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Influence of Biopolymer Synthesized from Tamarind Seed Polysaccharide (TSP) on Physiological and Biochemical Parameters of Maize Hybrid COH(M) 8 Seeds

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

The experiment was conducted in Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu during 2021. Biopolymer was synthesized from Tamarind Seed Polysaccharide (TSP) and added with different additives and coated maize seeds to know how they effect their physiological and biochemical parameters. The seeds were given with four treatments *viz.,* T_0- control (untreated seed), T_1- biopolymer (B.P) - 10g kg^{-1}, T_2- B.P (10g) + Humic acid (0.3g) + Zimmu (*Allium cepa* × *Allium sativum*) leaf extract (0.5 ml) and T_3- T_2+ Ascorbic acid (0.2 g) and evaluated for seed quality parameters. The results of the present investigation revealed that T_3 was significantly superior *viz.,* higher percentage of germination (95%), rate of germination (31.64), seed metabolic efficiency (2.78), seedling root length (25.92 cm), seedling shoot length (15.57 cm), total biomass production (9.41 g), vigour index I (3942), vigour Index-II (94.15). This treatment also recorded highest value of biochemical parameters such as α-amylase (2.23), dehydrogenase (1.87 OD value), catalase (29.94) and peroxidase (7.00) activities. It

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reduced the abnormal seedlings (2%), dead seeds (3%), days to 50% germination (2.54), mean emergence time (3.02) and pathogen infection (0.25%). We concluded that maize seeds coated with T_3 had better seedling establishment and may be recommended as pre sowing seed treatment under organic agriculture.

Keywords: Tamarind seed polysaccharide; biopolymer; humic acid; zimmu leaf extract; seed germination and vigour.

1. INTRODUCTION

One of the most important cereals in the world is maize (*Zea mays* L.). It is used as a human food, animal feed and a raw ingredient in a variety of industrial products. Maize is a high-yielding, easily digestible crop and widely utilized in confectioneries. It's a key ingredient in the manufacture of starch, oil, protein, alcoholic drinks, food sweeteners and more recently in the biofuel industry.

Maize is a plant species adapted to a wide range of environmental conditions, but pest and disease outbreaks can lead to lower crop yield and quality. Moreover, seed quality is essential for success in agriculture, because each seed must germinate promptly and develop a healthy seedling, and profitable crop yield [1]. Seed coating technology has advanced rapidly over the last three decades, and it offers a cost-effective method of seed improvement. Seed coating is the act of applying a beneficial material directly on a seed to generate a thin, homogeneous coating without changing the seed's shape or size. Seed coating has presented promising results in many crops including cereals. Seed coating with synthetic polymers has gained rapid acceptance by the seed industry. It makes room for including all the required ingredients like inoculants, protectants, nutrients, plant growth promoters, hydrophobic / hydrophilic substances, herbicides, oxygen suppliers etc.

Seed coating with polymer enhances chemical adhesion to the seed and allows for dust-free handling of treated seed [2]. The polymer coating is easy to apply, diffuses promptly, and is non-toxic to seed germination. The polymer coating may operate as a physical barrier, preventing inhibitors leaching from seed coverings and restricting oxygen transport to the embryo [3]. Thereby, it provides protection from the stress imposed by ageing, improves plant stand and emergence of seedlings. Polymer acts as a temperature switch and protective coating by regulating the water uptake and subsequent germination of seed [4]. Coating results in more uniform and accurate seed sowing rate because of the smooth flow of the seed during the mechanical sowing. Increase in germination can also be observed in polymer coated seed. Addition of colourants helps in visual monitoring of placement accuracy, enhance the appearance, marketability and consumer preference.

