a. Plant height

The plant height of maize was recorded at 15, 30 and 45 DAS are presented in Table 1, and Plate 3. The plant height was found to be increased steadily with number of days after sowing due to different AMF formulations at 15, 30 and 45 DAS. In general, the plants inoculated with the AMF formulation were found to be superior over uninoculated control plants at all the stages of crop growth. However, the plant height differed significantly among the mycorrhizal plants. At 15 DAS, the plant height did not show any variation in the test crop and the results were found to be on par with each other with different treatments. At 30 DAS, AMF formulations have influenced the plant height when compared to uninoculated control. Among the formulations, sodium alginate bits with AMF product treatment recorded significantly the highest plant height (28.33 cm), followed by glycerol with AMF product (28.00 cm). Among the talc based formulations, talc with AMF product (1:3) recorded the highest plant height (24.37 cm), followed by talc with AMF product (1:2) (23.80 cm). Among the lignite-based formulations, lignite with AMF product (1:3) recorded the highest plant height (25.03 cm) followed by lignite with AMF product (1:2) (23.06 cm). Among the humic acid with AMF product (1:3) recorded the highest plant height (25.37 cm) followed by humic acid with AMF product (1:2) (23.87 cm). The lowest plant height was recorded with non-mycorrhizal maize plants (20.00 cm).

