



## ***In vitro* assessment of Seedling growth and Management of castor wilt and Groundnut collar rot through the combination of seed coat Polymers, fungicides and Bioagents**

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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. In the present study, effect of individual and combined application of seed coat polymers (synthetic polymer & chitosan), fungicides viz., carbendazim, vitavax+thiram (which were taken from poisoned food technique results) and two *Trichoderma* species viz., *Trichoderma asperellum* TaDOR 7316 and *Trichoderma harzianum* Th4d were used for seed coating and determined the effective combination to promote plant growth and control those soil borne diseases of castor and groundnut under *in vitro* conditions. In case of castor the treatment T13 (Chitosan + Carbendazim + *T. harzianum* Th4d) significantly increased the seedling growth and vigour in both uninoculated and pathogen inoculated condition than controls. In groundnut the treatment T12 (Chitosan + *T. asperellum* TaDOR 7316) exquisite in the seedling growth under uninoculated condition whereas under pathogen inoculation the treatment T13 (Chitosan + vitavax +thiram + *T. asperellum* TaDOR 7316) was the most effective.

**Keywords:** *Ricinus communis*, groundnut

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### **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 wilt caused by *Fusarium oxysporum* f. sp. *ricini* is an important soil borne disease. The disease assumed serious proportion in all the castor growing areas of India. Groundnut (*Arachis hypogaea* L.) is an important oilseed and ancillary food crop of the world. Of various biotic stresses, soil borne and foliar diseases account for reduced pod yields. Of those, collar rot and stem rot diseases are the major soil borne diseases with significant yield losses annually. In India, the collar rot disease on groundnut losses may account to 40-50 % in terms of seedling mortality due to *Aspergillus niger* (Ghewande *et al.*, 2002). Currently seed coating polymers are being used for seed coating along with active ingredients such as insecticides and fungicides. 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 especially in the sub-optimal range (Scott, 1989; Sherin & Susan John, 2003).

### **MATERIALS AND METHODS**

#### **Culture and Handling of Biological Control Preparations**

Seed coat polymers of synthetic, commercial biopolymer and chitosan were used in the present study to carry out the experiment. *Fusarium oxysporum* f. sp. *ricini* and *Aspergillus niger* was isolated from infected plant roots of castor and collar portions of groundnut respectively and placed on Petri plates containing PDA medium after surface sterilization (1% sodium hypochlorite) and incubated at 25-28°C for 4-5 days. The fungal growth emerging from diseased root pieces were picked up and the culture was further purified by single spore isolation method (Hansen, 1926). The pure cultures of the *Trichoderma asperellum* TaDOR 7316 and *Trichoderma harzianum* Th4d were obtained from Plant Pathology

laboratory, IIOR. *Trichoderma* spp. were grown on molasses-soy medium (Prasad and Rangeshwaran, 2000) and prepared the *Trichoderma* talc formulation.

### Seed Coating

Synthetic seed coat polymer solution was prepared by mixing the polymer @ 0.3% per kg in 5 ml of sterile distilled water in clean, dry Eppendorff tube (10 ml) by pipette. The polythene bag was closed tightly and shaken till the seeds are uniformly coated (Basavaraj *et al.*, 2008). For the preparation of chitosan solution, chitosan @ 0.25% per kg was weighed, mixed with water and to this 5 ml of 1% acetic acid was added to dissolve chitosan and soaked the seeds for 8 h (Hameed *et al.*, 2014). The synthetic polymer and chitosan coated castor and groundnut seed was again coated with fungicides *viz.*, carbendazim (castor), vitavax+thiram (groundnut) and biocontrol agents *viz.*, *Trichoderma harzianum* Th4d (castor) and *Trichoderma asperellum* TaDOR 7316 (groundnut) against *Fusarium oxysporum* f. sp. *ricini* and *Aspergillus niger* respectively. The treated seeds were tested for their germination by rolled paper towel method (ISTA). Proper check was maintained for each treatment. The seed quality parameters (inoculated & uninoculated condition) and per cent incidence in inoculated condition were recorded. The design of the experiment adopted was Completely Randomized Block Design with five replications.

