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The Antimicrobial Effect of Seed Coat Polymers on Soil Borne Pathogens of Castor and Groundnut

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ABSTRACT

Castor (Ricinus communis L.) and groundnut (Arachis hypogaea L.) are the two important oilseed crops grown in India. Of the various soil borne diseases, the wilt caused by Fusarium oxysporum f. sp. ricini in castor and the collar rot caused by Aspergillus niger in groundnut are the major diseases. The effect of polymers was tested on wilt pathogen of castor and collar rot pathogen of groundnut separately by soil inoculation in pot culture studies. The chitosan (0.25%) coated castor and groundnut seeds were grown in F. oxysporum f. sp. ricini and Aspergillus niger inoculated soil respectively there was significant difference in per cent germination, vigou index-I and vigour index-II with minimum per cent disease incidence of 34.60 and 68.80 respectively, when compared to synthetic polymer-II and control.

Key words: *Castor, germination, Aspergillus niger, Fusarium oxysporum.*

INTRODUCTION

Castor is known to suffer from many diseases at different stages of crop growth and about 150 organisms are reported as pathogenic on castor plant. The crop is mainly affected by wilt, seedling blight, *Alternaria* blight, *Cercospora* leaf spot, root rot, powdery mildew and also bacterial leaf spot. Among these, the wilt caused by *Fusarium oxysporum* f.sp. *ricini* is an important soil borne disease. The extent of yield loss depends on the stage at which the plants wilt which is about 77% at flowering stage, 63% at 90 days and 39% at later stages. The disease assumed serious proportion in all the castor growing areas of India including Telangana state because of long duration survival of the pathogen in the soil and susceptibility of prevalent cultivars. Groundnut (*Arachis hypogaea* L.) is an important oilseed and ancillary food crop of the world. Groundnut cultivation in India as a rainfed crop is often subjected to significant yield losses annually due to insufficient and uneven distribution of rainfall during the crop growth period, non-availability and inaccessibility of high yielding cultivars and fertilizers to farmers. Of various biotic stresses, soil borne and foliar diseases account for reduced pod yields.

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Fungal diseases such as collar rot (*Aspergillus niger* Van Tieghem), stem rot (*Sclerotium rolfsi* Sacc.), root rot (*Rhizoctonia solani* Kuhn), dry root rot (*Macrophomina phaseolina* (Tassi) Goid) and seedling blight (*Fusarium oxysporum* Schlecht) are causing major havoc in all crop growing areas. Of these, collar rot and stem rot diseases are the major soil borne diseases with significant yield losses annually. Polymer coating acts as a temperature switch, regulating intake of water by seed coat, the stress imposed by accelerated ageing, which includes fungal invasion and improves the seedling emergence at changing soil moisture regime. The positive charge of chitosan confers the numerous and unique physiological and biological properties with great potential in a wide range of industries such as pharmacology, medicine, and agriculture[6]. Another important attribute of this natural compound is associated with its fungistatic or fungicidal properties against pathogens of various crops[3].

MATERIAL AND METHODS

Isolation of the test fungal pathogen was made from infected plant roots of castor wilt (*Fusarium oxysporum* f. sp. *ricini*) and infected collar portions of the diseased ground plants which were collected from the IIOR, Rajendranagar, Hyderadad. The infected roots were thoroughly washed under running tap water and transferred to blotting paper. They were cut into 0.20 cm thick pieces and surface sterilized with 1% sodium hypochlorite solution for 1 minute followed by three washings with sterile distilled water and were placed on Petri plates containing PDA medium. The plates were incubated at 25-28ºC for 4 to 5 days. The fungal growth emerging from diseased root pieces were picked up and the culture was further purified by single spore isolation method and incubated at 28±2ºC for 7 to 8 days. The pure culture of the pathogen was maintained on PDA medium by periodical transfers. The test pathogen was mass multiplied on sorghum grains[2]. Sorghum grains were soaked in water overnight. Later, the excess water was removed and soaked grains were transferred into 500 ml flasks @ 200 g and autoclaved at 15 lb *psi* (121ºC) for 20 min. The flasks were allowed to cool at room temperature and inoculated with 5 mm discs of actively growing 3-4-day-old culture of *Aspergillus niger*. Five to six discs per flask were added and the flasks were later incubated in growth room for 10 days at 28±2ºC.

