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Innovative delivery methods for *Trichoderma* formulations

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Abstract
The present investigation was carried out to evaluate the shelf life in *T. asperellum* tablets and capsules. Four types of tablets *viz*. T_1 (*T. asperellum* talc tablet), T_2 (*T. asperellum* charcoal tablet), T_3 (*T. asperellum* lignite tablet), T_4 (*T. asperellum* flyash tablet) and Six types of capsules *viz*. T_5 (*T. asperellum* talc capsule), T_6 (*T. asperellum* gelatin capsule), T_7 (*T. asperellum* alginate capsule), T_8 (*T. asperellum* alginate + charcoal capsule), T_9 (*T. asperellum* gelatin + flyash capsule), T_{10} (*T. asperellum* Frozen culture mass along with disc capsule) were prepared and compaired with sorghum grains based carrier as control T_{11}. Then observe the shelf life upto 180 days from the date of manufacturing at regular interval of 30 days. The study revealed that the desired minimum count i.e. 1 x 10^7 was noticed in all the formulations upto 180 days storage. Maximum cfu/g (76.33 x 10^7) was obtained inT_{10} i.e. frozen culture mass along with medium disc capsule followed by T_4, *T. asperellum* fly ash tablet (39 × 10^7cfu/g). However, least cfu/g recorded in T_9 *T. asperellum* alginate + charcoal capsules (20 × 10^7cfu/g). In the present study tablet and capsule delivery system of various formulations was also tried by using jaggery treatment and maximum population of *T. asperellum* obtained (77.66 × 10^7cfu/ml) in T_{10},*T. asperellum* Frozen culture mass along with medium disc capsule followed by (53 × 10^7cfu/ml) in T_7, *T. asperellum* alginate capsule.

Keywords: *T. asperellum*, shelf life, formulation, tablet, capsule, jaggery treatment

Introduction
Some of the most widely used biocontrol agents in the world belong to the fungal genus *Trichoderma*. *Trichoderma* spp. are found in almost all types of soils and diverse habitats and contribute to control of many soil borne plant diseases caused by fungi (Mishra *et al*. 2011) [4]. One of the popular species *Trichoderma asperellum* is effective against all soil borne fungal pathogen. It have been exploited on about 87 different crops and about 70 soil borne and 18 foliar pathogens (Sharma *et al*. 2014) [9]. More than 50% crop losses are due to soil inhabiting microorganisms, such as *Fusarium*, *Sclerotium*, *Rhizoctonia*, *Verticillium*, *Phytophthora*, *Pythium* (Elad *et al*. 1982) [3].
Chemical control is offer nonspecific in its effect, killing beneficial organisms and it may have undesirable health and environmental pollution risk. Besides that, it is not cost effective to use chemical pesticides in the long run. Biological control of these pathogens by microorganisms has been considered a more natural and environmentally acceptable alternative to the existing chemical treatment methods (Baker and Paulitz, 1996) [1].
Biological control of plant diseases appears to be an effective and ecofriendly approach being practiced world over. Further biological control strategy has a major role to play as a component of integrated pest management (IPM). Large scale production, along with shelf life and establishment of bioagent in targeted niche, determine the success of biological control (Tewari and Bhanu 2004) [10].
In present investigation efforts has been made to improve the awareness and application technology along with shelf life of different formulation of *Trichoderma asperellum* culture. The shelf life of the different formulation has been tested for six months from date of manufacturing at regular intervals of 30 days.

Materials and Methods

Evaluation of shelf life of *T. asperellum* in different formulations

Eleven carrier based formulations of *T. asperellum* were evaluated for viability from date of manufacturing to 6 months and observed shelf life at 30 days interval by adopting the following methods.

One gram sample or one tablet or one capsule was drawn from each formulation and transferred in 10 ml sterilized distilled water in test tube and shaked thoroughly for 3 minutes to make 10^{-1} dilution. One ml suspension of stock solution was transferred in next test tube containing 9 ml sterilized 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 observation 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) [7].

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

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 and four tablet form *viz*. talc, lignite, charcoal, fly ash were used for present study.

Distilled water was filled in 500 ml capacity conical flask @ 250 ml per flask enriched with 10 g jaggery and sterilized in autoclave at 15 psi. After cooling each conical flask inoculated with one tablet and capsule separately and incubated at room temperature 30^{0}C 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 obtain10^{-7} concentration and 1 ml from 10^{-7} dilution factor was poured in sterilized petri-dish containing PDA medium and incubate that plate for 48 hours and observation of cfu was recorded. Three replication were maintain for each treatment. Colony forming units (CFU) of *T. asperellum* was calculated by the formula derived by Schmidt & Caldwell, 1967 [7].

