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Assessment of different formulations of *Trichoderma* for their shelf life

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Abstract
Eleven formulations of *Trichoderma asperellum* were evaluated in present investigation, among these four types of tablets formulation *viz*. talc tablets, charcoal tablets, lignite tablets, fly ash tablets, six capsules formulations *viz*. talc capsule, alginate capsules, gelatin capsules, alginate + charcoal capsules, gelatin + fly ash capsules and *T. asperellum* frozen culture mass along with medium disc capsules and one sorghum grains formulation as a control were used and observation on shelf life up to 270 days from date of manufacturing at regular intervals of 30 days were taken. Maximum cfu/g (42.00 x 10^7) was obtained in *Trichoderma* treatment frozen culture mass along with medium disc capsule and least cfu/g was recorded in *T. asperellum* lignite tablet (11.33 × 10^7 cfu/g) upto 270 days of storage. In the present study tablet and capsule delivery system of various formulations was tried by using jaggery treatment. Maximum population of *T. asperellum* obtained (45.00 × 10^7 cfu/ml) in *T. asperellum* frozen culture mass along with medium disc capsule and minimum were obtained in *T. asperellum* lignite tablets, (8.33 × 10^7cfu/ml).

Keywords: *Trichoderma asperellum,* formulations, shelf life, tablet, capsule

Introduction
Trichoderma spp. is the most common fungal biological control agents that have been comprehensively researched and deployed throughout the world. One of the popular species *Trichoderma asperellum* is effective against soil-borne fungal pathogens like *Phytophthora, Fusarium, Pythium, Rhizoctonia* and have been exploited on about 87 different crops and about 70 soil-borne and 18 foliar pathogens (Sharma *et al*.,2014) [13]. Thus, the culture of *Trichoderma* should be immobilized in certain carriers and should be prepared as formulations for easy application, storage, commercialization and field use (Kumar *et al.,* 2014) [5]. More than 50% crop losses are due to soil inhabiting microorganisms, such as *Fusarium, Sclerotium, Rhizoctonia, Verticillium, Phytophthora, Pythium* (Elad *et al.* 1982) [2]. Large scale production, along with shelf life and establishment of bioagent in targeted *niche,* determine the success of biological control (Lakshmi Tewari and Chandra Bhanu, 2004) [6]. Shelf life of the formulated product of a biocontrol agent plays a significant role in successful marketing. In general, the antagonists multiplied in an organic food base have longer shelf life than the inert or inorganic food bases. It has good effects of controlling *Sclerotinia*, wilt and seedling blight of vegetables, fruit trees, flowers and other crops (*Trichoderma* biocontrol capsule microbial inocula and preparation method there of CN 101496528 B). The use of fungicides for control of pathogens has met with moderate success and their future use is a question due to increased regulatory restrictions. In addition, a number of currently used fungicides, such as mercurials have been withdrawn from the market. Mass production is broad aspect of successful biological control techniques which include establishment of products, formulation and delivery system of fungi where used as an efficient disease control (Seema Solanki *et al*.,2016) [11] but some of the limitation in their use is carrier material have shelf life upto 4 to 5 month and contamination occurs during delivery methods. In present investigation the shelf life of the different formulations has been tested upto nine months from date of manufacturing at regular intervals of 30 days.

Material and Methods

Delivery system and application technology

Tablet and capsule form of *Trichoderma* culture were used to test to improve application technology. Six capsule form *viz*. talc, gelatin, alginate, alginate + charcoal (1:1), gelatin + fly ash (1:1) and pure *T. asperellum* frozen mycelia with spores capsule and four tablet form *viz*. talc, lignite, charcoal, fly ash were used for present study. Sterilized 1000 ml capacity conical flask contained 500 ml distilled water enriched with 10 g jaggery used for this study. After cooling each conical flask inoculated with one tablet/capsule separately and incubated at room temperature, 30ºC for 12 h. The initial population of *T. asperellum* from each formulation was assessed by serial dilution technique. After 12 h of incubation period, the culture was serially diluted to obtain 10^{-7} concentration and 1 ml from 10^{-7} dilution factor was poured in sterilized petri-dish containing PDA medium and incubated at 27 + 2ºC for 48 hours and observation was taken for the development of colonies of *T. asperellum*. Three replications were maintained for each treatment. Colony forming units (CFU) of *T. asperellum* was calculated by the formula derived by Schmidt & Caldwell, 1967 [10].