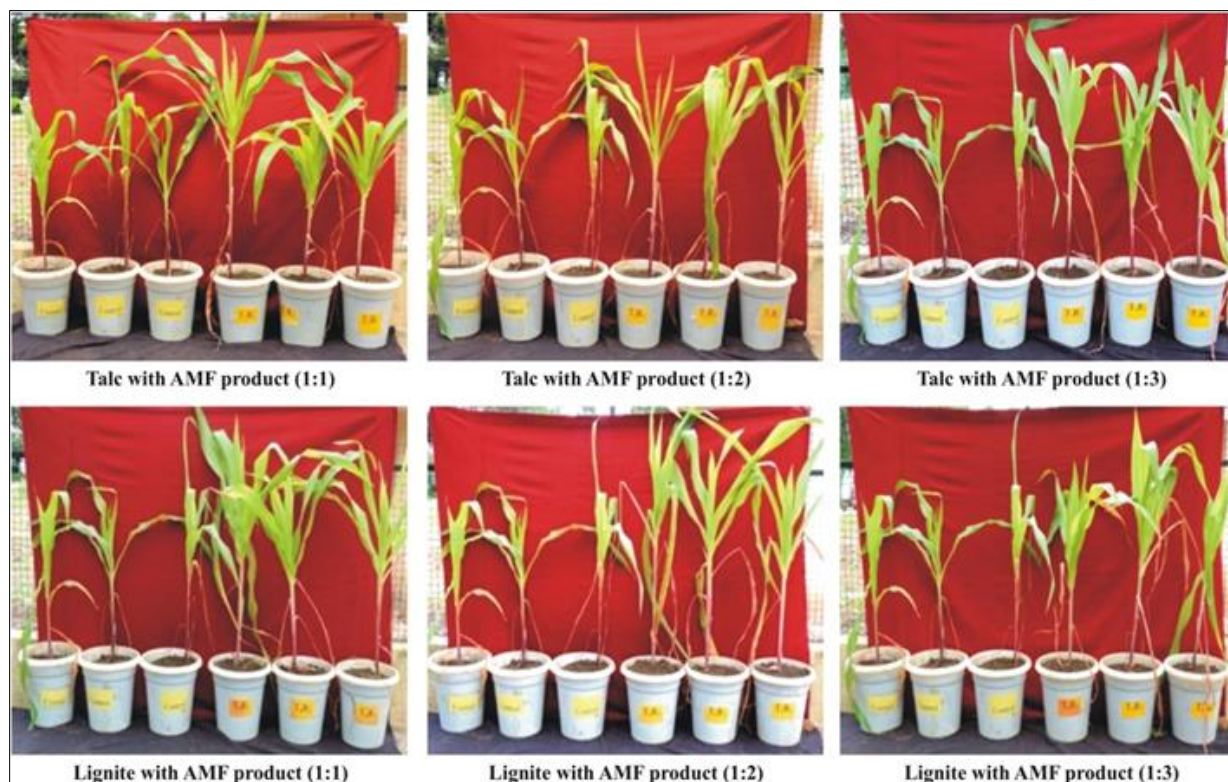


Plate 3: Influence of AMF formulations on growth parameters of maize

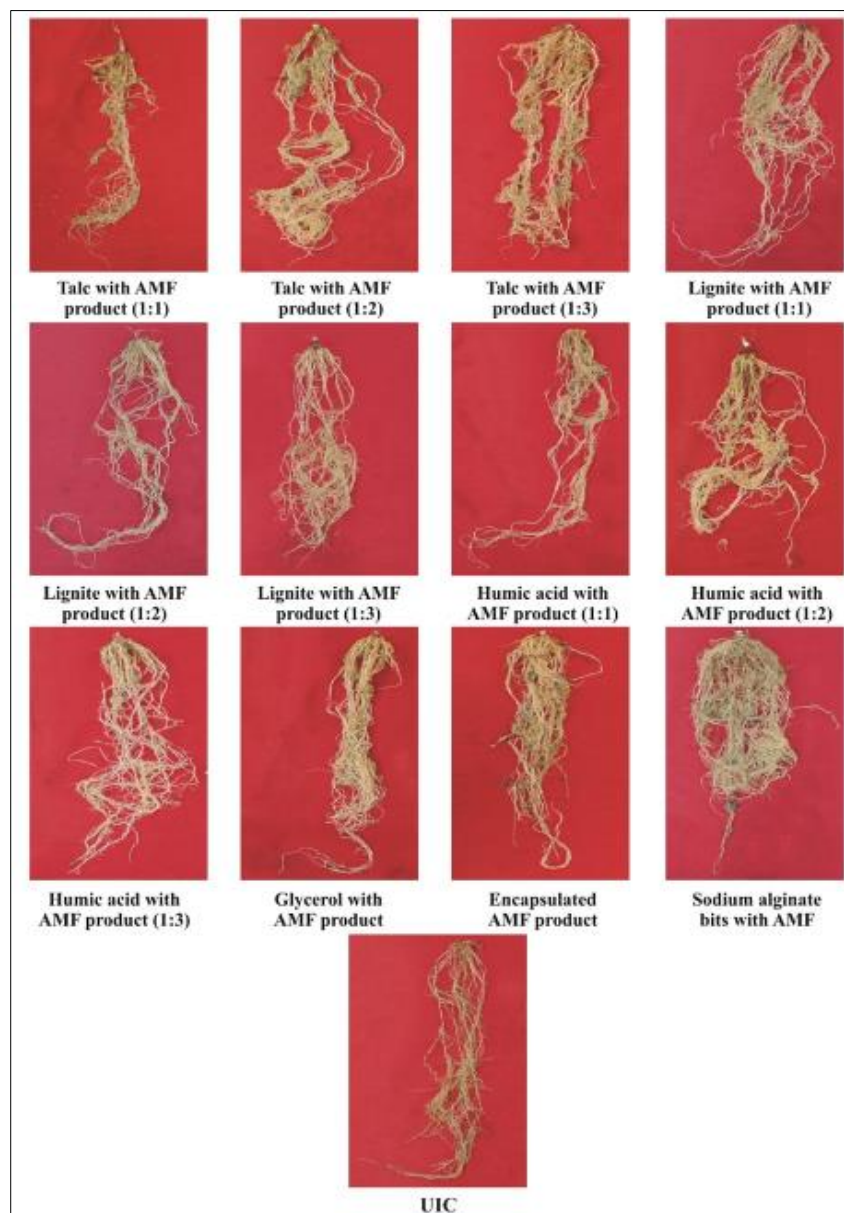
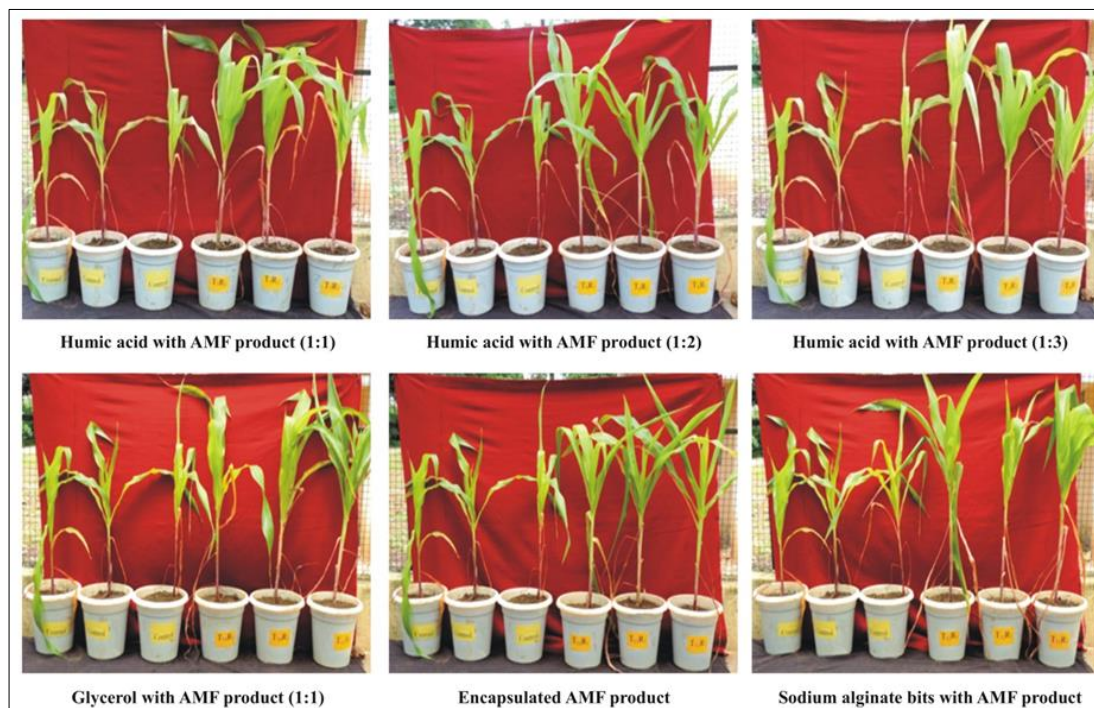