Results and Discussion

Four types of tablets and six types of capsules were evaluated, observation should be taken at 30 days interval period upto six month and data are presented in the table 1. It is evident from the data that maximum cfu/g (76.33 x 10^7) was obtained in *Trichoderma* treatment T_{10} i.e. frozen culture mass along with medium disc even after 180 days of storage followed by T_4, *T. asperellum* fly ash tablet (39 × 10^7cfu/g), T_2,*T. asperellum* charcoal tablet (37.33 × 10^7cfu/g), T_5,*T. asperellum* gelatin capsule (37 × 10^7cfu/g), T_8, *T. asperellum* gelatin + fly ash capsule (35.33 × 10^7cfu/g), T_6, *T. asperellum* alginate capsule (35 × 10^7cfu/g), T_7, *T. asperellum* talc capsule (32.33 × 10^7cfu/g). However least recorded in T_9, *T. asperellum* alginate + charcoal capsules (20 × 10^7cfu/g), T_3, *T. asperellum* lignite tablet (22.67 × 10^7cfu/g) and T_1, *T. asperellum* talc tablet (30.33 × 10^7cfu/g) after 180 days of storage.

Similar findings found by Baghel *et al*. (2014) [2] studied various formulation *viz*. capsules and tablet with various carrier and reported that charcoal powder has given maximum cfu/g i.e. 80 x 10^7upto 260 days. Sanjiv *et al*. (2013) [6] reported that sorghum grains charcoal based formulation of *T. asperellum* was better up to 120 days of storage and similar types of results were also worked out by Shafa *et al*. (2007) [8] obtained good result with talc and gypsum based formulation upto 195 - 250 days.

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

Treat. no.	Formulations	× 10^7 CFU/g 30 days after storage	10^7 CFU/g 60 days after storage	10^7 CFU/g 90 days after storage	10^7 CFU/g 120 days after storage	10^7 CFU/g 150 days after storage	10^7 CFU/g 180 days after storage
T_1	*T. asperellum* Talc tablets	106.67	89.33	86.33	75.33	44.67	30.33
T_2	*T. asperellum* Charcoal tablets	93.33	81.33	82.00	58.67	55.00	37.33
T_3	*T. asperellum* Lignite tablets	102.3	84.33	79.00	35.00	31.33	22.67
T_4	*T. asperellum* Flyash tablets	104.3	91.67	75.67	67.00	62.33	39.00
T_5	*T. asperellum* Gelatin capsules	114.6	109.6	96.00	76.67	44.67	37.00
T_6	*T. asperellum* Alginate capsule	118.6	129.6	84.67	76.33	75.00	35.00
T_7	*T. asperellum* Talc capsules	137.0	82.00	74.00	65.67	39.00	32.33
T_8	*T. asperellum* Gelatin + Flyash capsules	94.33	81.33	94.00	87.00	85.67	35.33
T_9	*T. asperellum* Alginate + Charcoal capsules	85.67	82.67	82.00	46.00	32.67	20.00
T_{10}	*T. asperellum* frozen culture mass along with disc capsules	200.6	174.0	178.0	123.3	89.33	76.33
T_{11}	Sorghum grains	217.0	185.6	145.6	108.6	80.00	75.67
	CD (P=0.01)	3.07	5.43	4.65	3.35	3.10	2.41
	CV %	1.07	2.17	2.06	1.95	2.31	2.45

Fig 1: Different types of capsules

Fig 2: *T. asperellum* Charcoal Tablets *T. asperellum* Talc Tablets

Fig 3: *T. asperellum* Lignite Tablets *T. asperellum* Flyash Tablets

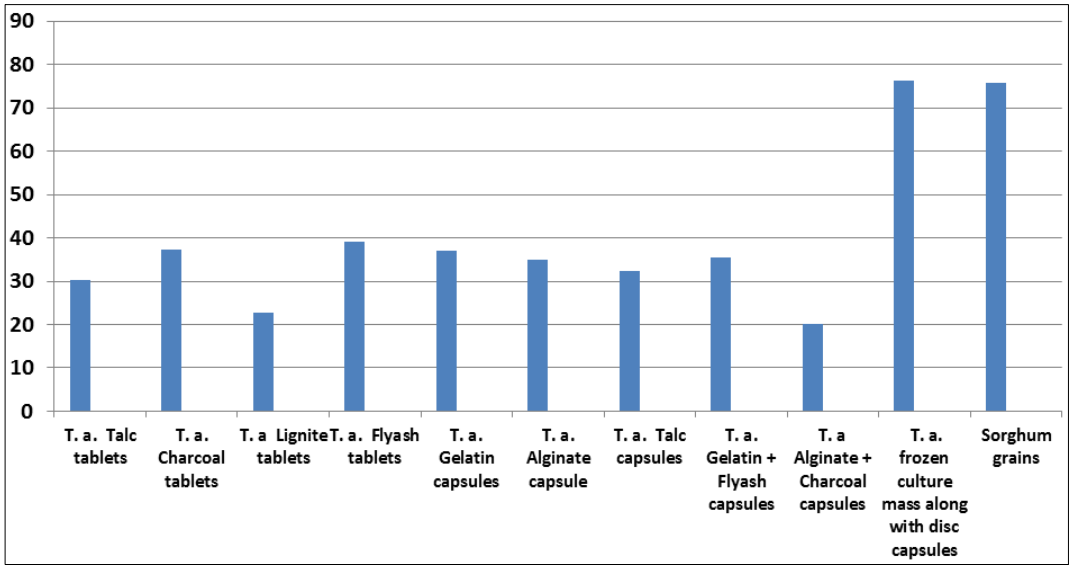

Fig 4: Evaluation of shelf life of *T. asperellum* in different formulations upto 6 months