### RESULTS

Effects of both synthetic polymer and chitosan and their combination with fungicide, biocontrol agent on castor and groundnut seeds were tested under *in vitro*. The results indicated that the castor seeds pretreated with chitosan, fungicide and biocontrol agent significantly affected the germination, seedling length (root length + shoot length), fresh weight and seedling vigour index (Table 1&2) in both uninoculated and inoculated condition and also reduces the per cent incidence (Table 2). The treatment T13 (chitosan+carbendazim+*T. harzianum* Th4d) was highly significant in increasing the per cent germination (98.00%) and vigour index-I (4437.60) and vigour index-II (210.32) when compared to control (germination- 84.60%, vigour index-I- 2549.54, vigour index-II- 133.50) under uninoculated conditions. Synthetic polymer-II+carbendazim+*T. harzianum* Th4d (T10) was also significant in recording germination of 93.40% and vigour index-I of 3857.34 and vigour index-II of 174.47. However treatment T5 *i.e.*, carbendazim+ *T. harzianum* Th4d, T8 (carbendazim+ synthetic polymer-II) and T9 (*T. harzianum* Th4d+synthetic polymer-II) were not significant in increasing per cent germination, vigour index-I and vigour index-II when compared to control.

**Table 1. Effect of seed coating polymers in combination with fungicide and biocontrol agent on the germination and vigour of castor *in vitro***

Treatment No.	Details of Treatment	Germination (%)	Root Length (cm)	Shoot Length (cm)	Vigour Index-I	Fresh Weight (g)	Dry Weight (g)	Vigour Index-II
T1	Synthetic Polymer-II	91.20 (72.77)*	18.44	17.44	3271.92	18.72	1.68	153.58
T2	Chitosan	93.20 (75.04)	19.14	20.10	3657.54	19.36	1.74	162.34
T3	Carbendazim	88.80 (70.44)	15.64	14.64	2689.04	17.60	1.60	141.90
T4	<i>T. harzianum</i> Th4d	87.60 (69.36)	15.34	13.52	2528.12	18.08	1.65	144.18
T5	Carbendazim+ <i>T. harzianum</i> Th4d	78.80 (62.57)	12.48	11.56	1894.32	14.60	1.23	97.25
T6	Synthetic Polymer-II + Carbendazim	91.00 (72.55)	17.54	18.56	3285.26	19.60	1.75	158.88
T7	Synthetic Polymer-II + <i>T. harzianum</i> Th4d	91.60 (73.16)	17.76	18.50	3321.28	19.90	1.81	165.99
T8	Carbendazim + Synthetic Polymer-II	83.60 (60.00)	14.52	15.42	2502.86	17.08	1.53	128.08
T9	<i>T. harzianum</i> Th4d + Synthetic Polymer-II	75.00 (66.10)	13.26	13.38	1998.44	16.66	1.48	111.13
T10	Synthetic Polymer-II + Carbendazim + <i>T. harzianum</i> Th4d	93.40 (75.11)	20.48	20.82	3857.34	20.48	1.87	174.47

T11	Chitosan + Carbendazim	94.60 (76.56)	20.78	21.60	4009.26	20.88	1.93	182.96
T12	Chitosan + <i>T. harzianum</i> Th4d	94.60 (76.56)	20.70	22.62	4097.80	21.42	2.04	192.79
T13	Chitosan + Carbendazim + <i>T. harzianum</i> Th4d	98.00 (82.79)	21.62	23.66	4437.60	22.60	2.15	210.32
T14	Untreated Seed	84.60 (66.91)	16.14	14.00	2549.54	16.36	1.58	133.50
CD (p = 0.05)		2.27	0.37	0.32	79.47	0.29	0.03	4.14
SE(d)		1.13	0.18	0.16	39.56	0.14	0.02	2.06
SE(m) ±		0.80	0.13	0.11	27.98	0.10	0.01	1.46
CV (%)		2.51	1.66	1.44	1.99	1.19	1.34	2.11

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

Data in Table 2 indicated that maximum per cent germination (95.80%) was observed in T13 when castor seed was treated with chitosan+carbendazim+*T. harzianum* Th4d and *F. oxysporum* f. sp. *ricini* when compared to control and also increased the vigour index-I (3542.58) and vigour index-II (152.90) and also reduced the per cent incidence (15.40%) than other individual, combinations and control.