Plate 4: Influence of AMF formulations on root biomass of maize

Table 1: Effect of AMF formulations on plant growth parameters in maize (15, 30 and 45 DAS)

Treatment	Plant height (cm)			Stem girth (cm)		
	15 DAS	30 DAS	45 DAS	15 DAS	30 DAS	45 DAS
Talc with AMF product (1:1)	10.15	23.43	74.67	0.26	1.02	1.23
Talc with AMF product (1:2)	10.65	23.80	75.50	0.27	1.07	1.38
Talc with AMF product (1:3)	10.15	24.37	78.60	0.25	1.11	1.40
Lignite with AMF product (1:1)	10.06	22.43	75.33	0.26	1.04	1.32
Lignite with LAM (1:2)	10.60	23.60	75.73	0.26	1.10	1.40
Lignite with LAM (1:3)	11.25	25.03	81.77	0.26	1.14	1.41
Humic Acid with AMF product (1:1)	10.05	23.43	75.17	0.25	1.07	1.33
Humic Acid with AMF product (1:2)	11.10	23.87	76.60	0.26	1.12	1.42
Humic Acid with AMF product (1:3)	10.40	25.37	81.95	0.26	1.15	1.43
Glycerol with AMF product (1:1)	10.25	28.00	84.00	0.25	1.23	1.47
Encapsulated AMF product	10.50	27.67	82.20	0.27	1.18	1.44
Sodium alginate with AMF product	10.20	28.33	86.67	0.27	1.24	1.48
Uninoculated control	10.05	20.00	55.67	0.24	0.85	1.20
S. Em±	0.27	0.68	3.23	0.008	0.09	0.07
CD (0.05)	0.78	1.96	9.40	0.022	0.254	0.20