Viability of *T. asperellum* in different formulations under *in vitro* condition

Eleven carrier based formulations of *Trichoderma asperellum* were evaluated for viability from date of manufacturing upto 270 days and observed at 30 days interval by adopting the following method. One tablet/capsule was drawn from each formulation and transferred in 9 ml sterilized water in test tube and shaked thoroughly for two minutes to make 10^{-1} dilution factor. One ml suspension of stock solution was transferred in next test tube containing 9 ml distilled water by using sterilized pipette and shaken to make 10^{-2} dilution and seven test tube to make up 10^{-7} dilution. One ml of suspension was taken from the dilution of 10^{-7} and transferred in petri plates containing 20 ml sterilized PDA and gently shaken to spread evenly. These petri plates were incubated at 27 + 2 ^{0}C for 72 h and periodic observations were taken for the development of colonies of *T. asperellum.*

Observations for colony forming units (CFU) were taken by using formula. (Schmidt, and Caldwell, 1967) [10]:

$$\text{CFU per gram} = \frac{\text{CFU per plate} \times \text{dilution factor}}{\text{Weight of substrate (g)} \times \text{amount plated (ml)}}$$

Results and Discussion

Evaluation of viability of *Trichoderma asperellum* in different formulation

Shelf life of all eleven formulations was studied up to 270 days of storage. (Table1) It is evident from the data that the maximum cfu/g (42.00 x 10^7) was obtained in *Trichoderma* treatment (T_{10}) i.e. frozen culture mass along with medium disc capsule even after 270 days of storage followed by (T_6), *T. asperellum* alginate capsule (29.67 × 10^7 cfu/g), (T_5) *T. asperellum* gelatin capsule (28.67 × 10^7 cfu/g), (T_8), *T. asperellum* gelatin + flyash capsule (21.00 × 10^7 cfu/g), (T_7), *T. asperellum* talc capsule (20.00 × 10^7 cfu/g), (T_9), *T. asperellum* alginate + charcoal capsule (19.33 × 10^7 cfu/g), (T_4), *T. asperellum* flyash tablets (18.00 × 10^7 cfu/g), (T_2), *T. asperellum* charcoal tablet (17.67 × 10^7 cfu/g) and minimum cfu was recorded in (T_1), *T. asperellum* talc tablet (12.33 × 10^7 cfu/g) and (T_3) *T. asperellum* lignite tablet (11.33 × 10^7 cfu/g) after 270 days of storage. The present findings are in accordance with the results of Baghel *et al*. (2014) [1], studied various formulation *viz*. capsules and tablet with various carrier upto 260 days. *T. asperellum* lignite tablet gave minimum cfu counts at 270 days of storage i.e. 11.33 x 10^7 cfu/g. Shafa khan *et al.* (2011) [12] obtained good result with talc and gypsum based formulation upto 195 - 250 days. However, in the present study gypsum based formulation was not tried but talc based tablet have shown inferiority over talc based capsule. Kumar *et al.* (2013) [4] tried carrier based formulation.