Synthetic seed coat polymer solution was prepared by mixing 3 ml of polymer solution in 5 ml of sterile distilled water in clean, dry Eppendorff tube (10 ml) by pipette. For the preparation of chitosan solution, 2.5 g of chitosan was weighed, mixed with water and to this 5 ml of 1% acetic acid was added to dissolve chitosan. Prior to coating initial seed moisture content, germination percentage, seedling dry weight and seedling vigour index were recorded. One hundred gram of seeds was taken in a polythene bag and added polymer @ 3 ml kg^{-1} of seeds. The polythene bag was closed tightly trapping air in it to form of a balloon then polythene bag was vigorously shaken till the seeds are uniformly coated, later the treated seeds were spread on a sheet under the shade and dried completely[7].

Seed coating with chitosan was done as per the procedure of Paulin *et al*.[5]. One hundred grams of the clean, dry seeds were dipped in a solution of acetylated chitosan @ 2.5 g kg^{-1} of seeds in a conical flask and kept on magnetic stirrer about 12 h at 100 rpm (25ºC). After 12 h of incubation, the uniformly coated seeds were spread on a sheet under the shade and dried completely. The dried seeds were used for sowing.

RESULTS

An experiment was conducted to test the effect of synthetic and biopolymers on castor wilt and collar rot of groundnut. One kilogram of sterilized soil was weighed and filled in black polythene planting bags. Inoculum of *Fusarium oxysporum* f. sp. *ricini* and *Aspergillus niger* mixed in the sterilized soil separately. Castor and groundnut seed were coated with synthetic polymer-II @ 0.3% and chitosan @ 0.25% and sown in the inoculated bags separately. Uninoculated bags for each

treatment served as control. Data on per cent germination, vigour index-I, II and growth parameters were recorded at 30 days after sowing and results are presented in Table 1 and 2.

Castor

Data in Table 1 indicated that there was a significant increase in per cent germination (95.20%), vigour index-I (5656.38), vigour index-II (197.96) and growth parameters when the castor seeds were treated with chitosan alone when compared to control. But a significant difference was not observed in per cent germination (88.20%), vigour index-I (4471.98), vigour index-II (99.10) and growth parameters when treated with synthetic polymer-II compared to control (Plate A).

However when chitosan and synthetic polymer-II coated castor seeds were grown in *Fusarium oxysporum* f. sp. *ricini* inoculated soil there was significant difference in per cent germination when compared to control. The chitosan treated seed recorded germination of 84.60% while in synthetic polymer-II coated seed the germination was 70.60 when compared to control. Similarly the vigour index-I and vigour index-II of castor seed coated with chitosan was 2956.18 and 161.25 respectively and with synthetic polymer-II, it was 1164.90 and 28.05 respectively.

The per cent disease incidence of *Fusarium oxysporum* f. sp. *ricini* was also calculated (Table 1.1) and it was observed that chitosan treated seed was highly significant (34.60%) in reducing the disease incidence when compared to synthetic polymer-II (63.60%).