b. Stem girth

The stem girth of maize was recorded at 15, 30 and 45 DAS are presented in the Table 1. The stem girth was found to be increased steadily with number of days after sowing due to the influence of different AMF formulations at 15, 30 and 45 DAS. The plants inoculated with the AMF formulation were found to be superior over uninoculated control plants at all the growth stages of crop. However, the stem girth differed significantly among the mycorrhized plant over non mycorrhized plants. At 15 DAS, the stem girth did not show any variation in test crop and the results were found to be statically at par with each other.

At 30 DAS, AMF formulations have significantly influenced the stem girth compared to uninoculated control. The treatment received sodium alginate bits with AMF product recorded significantly the highest stem girth (1.24 cm), followed by glycerol with AMF product (1.23 cm). Among the talc based formulation, talc with AMF product (1:3) recorded highest stem girth (1.11 cm), followed by talc with AMF product (1:2) (1.07 cm). Among the lignite based formulations, lignite with AMF product (1:3) recorded highest stem girth (1.14 cm) followed by lignite with AMF product (1:2) (1.10 cm). In case of humic acid with AMF product (1:3) recorded the highest stem girth (1.15 cm) followed by humic acid with AMF product (1:2) (1.2 cm). The lowest stem girth was with uninoculated control maize plants (0.85 cm). Similar observations were also noticed at 45 DAS.

c. Effect of AMF formulations on total dry biomass in maize (45 DAS)

The total dry biomass was recorded at 45 DAS as influenced by different AMF formulation are presented in Table 2. The highest total dry biomass was recorded in the plants which received the AMF formulations over compared to UIC.

Among the AMF formulations, sodium alginate with AMF product recorded the highest total dry matter (89.94g p⁻¹), followed by glycerol with AMF product (89.87g p⁻¹). Among the talc based AMF formulations, talc with AMF product (1:3) recorded highest total dry matter (88.47g p⁻¹), while lignite with AMF product (1:3) recorded highest total dry matter (88.74g p⁻¹), followed with humic acid with AMF

product (1:3) recorded highest total dry matter (89.04g p⁻¹). The lowest total dry matter was recorded with non mycorrhized maize plants (69.20g p⁻¹).

Table 2: Effect of AMF formulations on total dry biomass in maize (45 DAS)

Treatment	Total biomass (g plant ⁻¹)
Talc with AMF product (1:1)	80.99
Talc with AMF product (1:2)	88.11
Talc with AMF product (1:3)	88.47
Lignite with AMF product (1:1)	84.09
Lignite with LAM (1:2)	88.53
Lignite with LAM (1:3)	88.74
Humic Acid with AMF product (1:1)	88.20
Humic Acid with AMF product (1:2)	88.69
Humic Acid with AMF product (1:3)	89.04
Glycerol with AMF product (1:1)	89.87
Encapsulated AMF product	89.52
Sodium alginate with AMF product	89.94
Uninoculated control	69.20
S. Em±	1.06
CD (0.05)	3.08

2. Effect of AMF formulation on mycorrhizal parameters in maize (30 and 45 DAS)

The data on mycorrhization parameters viz, spore count and per cent root colonization as influenced by different AMF formulations at 30 and 45 DAS are presented in Table 3.

a. AMF spore count (Spore count/50 g of soil)

At 30 DAS, the highest mycorrhizal spore count was enumerated in the maize rhizosphere received sodium alginate with AMF product (67.33), followed by glycerol with AMF product (64.00). Among the talc based formulations, talc with AMF product (1:3) recorded the highest spore load (51.33). Similarly the lignite based AMF formulation (1:3) recorded the highest spore load (54.00) compared to rest of the dilutions. Similar results were also recorded in the plants which received humic acid with AMF product (1:3) (56.7). Similar observations were also recorded at 45 DAS, wherein the plants treated with sodium alginate with AMF product harbored the highest spore count (81.50) (Table 3).