Despite the polymer coating has number of benefits on seed quality parameters and agriculture, the continuous use of synthetic polymer and synthetic colourants may degrade the soil quality and ultimately reduces the crop yield, because most of the seed coating polymers are synthetic and slowly degradable in nature. Continuous use of polymer may leads to its accumulation in the soil profile and they may cause negative effect on the soil microorganisms and soil health. Eventually they may impact the crop growth and yield, and may pollute the water bodies and environment conditions. Considering this issue, we synthesized the biopolymer from tamarind seed polysaccharide and studied its influence on seed physiological and biochemical characteristics in one hybrid of maize.

2. MATERIALS AND METHODS

We conducted a laboratory experiment conducted to study “The Effect of biopolymer coating on the seed quality parameters of the hybrid maize COH(M) 8 (*Zea mays* L.), at the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore, during 2021. The treatments were T_0- Control (untreated seed), T_1- biopolymer (B.P) 10g kg^{-1}, T_2- B.P (10g) + Humic acid (0.3g) + Zimmu leaf extract (0.5 ml) and T_3- B.P (10g) + Humic acid (0.3g) + Zimmu leaf extract (0.5 ml) + ascorbic acid (0.2 g). Specified dose of polymer and additives were mixed with 15 ml of water and treated the one kilogram of seeds. Treated seeds were shade dried for one hour and evaluated for seed quality parameters.

2.1 Preparation of Seed Coating TSP Polymer

TSP polymer was prepared from defatted tamarind kernel powder as per the protocol described by Sivasakthi and Renganayaki, 2022 [5].

2.2 Design of the Experiment

With five replications, the experiment was done in a completely randomized block design.

2.3 Observations

2.3.1 Seed physical characters

2.3.1.1 One hundred seed weight

Seed weight was estimated by weighing 100 seeds from eight replication and the mean values were expressed in gram.

2.3.1.2 Seed moisture content

Moisture estimation was carried out by high constant temperature method based on ISTA protocol [6].

2.3.2 Seed physiological characters

2.3.2.1 Percentage of seed germination

The germination test was carried out by using the procedure prescribed by ISTA [6] in roll towel paper method. The test conditions were 25±2ºC and 95±5% RH maintained in a germination room illuminated with fluorescent light. After seven days, the number of normal seedlings was counted and germination percentage (GP) was calculated, according to the formula:

Germination percentage (GP) = (Ng / Nt) × 100

Where Ng is a total number of normal seedlings germinated, Nt is a total number of seeds

2.3.3 Days to fifty per cent germination and maximum germination

In sand media, the number seeds germinated was recorded daily up to final count and number of days required to 50 per cent germination and number of days required to maximum germination was computed according to Heydecker & Coolbear [7] and Mauromicale & Cavallaro [8] respectively.

2.3.4 Rate of germination

Numbers of seeds germinated were counted daily up to seven days at the same time of day. From the number of seeds germinated on each counting day, the rate of germination was computed adopting the formula given by Maguire [9].

2.3.5 Mean germination time

Mean germination time (MGT) was calculated according to Bailly et al. [10] using the formula:

$$MGT = \Sigma (Dn) / \Sigma n$$

Where, n is the number of seeds germinated on each day and D is the day of counting

2.3.6 Endosperm and embryo degradation (Seed Metabolic Efficiency)

Amount of seed respired (SMR) was calculated as

$$SMR = SDW - (SHW + RTW + RSW)$$

Where,

SDW - Seed dry biomass before germination
SHW - Shoot dry biomass
RTW - Root dry biomass
RSW - Remaining seed dry biomass

Seed Metabolic Efficiency (SME) was calculated using the formula [11]

$$SME = (SHW + RTW) / SMR$$

2.3.7 Root length and shoot length (cm)

Ten normal seedlings from the standard germination test were randomly selected and the root and shoot length was measured from the collar region to the tip of the primary root and tip of the shoot respectively. The average value was expressed in centimeter.