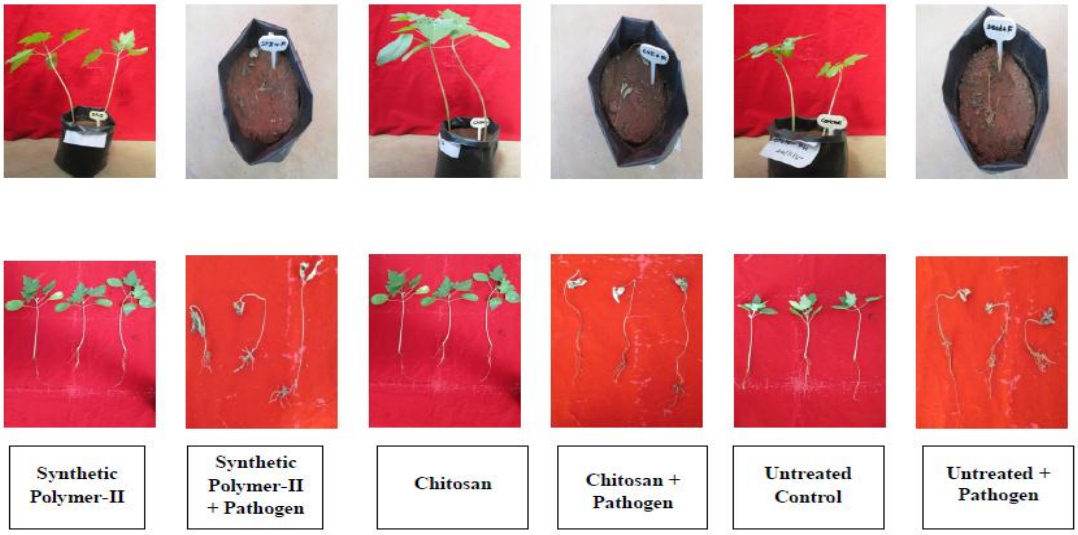

Plate A: Effect of synthetic polymer and biopolymer chitosan treated seed on growth parameters and antifungal activity against wilt of castor in pot culture

Table 1: Effect of seed coat polymers on wilt of castor in pot culture studies

Treatment	Germination (%)	No. of Leaves	Root Length (cm)	Shoot Length (cm)	Vigour Index-I	Fresh Weight (g)	Dry Weight (g)	Vigour Index-II
Synthetic Polymer-II	88.20 (69.92)**	13.60	18.88	31.82	4471.98	4.12	1.12	99.10
Synthetic Polymer-II + Pathogen*	70.60 (57.21)	4.00	5.40	11.06	1164.90	1.22	0.39	28.05
Chitosan	95.20 (78.78)	13.20	22.98	36.38	5656.38	5.96	2.07	197.96
Chitosan + Pathogen	84.60 (66.91)	8.00	13.28	21.66	2956.18	3.74	1.91	161.25
Untreated	88.20 (69.92)	12.40	17.54	27.30	3723.68	3.49	1.21	100.53
Untreated + Pathogen	42.40 (40.59)	4.00	4.46	9.52	596.10	0.39	0.09	3.97
CD (p = 0.05)	4.84	1.32	0.55	1.71	289.31	0.47	0.23	22.77
SE(d)	2.41	0.67	0.26	0.82	139.35	0.22	0.11	10.97
SE(m) ±	1.70	0.47	0.19	0.58	98.53	0.16	0.08	7.76
CV (%)	6.09	15.38	3.04	5.66	7.12	11.25	15.11	17.61

*** Pathogen** = *Fusarium oxysporum* f. sp. *ricini*

****** Values in the parentheses are angular transformed and are the means of Five replications

Table 1.1 Incidence of *Fusarium oxysporum* f. sp. *ricini* in pot culture of polymer treated castor seed

Treatment	Incidence (%)
Synthetic Polymer-II + Pathogen*	63.60 (52.89)**
Chitosan + Pathogen	34.60 (36.01)
Untreated + Pathogen	90.20 (71.78)
CD (p = 0.05)	2.32
SE(d)	1.05
SE(m) ±	0.74
CV (%)	3.10

*** Pathogen** = *Fusarium oxysporum* f. sp. *ricini*

****** Values in the parentheses are angular transformed and are the means of seven replications

Groundnut

Data in Table 2 indicated that there was significant difference in per cent germination of groundnut seeds (89.40%) and also in case of vigour index-I (4447.86), vigour index-II (166.87) and growth parameters when the groundnut seeds were treated with chitosan alone when compared to control. But there was no significant difference in per cent germination (84.60%) but some degree of significant difference was observed in vigour index-I (3850.56), vigour index-II (122.70) and growth parameters when treated with synthetic polymer-II compared to control (Plate B).