Table 3: Effect of AMF formulations on mycorrhizal parameters in maize (30 and 45 DAS)

Treatment	Spore density/ 50 g of soil		Root colonization (%)	
	30 DAS	45 DAS	30 DAS	45 DAS
Talc with AMF product (1:1)	40.00	63.00	30.00	49.33
Talc with AMF product (1:2)	48.00	67.50	30.67	51.33
Talc with AMF product (1:3)	51.33	69.50	32.00	52.67
Lignite with AMF product (1:1)	42.00	61.50	31.33	50.00
Lignite with LAM (1:2)	48.67	67.00	30.67	52.00
Lignite with LAM (1:3)	54.00	71.50	32.67	54.67
Humic Acid with AMF product (1:1)	42.67	63.50	32.00	52.67
Humic Acid with AMF product (1:2)	50.00	69.00	36.00	56.00
Humic Acid with AMF product (1:3)	56.67	74.00	35.33	60.00
Glycerol with AMF product (1:1)	64.00	79.50	44.67	60.67
Encapsulated AMF product	60.00	76.00	42.00	60.00
Sodium alginate with AMF product	67.33	81.50	45.33	61.33
Uninoculated control	0.00	0.00	0.00	0.00
S. Em±	1.86	2.11	1.76	1.93
CD (0.05)	5.40	6.14	5.13	5.61

b. Per cent root colonization

At 30 DAS, AMF root colonization in maize root was the highest in the plants inoculated with AMF formulations. The highest per cent root colonization was observed in the treatment which received sodium alginate with AMF product (45.33%), followed by glycerol with AMF product (44.67%).

3. Dehydrogenase and phosphatase activity as influenced by AMF formulations in maize rhizosphere at 45 DAS

The dehydrogenase and phosphatase activity was recorded at 45 DAS and the data are presented in Table 4.

At 45 DAS, highest dehydrogenase and phosphatase enzyme activity was recorded in the plants received sodium alginate with AMF product (5.12 µg) followed by glycerol based (5.03 µg). Among the talc based formulations, talc with AMF product (1:3) recorded the highest dehydrogenase activity (4.60 µg), while lignite with AMF product (1:3) recorded highest dehydrogenase activity (4.78 µg). The humic acid

with AMF product (1:3) showed highest dehydrogenase activity (4.93 µg). Least soil enzyme activity was measured in the maize rhizosphere not received AMF formulation (2.35 µg).

4. The phosphatase activity (45 DAS)

The phosphatase activity was recorded at 45 DAS and are presented in Table 4.

The phosphatase activity was highest in the treatments which received AMF formulations compared to UIC.

At 45 DAS, the highest phosphatase activity was recorded in the plants received sodium alginate with AMF product (16.04 µg) followed by glycerol with AMF product (15.64 µg). Among the carrier based formulations, humic acid with AMF product (1:3) recorded the highest phosphatase activity, followed by lignite with AMF product (1:3) and talc with AMF product (1:3) (15.23, 14.63 and 11.63, respectively).