**Table 2. Effect of seed coating polymers in combination with fungicide and biocontrol agent on the germination and vigour of castor by *Fusarium oxysporum* f. sp. *ricini* inoculation *in vitro***

Treatment No.	Details of Treatment	Germination (%)	Root Length (cm)	Shoot Length (cm)	Vigour Index-I	Fresh Weight (g)	Dry Weight (g)	Vigour Index-II	Per cent Incidence
T1	Synthetic Polymer-II	62.60 (52.28)*	12.50	9.92	1404.02	12.20	0.85	53.08	72.60 (58.42)*
T2	Chitosan	77.40 (61.60)	15.92	14.02	2317.42	16.08	1.14	88.22	52.40 (46.36)
T3	Carbendazim	83.20 (65.79)	14.82	13.70	2372.72	15.46	1.25	103.68	43.00 (40.96)
T4	<i>T. harzianum</i> Th4d	85.80 (67.87)	14.66	13.44	2410.38	15.66	1.34	115.33	44.20 (41.65)
T5	Carbendazim + <i>T. harzianum</i> Th4d	62.60 (52.30)	11.86	11.08	1436.10	10.70	1.08	67.84	67.40 (55.16)
T6	Synthetic Polymer-II + Carbendazim	88.00 (69.70)	16.34	14.76	2736.80	16.56	1.39	122.14	38.80 (38.51)
T7	Synthetic Polymer-II + <i>T. harzianum</i> Th4d	88.60 (70.24)	16.52	15.00	2792.70	15.34	1.44	127.41	32.60 (34.80)
T8	Carbendazim + Synthetic Polymer-II + Pathogen	65.40 (53.95)	12.48	11.44	1564.52	13.74	1.06	69.59	43.80 (41.42)
T9	<i>T. harzianum</i> Th4d + Synthetic Polymer-II	58.20 (49.70)	10.48	12.42	1333.14	13.14	0.92	53.54	45.60 (42.46)
T10	Synthetic Polymer-II + Carbendazim + <i>T. harzianum</i> Th4d	89.80 (71.39)	16.96	15.88	2949.38	16.94	1.50	134.34	27.40 (31.54)
T11	Chitosan + Carbendazim	93.20 (74.90)	17.40	16.16	3127.82	17.62	1.55	144.47	24.00 (29.31)
T12	Chitosan + <i>T. harzianum</i> Th4d	93.80 (75.60)	18.00	16.58	3243.50	17.30	1.56	146.33	20.80 (27.12)
T13	Chitosan + Carbendazim + <i>T. harzianum</i> Th4d	95.80 (78.23)	19.12	17.86	3542.58	18.44	1.60	152.90	15.40 (23.09)
T14	Untreated Seed	56.20 (48.54)	12.56	8.94	1208.70	9.48	0.48	26.83	92.20 (73.80)
CD (p = 0.05)		1.37	0.44	0.26	62.26	0.32	0.03	3.05	1.19
SE(d)		0.68	0.22	0.13	31.00	0.16	0.01	1.52	0.59
SE(m) ±		0.48	0.16	0.09	21.92	0.11	0.01	1.07	0.42
CV (%)		1.70	2.34	1.50	2.11	1.68	1.83	2.39	2.24

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

In groundnut seed coating with chitosan+*T. asperellum* TaDOR 7316 treatment (T12) enhanced the germination and all growth parameters compared with uncoated and other treated seeds in uninoculated condition (Table 3). The treatment T12 (chitosan+*T. asperellum* TaDOR 7316) was highly significant in increasing the per cent germination (94.60%) and vigour index-I (3293.98) and vigour index-II (347.56) when compared to control (germination-85.00%, vigour index-I- 2026.68 and vigour index-II- 254.16). The treatment T13 (chitosan+vitavax+thiram+ *T. asperellum* TaDOR 7316) was next best with the per cent germination of 92.80%, vigour index-I of 3118.28 and vigour index-II of 330.53. The per cent germination of treatments T10 (synthetic polymer-II+vitavax+thiram+*T. asperellum* TaDOR 7316) and T11 (chitosan+vitavax+thiram) were on par with each other recording 92.80 and 92.60 respectively. However vigour index-I (3059.56) and vigour index-II (323.17) was higher in T11 (chitosan+vitavax+thiram) than the T10 (synthetic polymer- II+vitavax+thiram+ *T. asperellum* TaDOR 7316) (2865.66 and 319.97).