2.3.8 Dry biomass production (g seedlings^{-10}) and total dry biomass production (g) [6]

The seedlings used for growth measurement and the remaining normal seedlings were placed in a butter paper cover separately and dried under shade for 24 h and then kept in an oven maintained at 85 ± 2^{o}C for 24 h. Dry biomass was recorded and the mean values expressed in

g. The total dry biomass production was calculated by adding dry weight of ten seedlings and remaining normal seedlings.

2.3.9 Vigour index [12]

Vigour index values were computed using the following formula and the mean values were expressed in whole number.

Vigour index I = Germination (%) x Total seedling length (cm)

Vigour index II = Germination (%) x Dry biomass production (g seedling^{-10})

2.4 Biochemical Parameters

2.4.1 Dehydrogenase activity (OD value)

Dehydrogenase activity was estimated by the procedure described by Kittock and Law [13].

2.4.2 α-amylase activity (mg maltose liberated/ minute)

α- amylase activity in pre germinated seeds were carried out according to the method suggested by Simpson and Naylor [14].

2.4.3 Peroxidase activity (∆ A436 / min/ g of seed)

Peroxidase activity was estimated as per the procedure described by Singh et al. [15].

2.4.4 Catalase activity (mMol H_2O_2 degraded/ minute)

Catalase activity was measured by an assay of hydrogen peroxide based on formation of its stable complex with ammonium molybdate [16].

2.5 Seed Health

2.5.1 Pathogen infection (%)

Pathogen infection was assessed by the protocol given by ISTA [6].

2.6 Data Analysis

Statistical analyses of the experimental data were performed using the SPSS software (ver. 18.0). All of the data presented are the averages of five replicates, with deviations calculated as the standard error of the mean (SEM). Analysis of variance (ANOVA) was used for statistical processing. Duncan test post hoc analysis was performed to define which specific mean pairs were significantly different. A significant level was defined as a probability of 0.05 or less.

3. RESULTS AND DISCUSSION

The result revealed that, seeds coated with bio polymer (B.P) and B.P with additives shows non-significant difference for 100 seed weight and moisture content (Table 1). Polymer coating forms a very thin layer around the seeds without obscuring size and shape; hence it did not alter the seed weight. After seed coating the seeds were dried under shade for one hour, hence the polymer coating did not change the moisture content significantly in the seeds.

According to the results, all studied traits were affected by the treatments and there was completely significant difference observed among treatments. Seed physiological characters *viz.,* germination percent (95%), rate of germination (31.64), seed metabolic efficiency (2.78), seedling root length (25.92 cm), seedling shoot length (15.57 cm), dry biomass production/10 seedlings (0.991 g), total dry biomass production (9.41 g), vigour index-I (3942), and vigor index-II (94.15) were significantly recorded maximum in T_3 whereas found lowest in control (Table 1,2 and Fig. 1,2). It significantly reduced days for 50% germination (2.54), mean emergence time (3.02), abnormal seedling (2%) and dead seeds (3%) compare to other treatments (Table 1).

The improvement in seed physiological parameters in T_3 is due to additives such as humic acid and ascorbic acid present in the polymer formulation. Humic acid is one of bio-stimulants that are known as the organic substances which promote plant growth [17]. Humic acid improves the nutrient availability especially microelements in soils because it promotes nutrient uptake in the form of chelating agent. Moreover, humic substances may increase root growth in a similar manner to auxins [18,19]. The present results similar to the findings of Asgharipour and Rafiei [20] and Basalma [21] who reported that seed treated with HA recorded maximum germination, seedling length, seedling fresh weight, seedling dry weight and vigour index in barley and safflower respectively. Likewise, HA seed treatment increased the shoot fresh and dry weight of seedlings in tomato [22], maize [23], pea [24], wheat [25], cucumber, squash and marigold [26].