Table 4: Soil enzyme activity as influenced by AMF formulations in maize rhizosphere at 45 DAS

Treatment	Dehydrogenase activity (µg TPF formed g ⁻¹ soil d ⁻¹)	Phosphatase activity (µg pnp released g ⁻¹ soil h ⁻¹)
Talc with AMF product (1:1)	3.72	10.18
Talc with AMF product (1:2)	3.89	10.89
Talc with AMF product (1:3)	4.60	11.63
Lignite with AMF product (1:1)	4.06	12.43
Lignite with LAM (1:2)	4.26	13.86
Lignite with LAM (1:3)	4.78	14.63
Humic Acid with AMF product (1:1)	4.32	12.70
Humic Acid with AMF product (1:2)	4.44	13.20
Humic Acid with AMF product (1:3)	4.93	15.23
Glycerol with AMF product (1:1)	5.03	15.64
Encapsulated AMF product	4.94	15.27
Sodium alginate with AMF product	5.12	16.04
Uninoculated control	2.35	6.27
S. Em±	0.25	0.94
CD (0.05)	0.72	2.73

5. Influence of AMF formulations on relative chlorophyll content (SPAD) and biophysiological parameters of maize plants (IRGA)**a. Relative Chlorophyll content (SPAD)**

The result of the relative chlorophyll content of the maize phyllo sphere at 45 DAS are presented in Table 5.

At 45 DAS, inoculation of sodium alginate with AMF product significantly improved the relative chlorophyll content

(19.88) followed by glycerol with AMF product (19.77). Among the carrier-based formulations, humic acid with AMF product (1:3) recorded the highest relative chlorophyll content, followed by lignite with AMF Product (1:3) and talc with AMF product (1:3) (17.53, 16.93 and 16.50 respectively). The lowest relative chlorophyll content was recorded in UIC (13.93).

Table 5: Influence of AMF formulations on relative chlorophyll content (SPAD) and biophysical parameters of maize plants (IRGA)

Treatment	SPAD	Photosynthetic rate ($\mu\text{mol}/\text{m}^2/\text{sec}$)	Stomatal conductance ($\mu\text{mol}/\text{m}^2/\text{sec}$)	Transpiration rate ($\text{mmol}/\text{m}^2/\text{sec}$)	Leaf temperature ($^{\circ}\text{C}$)
Talc with AMF product (1:1)	15.17	14.43	0.47	5.10	26.01
Talc with AMF product (1:2)	15.70	14.53	0.50	5.01	25.52
Talc with AMF product (1:3)	16.50	14.64	0.53	4.97	25.32
Lignite with AMF product (1:1)	15.63	14.52	0.48	5.08	25.89
Lignite with LAM (1:2)	15.73	14.63	0.51	5.03	25.62
Lignite with LAM (1:3)	16.93	14.72	0.53	5.00	25.50
Humic Acid with AMF product (1:1)	16.17	14.46	0.48	5.04	25.69
Humic Acid with AMF product (1:2)	16.33	14.62	0.50	5.02	25.58
Humic Acid with AMF product (1:3)	17.53	14.82	0.53	5.01	25.52
Glycerol with AMF product (1:1)	19.77	14.85	0.57	4.95	25.20
Encapsulated AMF product	19.63	14.79	0.55	4.95	25.24
Sodium alginate with AMF product	19.88	14.94	0.59	4.66	23.73
Uninoculated control	13.93	13.55	0.44	5.13	26.15
S. Em±	0.89	0.26	0.02	0.08	0.43
CD (0.05)	2.58	0.76	0.06	0.24	1.25

b. The influence of AMF formulations on biophysical parameters of maize plants photosynthetic rate (μmol of $\text{CO}_2/\text{m}^2/\text{sec}$)

In general, rate of photosynthesis was significantly higher in the maize plants which received AMF formulations. The treatment which received sodium alginate with AMF product recorded the highest photosynthetic rate (14.94) followed by glycerol with AMF product (14.85). Among the carrier-based formulations, humic acid with AMF product (1:3) recorded the highest photosynthetic rate, followed by lignite with AMF Product (1:3) and talc with AMF product (1:3) (14.82, 14.72 and 14.64 respectively). The lowest photosynthetic rate was recorded in UIC (13.55).