**Table 3. Effect of seed coating polymers in combination with fungicide and biocontrol agent on the germination and vigour of groundnut *in vitro***

Treatment No.	Details of Treatment	Germination (%)	Root Length (cm)	Shoot Length (cm)	Vigour Index-I	Fresh Weight (g)	Dry Weight (g)	Vigour Index-II
T1	Synthetic Polymer-II	86.40 (68.38)*	14.32	10.30	2127.00	33.60	3.04	262.69
T2	Chitosan	90.60 (72.30)	16.30	13.66	2715.18	38.30	3.27	296.60
T3	vitavax+thiram	89.20 (70.81)	14.38	10.68	2235.24	35.52	3.15	280.81
T4	<i>T. asperellum</i> TaDOR 7316	84.80 (67.12)	14.02	10.36	2066.92	32.90	3.07	260.54
T5	vitavax+thiram + <i>T. asperellum</i> TaDOR 7316	87.40 (69.18)	14.88	11.10	2270.62	35.72	3.18	278.11
T6	Synthetic Polymer-II + vitavax+thiram	91.20 (72.75)	16.18	11.66	2538.84	37.36	3.32	302.42
T7	Synthetic Polymer-II + <i>T. asperellum</i> TaDOR 7316	91.60 (73.13)	16.46	12.16	2621.58	38.66	3.36	308.14
T8	vitavax+thiram + Synthetic Polymer-II	87.60 (69.38)	15.50	9.66	2203.92	34.04	2.96	259.28
T9	<i>T. asperellum</i> TaDOR 7316+ Synthetic Polymer-II	84.80 (67.04)	15.34	9.50	2106.26	33.46	2.86	242.35
T10	Synthetic Polymer-II + vitavax+thiram + <i>T. asperellum</i> TaDOR 7316	92.80 (74.41)	16.68	14.20	2865.66	39.36	3.45	319.97
T11	Chitosan + vitavax+thiram	92.60 (74.19)	18.38	14.66	3059.56	40.52	3.49	323.17
T12	Chitosan + <i>T. asperellum</i> TaDOR 7316	94.60 (76.56)	19.52	15.30	3293.98	42.72	3.67	347.56
T13	Chitosan + vitavax+thiram + <i>T. asperellum</i> TaDOR 7316	92.80 (74.47)	18.66	14.94	3118.28	42.08	3.56	330.53
T14	Untreated Seed	85.00 (67.20)	14.80	9.04	2026.68	33.08	2.99	254.16
CD (p = 0.05)		1.90	0.30	0.32	69.60	0.25	0.02	6.78
SE(d)		0.94	0.15	0.16	34.65	0.12	0.01	3.38
SE(m) ±		0.67	0.11	0.11	24.50	0.09	0.01	2.39
CV (%)		2.10	1.47	2.09	2.18	0.53	0.56	1.84

\* Values in the parentheses are angular transformed and are the means of five replication

In *Aspergillus niger* inoculated condition the results indicated that all the treatments except synthetic polymer-II were significant in increasing per cent germination and vigour index-I, vigour index-II of groundnut under *in vitro* condition (Table 4). Maximum per cent germination (92.80%) was observed in T13 when groundnut seed was treated with chitosan+vitavax+thiram+*T. asperellum* TaDOR 7316 when compared to control and the other treatments and also increased the vigour index-I (2332.98) and vigour index-II (200.45). The treatment T12 (chitosan + *T. asperellum* TaDOR 7316+pathogen) was on par with a per cent germination of 90.60% and recorded the vigour index-I of 2131 and vigour index-II of 188.27 when compared to control. Chitosan in combination with vitavax+thiram (T11) was the next best treatment in recording a germination of 89.60% and vigour index-I of 1956.84 and vigour index-II of 180.28 under pathogen inoculated conditions.

**Table 4. Effect of seed coating polymers in combination with fungicide and biocontrol agent on the germination and vigour of groundnut by *Aspergillus niger* inoculation *in vitro***