Innovative delivery system and application technology

Very meager work could be traced during the literature hunt on various formulations and delivery system. In the present study tablet and capsule delivery system of various formulations was tried.

Six types of capsules and four types of tablets was used. 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 (77.66 × 10^7cfu/ml) in T_{10},*T. asperellum* Frozen culture mass along with medium disc capsule followed by (53 × 10^7cfu/ml) in T_7, *T. asperellum* alginate capsule, (36.66 × 10^7cfu/ml) in T_8, *T. asperellum* alginate + charcoal capsule, (31 × 10^7cfu/ml) in T_5, *T. asperellum* talc capsule, (27.66 × 10^7cfu/ml) in T_6, *T. asperellum* gelatin capsule, (27.3 × 10^7cfu/ml) in T_4, *T. asperellum* fly ash tablet, (24.6 × 10^7cfu/ml) in T_2, *T. asperellum* charcoal tablet.

However least cfu was recorded in T_1, *T. asperellum* talc tablet (16.33 × 10^7cfu/ml), T_3, *T. asperellum* lignite tablet (18 × 10^7cfu/ml) and T_9, *T. asperellum* gelatin + fly ash capsule (23.66 × 10^7cfu/ml), however which is more than recommended cfu/ml, that means one gram of capsule or tablet form of *T. asperellum* formulation when dissolved in 250 ml broth it was more than 500 g carrier based culture when mixed with suitable carrier at farm level.

There was no evidence or references noticed during literature hunt. Therefore it is to be considered as new evidence which might be beneficial not only for marketing but to all farming community. In hypothesis one gram of capsule form of *T. asperellum* of treatment T_{10} (77.66 × 10^7cfu/ml) which is sufficient as compared with the available formulations in the market. Some strains of *Trichoderma* are nice lingocellulolytic degraders and are also included in decomposting cultures. Hence the better delivery system and formulate can be made applicable for supply of decomposting cultures and there is still a scope for exploring more applicability. Saili *et al.* (2014) [5], revealed that soil prevalent fungus *Trichoderma* produces lignocellulolytic enzymes. That assist the degradation of woody lignocellulose materials.

In present investigation so as to develop the innovative delivery system of *T. asperellum*, maximum population i.e. 77.66 × 10^7cfu/ml was observed when one capsule that has 76.33 × 10^7 cfu/g population even after 180 days of storage dispersed in 250 ml sterilized water amended with 10 g jaggery solution and incubated for 12 h i.e. 1000 mg capsulated *T. asperellum* (frozen culture mass along with medium disc) gives 77.66 × 10^7cfu/ml in 250 ml volume. It is revealed from present investigation that in treatment T_{10} of *T. asperellum* capsule, some nutrition comes along with mycelial mat and spores, there is possibility that some of the nutrient media along with mycelial mat and spores would have supported the further growth and reproduction. More over jaggery added to solution also serves as source of minerals and carbohydrates to enhance cfu. Certainly the pure frozen mycelial mat along with medium disc gelatin capsule in treatment T_{10} supported the fungus for maximum cfu by providing the essential nutrients, proteins and carbohydrates during 12 h suspension in sterile water.

Table 2: Shelf life of *T. asperellum* in tablet and capsule formulation with jaggery treatment after 180 days of storage

Treat. no.	Formulation	10^7cfu/ml R_1	10^7cfu/ml R_2	10^7cfu/ml R_3	Mean
T_1	*T. asperellum* talc tablet	17	16	16	16.3
T_2	*T. asperellum* charcoal tablet	25	25	24	24.6
T_3	*T. asperellum* lignite tablet	19	17	18	18
T_4	*T. asperellum* fly ash tablet	27	28	27	27.3
T_5	*T. asperellum* talc capsule	32	30	31	31
T_6	*T. asperellum* gelatin capsule	29	26	28	27.6
T_7	*T. asperellum* alginate capsule	53	54	52	53
T_8	*T. asperellum* alginate + charcoal capsule	36	38	36	36.6
T_9	*T. asperellum* gelatin + flyash capsule	23	24	24	23.6
T_{10}	*T. asperellum* frozen culture mass along with disc capsule	78	77	78	77.6
	CD P=0.01 CV%				2.12 2.71

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