Table 1: Evaluation of shelf life of *T. asperellum* in different formulations

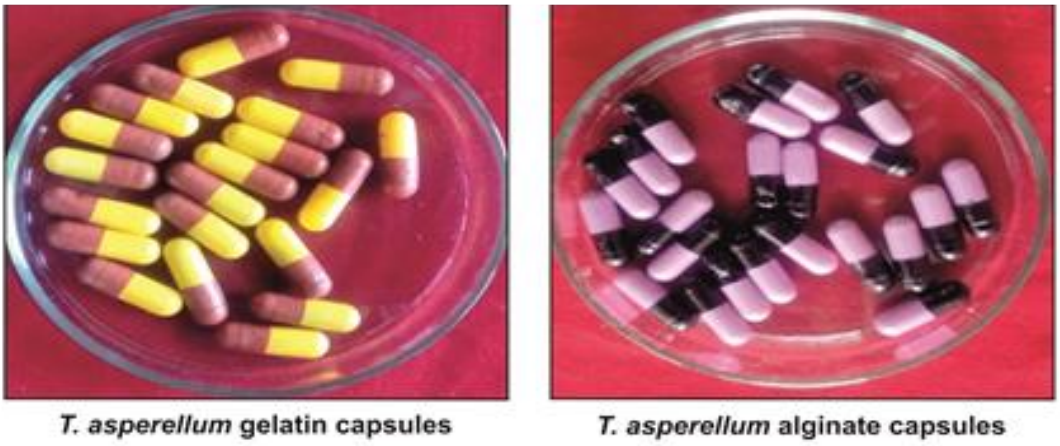
Tre. no.	Formulations	10^7CFU/g 30 days after storage	10^7 CFU/g 60 days after storage	10^7CFU/g 90 days after storage	10^7 CFU/g 120 days after storage	10^7CFU/g 150 days after storage	10^7CFU/g 180 days after storage	10^7CFU/g 210 days after storage	10^7CFU/g 240 days after storage	10^7CFU/g 270 days after storage
T_1	*T. asperellum* talc tablets	107.33	91.33	88.00	76.67	43.33	32.67	28.33	20.00	12.33
T_2	*T. asperellum* charcoal tablets	95.00	83.33	82.33	60.33	56.67	38.67	35.00	24.67	17.67
T_3	*T. asperellum* lignite tablets	104.33	85.67	81.33	36.67	33.33	28.33	22.00	15.00	11.33
T_4	*T. asperellum* flyash tablets	105.33	92.67	77.00	68.33	63.33	47.33	41.00	29.67	18.00
T_5	*T. asperellum* gelatin capsules	115.67	110.67	97.33	78.67	42.67	40.33	42.67	33.00	28.67
T_6	*T. asperellum* alginate capsule	121.33	130.67	85.67	78.00	76.67	63.00	59.33	43.67	29.67
T_7	*T. asperellum* talc capsules	135.33	83.67	75.33	66.67	40.00	35.00	34.67	21.33	20.00
T_8	*T. asperellum* gelatin + flyash capsules	95.67	82.33	95.67	85.67	87.33	42.00	37.33	23.33	21.00
T_9	*T. asperellum* alginate + charcoal capsules	84.33	83.67	83.33	45.33	31.67	27.67	24.33	15.33	19.33
T_{10}	*T. asperellum* frozen culture mass along with disc capsules	205.00	176.67	179.67	125.00	90.33	80.33	75.33	68.67	42.00
T_{11}	Sorghum grains	221.33	187.00	147.33	110.00	82.00	78.67	74.67	58.67	30.67
	S.E (M)±	1.206	1.307	1.030	0.810	0.835	0.882	1.124	0.980	0.785
	CD (P=0.01)	4.807	5.208	4.105	3.230	3.327	3.515	4.479	3.904	3.129