The activity mechanism of humic acid in promoting plant growth is not fully understood, and the beneficial effects to plants are difficult to comprehend due to its chemical heterogeneity [27,28]. The most established explanations for the beneficial effects of HA are related to their positive influence on ion transport, which improves cell permeability, thereby affects absorption. They also promote increased respiration and speed of enzymatic reactions of the Krebs cycle, resulting in increased ATP production, altering directly plant metabolism and consequently may influence growth and development [29,30]. The increase in absorption rates can be explained by the activation of ATPase present in the plasma membrane [31], acting on two mechanisms essential to plant growth, through the energy supply to the secondary systems in the translocation of ions and by increasing plasticity of the cell wall, thus allowing cell growth and division [32].

The reason for improved seed physiological parameters in T_3 may also be due to ascorbic acid present in the polymer formulation. Ascorbic acid (AsA), also known as ascorbate or vitamin C, is a low molecular weight water-soluble antioxidant both in plants and animals. And AsA is a universal non-enzymatic antioxidant having a substantial potential of not only scavenging reactive oxygen species (ROS), but also modulating many fundamental functions in plants both under stress and nonstress conditions [33,34,35]. Burguieres et al. [36] and Chen et al. [37] reported that seeds treated with AsA increased the germination, seedling length, fresh weight and dry weight in pea and alfalfa respectively.

Table 1. Effect of biopolymer and additives on stand establishment traits of hybrid maize COH(M) 8

Treatments	100 seed weight (g)	Seed moisture content (%)	Abnormal seedling (%)	Dead seeds (%)	Days for 50 per cent germination	Rate of germination	Mean emergence time
T_0	31.27	10.22	5	5	3.16	27.31	3.37
T_1	31.44	10.24	5	5	3.14	27.66	3.34
T_2	31.43	10.23	2	5	2.62	28.42	3.08
T_3	31.44	10.22	2	3	2.54	31.64	3.02
Mean	31.40	10.23	4	5	2.87	28.76	3.20
SEd	0.26	0.06	0.03	0.06	0.04	0.40	0.03
CD (P=0.05)	0.55	0.13	0.06	0.12	0.08	0.85	0.06

T_0- Control (untreated seed), T_1- biopolymer (B.P) @ 10g kg^{-1}, T_2- B.P (10g) + Humic acid (0.3g) + Zimmu leaf extract (0.5 ml) and T_3- B.P (10g) + Humic acid (0.3g) + Zimmu leaf extract (0.5 ml) + ascorbic acid (0.2 g)

Table 2. Effect of biopolymer and additives on seedling vigour parameters of hybrid maize COH(M) 8

Treatments	Seed metabolic efficiency	Shoot length (cm)	Root length (cm)	Dry biomass production (g/10 seedlings)	Total dry biomass production (g)	Vigour index II
T_0	2.24	12.85	23.48	0.908	8.17	81.72
T_1	2.26	13.14	23.85	0.912	8.21	82.08
T_2	2.65	15.14	25.65	0.956	8.89	88.91
T_3	2.78	15.57	25.92	0.991	9.41	94.15
Mean	2.48	14.18	24.73	0.942	8.67	86.72
SEd	0.02	0.13	0.39	0.01	0.06	1.03
CD (P=0.05)	0.04	0.28	0.83	0.02	0.13	2.18

T_0- Control (untreated seed), T_1- biopolymer (B.P) @ 10g kg^{-1}, T_2- B.P (10g) + Humic acid (0.3g) + Zimmu leaf extract (0.5 ml) and T_3- B.P (10g) + Humic acid (0.3g) + Zimmu leaf extract (0.5 ml) + ascorbic acid (0.2 g)