c. Stomatal conductance ($\mu\text{mol}/\text{m}^2/\text{sec}$)

The highest stomatal conductance was recorded in the plants which received sodium alginate with AMF Product (0.59), followed by glycerol with AMF product (0.57). Among the carrier-based formulations, humic acid with AMF product (1:3) recorded the highest stomatal conductance, followed by lignite with AMF Product (1:3) and talc with AMF product (1:3) (0.53, 0.53 and 0.52, respectively).

d. Transpiration rate (μmol of $\text{H}_2\text{O}/\text{m}^2/\text{sec}$)

The data clearly indicated that the uninoculated maize plants recorded the highest rate of transpiration (5.13). Among the talc-based formulations, talc with AMF product (1:1) recorded the highest transpiration rate (5.10) and the lowest transpiration rate was recorded in the treatment receiving talc with AMF product (1:3) (4.97). Among the lignite-based formulations, lignite with AMF product (1:1) recorded the highest transpiration rate (5.08) and the lowest transpiration rate (5.00) was recorded in the treatment receiving lignite

with AMF product (1:3). The humic acid with AMF product (1:1) recorded highest transpiration rate (5.04), while the lowest transpiration rate (5.01) was recorded in the treatment received humic acid with AMF product (1:3) followed by glycerol with AMF product (5.01). However, the lowest transpiration rate was measured in sodium alginate with AMF product (4.66).

e. Leaf temperature ($^{\circ}\text{C}$)

The lowest leaf temperature was recorded in the treatments which received sodium alginate with AMF product (23.73) followed by glycerol with AMF product (25.20). Further, the maize plant received which talc with AMF product (1:1) recorded the highest leaf temperature (26.01) and the lowest in talc with AMF product (1:3) (25.32). Among the lignite-based AMF formulations, lignite (1:1) recorded the highest leaf temperature (25.89) and the lowest in lignite with AMF product (1:3) (25.50). Among the humic acid-based formulations, humic acid with AMF product (1:1) recorded the highest (25.69) and the lowest in humic acid with AMF product (1:3) (25.58).

6. Influence of AMF formulations on P content and uptake of maize plant at 45 DAS

The data on the effect of AMF inoculum on phosphorus content and uptake in maize are presented in Table 6.

The P content and uptake in the plants inoculated with AMF formulations was found to be increased significantly over UIC. Among the different formulations, sodium alginate-based formulation recorded the highest P content (0.56%) and uptake, followed by glycerol with AMF product (0.54%). The lowest P content and uptake was recorded in the UIC (0.32%).

Table 6: Influence of AMF formulation on P content and uptake of maize plant at 45 DAS

Treatment	P content (%)	P uptake (g plant^{-1})
Talc with AMF product (1:1)	0.39	0.32
Talc with AMF product (1:2)	0.40	0.36
Talc with AMF product (1:3)	0.41	0.37
Lignite with AMF product (1:1)	0.40	0.34
Lignite with LAM (1:2)	0.42	0.38
Lignite with LAM (1:3)	0.42	0.38
Humic Acid with AMF product (1:1)	0.41	0.37
Humic Acid with AMF product (1:2)	0.42	0.38
Humic Acid with AMF product (1:3)	0.43	0.39
Glycerol with AMF product (1:1)	0.54	0.49
Encapsulated AMF product	0.51	0.46
Sodium alginate with AMF product	0.56	0.50
Uninoculated control	0.32	0.22
S. Em±	0.03	0.03
CD (0.05)	0.09	0.08

Discussion

Development of successful AM formulations requires a suitable economically viable, carrier material. It needs to be farmers friendly with respect to handling and application at the field sites. Several researchers across the world proposed different inert carrier material used for developing AM formulation *viz.*, glass beads (Redecker *et al.*, 1995) [24] expanded clay (Plenchette *et al.*, 1983) [23] sand, vermiculite and soil-rite (Millner and Kitt 1992) [19], alginate beads (Declereck *et al.*, 1996) [6]. Gentry *et al.* (2004) [10] suggested bentonite clay as an ideal carrier material that protection for the cells against toxic and adverse environmental condition. Malusa *et al.* (2012) [18] also reported that the inert carrier material like vermiculite, perlite, kaolin, bentonite, silicates could be used for *in vitro* produced AM inoculum formulation.