T. asperellum talc Tablets

T. asperellum charcoal Tablets

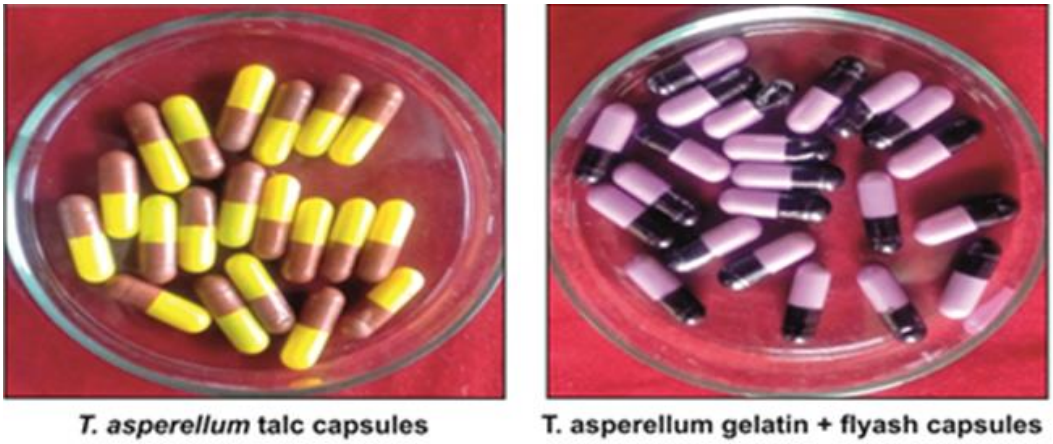
T. asperellum lignite Tablets

T. asperellum flyash Tablets



T. asperellum gelatin capsules

T. asperellum alginate capsules



T. asperellum talc capsules

T. asperellum gelatin + flyash capsules

Innovative delivery system and application technology

In the present study tablet and capsule delivery system of six types of capsules and four types of tablets were studied. It is revealed from data presented in Table 2 that the population of *T. asperellum* gradually improved within 12 h of incubation. One gram of capsulated or tablet form of *T. asperellum* culture retained more than sufficient population. It is clear from data that maximum population of *T. asperellum* obtained (45.00×10^7 cfu/ml) in (T_{10}), *T. asperellum* Frozen culture mass along with medium disc capsule followed by (31.00×10^7 cfu/ml) in (T_6), *T. asperellum* alginate capsule, (21.33×10^7cfu/ml) in (T_9), *T. asperellum* alginate + charcoal capsule, (20.66×10^7cfu/ml) in (T_7), *T. asperellum* talc capsule, (20.33×10^7 cfu/ml) in (T_5), *T. asperellum* gelatin capsule, (15.00×10^7cfu/ml) in (T_4), *T. asperellum* fly ash tablet, (14.33×10^7cfu/ml) in (T_8), *T. asperellum* gelatin + flyash capsule, (T_2), *T. asperellum* charcoal tablets, (14.00×10^7cfu/ml). However least cfu was recorded in (T_3,) *T. asperellum* lignite tablet (8.33×10^7cfu/ml), (T_1) and *T. asperellum* talc tablet (8.66×10^7cfu/ml). The present findings are in accordance with the studies of Kose and Totawar (2017) [3] tried six types of capsules of *T. asperellum viz.* talc, gelatin, alginate, alginate + charcoal, gelatin + flyash and frozen mass culture along with medium disc capsules and four types of tablets of *T. asperellum viz.* talc, charcoal, lignite and flyash for delivery system and application revealed that when one capsule/tablet from each formulation dispersed separately in 500 ml capacity conical flask containing 250 ml sterilized water enriched with 10 g jaggery and incubated for 12 h given maximum population of *T. asperellum.* Also they recorded the increased cell count by this technology was due to composition of hard gelatin capsule. In hypothesis one gram of capsule form of *T. asperellum* of treatment T_{10} (45.00×10^7 cfu/ml) which is sufficient as compared with the available formulations in the market.

Table 2: Evaluation of *T. asperellum* population for innovative delivery system and application technology

Treat. no.	Formulation	10^7cfu/ml R_1	10^7cfu/ml R_2	10^7cfu/ml R_3	Mean
T_1	*T. asperellum* talc tablets	9	9	8	8.66
T_2	*T. asperellum* charcoal tablets	14	13	15	14.00
T_3	*T. asperellum* lignite tablets	9	8	8	8.33
T_4	*T. asperellum* flyash tablets	16	15	14	15.00
T_5	*T. asperellum* gelatin capsules	21	20	20	20.33
T_6	*T. asperellum* alginate capsule	33	30	30	31.00
T_7	*T. asperellum* talc capsules	22	21	19	20.66
T_8	*T. asperellum* gelatin + flyash capsules	13	14	16	14.33
T_9	*T. asperellum* alginate + charcoal capsules	20	21	23	21.33
T_{10}	*T. asperellum* frozen culture mass along with disc capsules	44	45	46	45.00
	S.E (M)±	-	-	-	0.591
	CD (P=0.01)	-	-	-	2.017

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