Treatment No.	Details of Treatment	Germination (%)	Root Length (cm)	Shoot Length (cm)	Vigour Index-I	Fresh Weight (g)	Dry Weight (g)	Vigour Index-II	Per cent Incidence
T1	Synthetic Polymer-II	36.80 (37.33)*	6.44	3.92	381.56	6.06	0.57	20.83	98.80 (84.43)*
T2	Chitosan	75.20 (60.11)	11.30	6.52	1340.24	17.58	1.84	138.37	75.40 (60.25)
T3	vitavax +thiram	80.60 (63.86)	11.52	6.76	1473.32	17.98	1.87	150.57	71.20 (57.52)
T4	<i>T. asperellum</i> TaDOR 7316	77.80 (61.88)	10.66	6.20	1311.72	17.30	1.73	134.92	68.40 (55.78)
T5	vitavax +thiram + <i>T. asperellum</i> TaDOR 7316	80.80 (64.00)	11.14	6.60	1433.42	17.82	1.79	144.31	62.80 (52.40)
T6	Synthetic Polymer-II + vitavax +thiram	82.80 (65.47)	11.92	7.08	1573.20	18.30	1.93	159.64	61.60 (51.69)
T7	Synthetic Polymer-II + <i>T. asperellum</i> TaDOR 7316	85.00 (67.19)	12.46	7.52	1698.40	18.76	1.95	165.58	57.00 (49.01)
T8	vitavax +thiram + Synthetic Polymer-II	80.40 (63.70)	10.48	6.02	1326.60	15.40	1.63	131.22	67.00 (54.92)
T9	<i>T. asperellum</i> TaDOR 7316+ Synthetic Polymer-II	76.60 (61.05)	10.14	5.70	1213.42	14.58	1.55	118.88	70.20 (56.89)
T10	Synthetic Polymer-II + vitavax +thiram + <i>T. asperellum</i> TaDOR 7316	87.80 (69.54)	12.82	7.94	1822.76	18.98	1.97	173.14	50.80 (45.44)
T11	Chitosan + vitavax +thiram	89.60 (71.17)	13.46	8.38	1956.84	19.44	2.01	180.28	49.20 (44.52)
T12	Chitosan + <i>T. asperellum</i> TaDOR 7316	90.60 (72.12)	14.56	8.96	2131.00	20.82	2.08	188.27	43.40 (41.19)
T13	Chitosan + vitavax +thiram + <i>T. asperellum</i> TaDOR 7316	92.80 (74.41)	15.52	9.62	2332.98	21.88	2.16	200.45	32.60 (34.80)
T14	Untreated Seed	25.80 (30.51)	4.38	2.38	174.36	2.62	0.23	5.94	99.40 (86.54)
CD (p = 0.05)		0.97	0.19	0.19	34.02	0.23	0.02	2.74	1.77
SE(d)		0.48	0.09	0.10	16.94	0.11	0.01	1.38	0.88
SE(m) ±		0.34	0.07	0.07	11.98	0.08	0.01	0.97	0.63
CV (%)		1.24	1.32	2.24	1.86	1.11	0.74	1.58	2.52

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

**DISCUSSION**

Polymer coating prior to fungicide seed treatment improve the efficacy of fungicide maintaining higher germination. These results are in agreement with Natarajan *et al.* (2012) who also reported higher germination (98.00 %) and vigour index (82.91) as compared to control (93.00 % and 60.54, respectively) in maize with pink polykote @ 3 g kg<sup>-1</sup> of seeds + fungicide + insecticide treatment. The findings of present investigation are also in line with Ramesh *et al.* (2011) and Chandravathi *et al.* (2012) in pearl millet and Shakuntala *et al.* (2010) in sunflower. The combination treatments synthetic polymer-II+ Fungicide (T6) and synthetic polymer-II+ Biocontrol agent (T7) were effective than the combinations of Fungicide + synthetic polymer-II (T8) and Biocontrol agent + synthetic polymer-II (T9) because the fungicide or biocontrol agent immediately available on surface and it inhibits external pathogens growth before they became active to infect the seed apart from the polymer coating enhances the growth internally. Whereas in fungicide / biocontrol agent + synthetic polymer-II combination treatment the fungicide or biocontrol agent may not be effectively inhibiting the pathogen growth because it takes time to pass through the polymer layer thus the vigour is reduced due to early pathogen infection.

The fungicide covered by polymer film enhanced the efficiency of fungicide. It forms a flexible film that adheres and protects the fungicide on the seed and preventing dusting off and loss of fungicide during handling. This, in the present study was reflected by reducing the seed infection by pathogen. The enhanced germination and quality parameters with treated seeds with fungicide and polymer coating is because of the combined favourable effects of these chemicals. The fungicide protected the seed deterioration by reducing the fungal invasion. The effectiveness of fungicides and polymer coating may be due to the compatibility and synergetic effect which reduced the growth of pathogen and favoured germination and other quality parameters (Omvir Singh *et al.*, 1973; Sindhan and Bose, 1981 and Sundaresh *et al.*, 1987).

This was the first report on combinations of fungicide, biocontrol agent and seed coat polymers and their role in plant growth promotion and pathogen inhibition in castor and groundnut. The investigation also revealed that the compatibility between biopolymer, fungicide and biocontrol agent and their combination treatment, antagonistic to pathogens (*Fusarium oxysporum* f. sp. *ricini* and *Aspergillus niger*) infection was due to direct protection by stimulating the defence responses within the castor and groundnut plant during germination and simultaneously showed the synergetic effect on growth promotion beyond the pathogen effect by influencing the critical germination activating enzymes present within the seed.

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