Plate B: Effect of synthetic polymer and biopolymer chitosan treated seed on growth parameters and antifungal activity against collar rot disease of groundnut in pot culture

Table 2: Effect of seed coat polymers on collar rot disease of groundnut in pot culture studies

Treatments	Germination (%)	No. of Leaves	Root Length (cm)	Shoot Length (cm)	Vigour Index-I	Fresh Weight (g)	Dry Weight (g)	Vigour Index-II
Synthetic polymer -II	84.60 (66.91)**	26.40	21.20	24.32	3850.56	5.50	1.45	122.70
Synthetic polymer -II + Pathogen*	0.00 (0.00)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chitosan	89.40 (71.00)	37.20	22.7	27.06	4447.86	6.192	1.87	166.87
Chitosan + Pathogen	75.80 (60.54)	16.00	16.58	19.16	2709.38	3.15	0.99	75.01
Untreated	85.80 (67.88)	30.40	20.56	23.3	3763.44	5.40	1.35	115.56
Untreated + Pathogen	0.00 (0.00)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CD (p = 0.05)	1.92	1.86	0.47	0.40	105.10	0.08	0.07	7.78
SE(d)	0.95	0.94	0.23	0.19	50.62	0.04	0.03	3.75
SE(m) ±	0.67	0.67	0.16	0.14	35.80	0.03	0.02	2.65
CV (%)	3.45	12.97	2.64	1.94	3.25	1.79	5.32	7.40

*** Pathogen** = *Aspergillus niger*
****** Values in the parentheses are angular transformed and are the means of five replications

However when the chitosan coated groundnut seeds were grown in *Aspergillus niger* inoculated soil, there was significant difference in per cent germination when compared to control. The chitosan treated seed recorded highest germination of 75.80% and lowest disease incidence of 68.80% (Table2.1). Similarly the vigour index-I and vigour index-II of groundnut seed coated with chitosan was 2709.38 and 75.01 respectively. Whereas total seedrotting (100%) and inhibiting germination was recorded in both synthetic polymer-II coated and untreated seeds of groundnut. The synthetic polymer-II coating also did not act as barrier against the collar rot pathogen and recorded the complete seed rotting as like as untreated seed infected with pathogen.

Table 2.1 Incidence of *Aspergillus niger* in pot culture of polymer treated groundnut seed

Treatment	Incidence (%)
Synthetic Polymer-II + Pathogen *	100.00 (90.00)**
Chitosan + Pathogen	68.80 (56.04)
Untreated + Pathogen	100.00 (90.00)
CD	1.51
SE(d)	0.69
SE(m) ±	0.49
CV	1.38

*** Pathogen** = *Aspergillus niger*
****** Values in the parentheses are angular transformed and are the means of seven replications

DISCUSSION

The synthetic polymer forms a thin oily film around the seed surface, act as barrier for direct contact between water and seed surface and enhanced the growth of plants. The chemical groups present in the synthetic polymer do not easily intermingle with the natural plant defence chemicals which are present within the plant. The biopolymer chitosan easily activates the defending mechanisms by lignifications of tissues along with growth promotion in plants. From the present investigation it was identified that the biopolymer chitosan is superior over the synthetic polymers in defending against the plant pathogens.

Yongxia *et al.*[8] observed highest germination rate, germination power, fresh weight of single plant, germination index, vigour index and resistance in soybean seeds treated by 1.0 ml l^{-1} chitosan against *Fusarium oxysporum* and observed a disease incidence upto 65.45%. Li *et al.*[4] showed antifungal activities of chitosan (0.1%) *in vitro* with complete inhibition (100%) of *Aspergillus niger*. Similar observations were made by Burrows *et al.*[1] who suggested that chitosan treatment resulted in best plant growth and showed antifungal activity.

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