In order to study the effect of different AMF formulation on the growth of maize, a pot culture experiment was conducted. In this study, a steady increase in AM spore load and root colonization percentage was observed in all the formulations at 45 DAS of maize. However, maximum root colonization (61.33 per cent) and spore load (81.50 spores per 50 g of soil) were recorded in the treatments received sodium alginate bits with AMF product.

G. intraradices formulations inoculated to the test plants resulted significantly better performance on growth parameters when compared to the UIC. The pot experiment showed a significant difference in plant growth, mycorrhizal parameters, enzyme activity and biophysical parameters. Improvement in the diameter and height has been observed in case of tomato which was inoculated with *Glomus mosseae* (Liasu and Ogundola, 2006) [16]. Inoculation of AMF formulations have shown a maximum increase in plant height over control plants.

The AMF formulation significantly improved the chlorophyll content compared to the uninoculated plants. Abdel and Mohamedin (2000) [1]; Franco and Garza (2006) [9] also observed increased chlorophyll content due to mycorrhizal application. This may be due to the increased balanced mineral nutrients like P and K content in the leaves of mycorrhizal plants (Giri and Mukerji, 2004) [12]. Zuccarini (2007) [29] reported an increased concentration of chlorophyll content and total foliar area due to mycorrhization in grapes. Further, they have also noticed an improvement in the chlorophyll contents (chlorophyll a, b and total chlorophyll). The biophysical parameters such as photosynthetic rate and stomatal conductivity of maize phyllosphere were all improved in the present study indicating the influence of AMF formulation on biophysical parameters in maize. Our findings are in agreement with the reports of Selvaraj and Chellapa (2006) [25]. They have also reported an increased photosynthetic activity in the leaves of *Prosopis julifera* when inoculated with *G. fasciculatum*.

In the present study, the AMF formulations showed a noticeable increase in the total dry matter of maize plants over uninoculated control. Growth parameters like plant height, chlorophyll content and stem girth in the inoculated plants, recorded higher in the present investigation; this led to increased dry matter of maize. The increase in growth and biomass of inoculated plants strongly depends on their ability to access minerals from the soil.

Therefore, positive effects of AM Formulations on P content could be related to the ability of the AMF to enhance soil P (Smith *et al.*, 1985; Mali *et al.*, 2009 and Somchit *et al.*, 2008) [26, 17, 27]. Diop (2003) [7] reported that the indigenous AM

fungi (*Glomus aggregatum*) significantly increased the shoot P in *Solanum aethiopicum* cultivars, resulting in increased growth and biomass of the inoculated plants. Similar findings were also reported by Cavagnaro *et al.* (2005) [4]. In numerous scientific studies, *Glomus intraradices* has been shown to increase phosphorus uptake in multiple plants as well as improve soil aggregation due to hyphal network.

Conclusion

Among the formulations, sodium alginate bits with AMF product received treatments recorded significantly the highest plant height (28.33 cm), followed by glycerol with AMF product (28.00 cm). Highest shoot and root biomass dry matter (81.56 g p⁻¹ and 8.36 g p⁻¹, respectively) and followed by glycerol with AMF product (81.55 g p⁻¹). Highest AMF spore load and per cent root colonization and soil enzyme activity. The plant physiological parameters *viz.*, relative chlorophyll content, stomatal conductance, transpiration rate, photosynthetic rate and leaf temperature were recorded maximum in sodium alginate bits with AMF product. The P content in the plants inoculated with AMF formulation was found to be increased significantly over UIC.

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