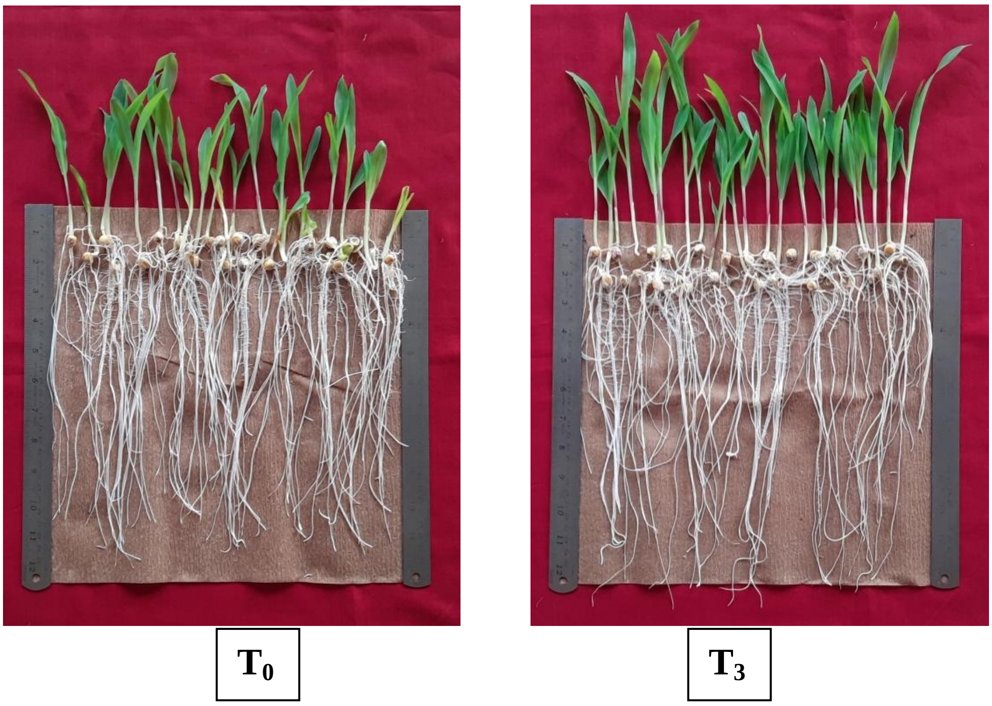

Fig. 1. Effect of biopolymer with additives on seed germination and seedling growth of hybrid maize COH(M) 8

T_0- Control (untreated seed), T_3- B.P (10g) + Humic acid (0.3g) + Zimmu leaf extract (0.5 ml) + ascorbic acid (0.2 g)

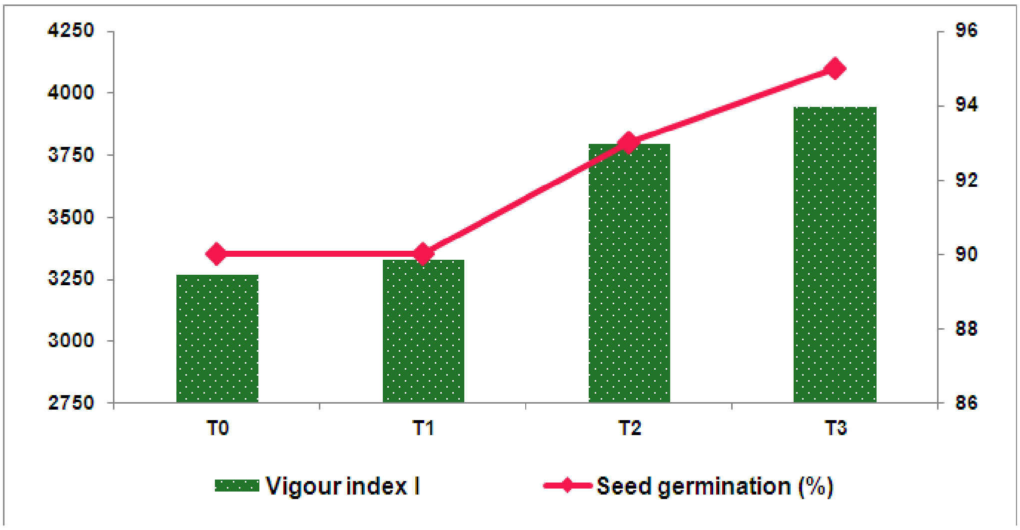

Fig. 2. Effect of biopolymer with additives on seed germination and seedling vigour index I of hybrid maize COH(M) 8

T_0- Control (untreated seed), T_1- biopolymer (B.P) @ 10g kg^{-1}, T_2- B.P (10g) + Humic acid (0.3g) + Zimmu leaf extract (0.5 ml) and T_3- B.P (10g) + Humic acid (0.3g) + Zimmu leaf extract (0.5 ml) + ascorbic acid (0.2 g)

Among the treatments T_3 provided significantly higher levels of enzymatic activities *viz.,* α-amylase (2.23 mg maltose/min), dehydrogenase (1.87 OD value), catalase (29.94 mMol H_2O_2 / min) and peroxidase (7.00 ∆ A436 /min/g) compared to other treatments (Table 3). The result similar to the findings of Burguieres *et al.* [36] who stated that seeds treated with AsA increased the guaiacol peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT) activities in pea. Similarly, Chen *et al.* [37] reported that seeds treated with AsA increased the α-amylase and protease activities in alfalfa seeds.

Table 3. Effect of biopolymer and additives on biochemical parameters and pathogen infection of hybrid maize COH(M) 8

Treatments	α-amylase activity (mg maltose min^{-1})	Dehydrogenase activity (OD value)	Catalase activity (μg H_2O_2/min/mg protein)	Peroxidase activity (ΔOD 430 mg^{-1} min^{-1})	Pathogen infection (%)
T_0	2.17	1.76	28.16	6.58	1.25
T_1	2.17	1.78	28.18	6.59	1.50
T_2	2.18	1.80	28.53	6.59	0.25
T_3	2.23	1.87	29.94	7.00	0.25
Mean	2.19	1.80	28.70	6.69	0.81
SEd	0.03	0.02	0.17	0.05	0.009
CD (P=0.05)	0.07	0.03	0.37	0.12	0.020

T_0- Control (untreated seed), T_1- biopolymer (B.P) @ 10g kg^{-1}, T_2- B.P (10g) + Humic acid (0.3g) + Zimmu leaf extract (0.5 ml) and T_3- B.P (10g) + Humic acid (0.3g) + Zimmu leaf extract (0.5 ml) + ascorbic acid (0.2 g)

The results shows that the seeds treated with T_2 and T_3 significantly reduced the pathogen infection (0.25%) than T_0 and T_1 (1.25 and 1.50% respectively) (Table 3). This may be the reason for reduced dead seed percentage in T_2 and T_3. Reduced pathogen infection is due to added antimicrobial agent (zimmu leaf extract) in the polymer. Satya et al. [38] found that the leaf extract of zimmu showed the maximum antifungal activity against *Rhizoctonia solani* and it also effective in inhibiting the growth of other fungal and bacterial pathogens *viz., Aspergillus flavus, Alternaria solani, Curvularia lunata, Xanthomonas campestris* pv. *Malvacearum, X. oryzae* pv. *oryzae,* and *X. oxonopodis* pv. *citri*. Thus the study indicates that the physiological and biochemical improvement in T_3 is due to synergetic effect of humic acid and ascorbic acid present in the polymer.

4. CONCLUSION

The biopolymer and additives had a significant effect on the physiological and biochemical seed performance in this hybrid maize. T_3 improved the stand establishment such as seed germination, rate of germination, seed metabolic efficiency, shoot length, root length, dry biomass production, vigour index I and vigour index II compared to other treatments. It also increased the dehydrogenase, α- amylase, catalase and peroxidase activities. It reduced the days to 50% germination, mean germination time, abnormal seedlings, dead seeds and pathogen infection compared to control. Thus the study highlighted that seeds coated with B.P (10g) + Humic acid (0.3g) + Zimmu leaf extract (0.5 ml) + ascorbic acid (0.2 g) improved the seed performance in this hybrid maize.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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