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Research Article

Studies on the shelf life of *Trichoderma* isolate in talc, prills, vermicompost, sago and dalia based formulations

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ABSTRACT: Biocontrol agents including microbials and macrobials are being used against several crop pests and diseases. The microbial bioagents should be formulated properly for making the product stable, effective, safer and obviously economic in case of storage and field level application. Several workers reported that amendments like chitin and ammonium sulphate will enhance the bioefficacy of *Trichoderma* isolates and shows positive effect on multiplication and storage of *Trichoderma* sp. In the present studies, an attempt was made to develop the *Trichoderma* sp. formulations in form of talc, sago, dalia and pellets using bentonite and wheat flour. Ammonium sulphate (0.5% and 1%) and chitin (01%) was used to increase the storage life and multiplication of the *Trichoderma* sp. Sago and dalia proved to be a good substrate of the bioagent under study in comparison to the conventional substrate talc. Among the amendments in the growth media, 1% chitin may be an effective supplement to the growth media for increasing its survival in storage. The interaction studies between different substrates and amendments selected to increase the shelf life, a combination of sago and 1% chitin was found to be effective for rapid multiplication of *Trichoderma* isolate. The rate of decline in population was found low in alginate prills both in bentonite and wheat and higher in organic substrates as vermicompost, sago and dalia with highest rate in dalia.

KEY WORDS: Ammonium sulphate, chitin, dalia, formulation, sago, *Trichoderma*, vermicompost
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INTRODUCTION

For making microbial biocontrol product stable, effective, safer and obviously economic in case of storage and field level application, biocontrol agent should be formulated properly. Formulation of biocontrol agent can be done in form of talc, charcoal, alginate pellets, agricultural byproducts, oil cakes, farmyard manures, wheat bran, poultry manure, dung, jaggery, ground nut cake, neem cake, niger cake and pongamia cake etc. Several workers reported that amendments like chitin and ammonium sulphate increases the bioefficacy of *Trichoderma* isolate and shows positive effect on multiplication and storage of *Trichoderma* spp. Amendment of three different soil types (loamy, clayey and sandy) with different carbon sources (glucose, cellulose, starch, chitin and sawdust) increased the total count of fungi in comparison with non-amended soil. Treatment with chitin increased densities of *Trichoderma harzianum* (Shaban *et al*., 1998). Ammonium sulphate enhanced the coloniozation and propagule number of *Trichoderma harzianum* (Jayaraj and Ramabadran, 1996). For mass production of *Trichoderma* use of ammonium sulphate has been recorded during fermentation as nitrogen source (Al-Tawain and Osman, 2009) and enhanced spore production was also observed (Gade *et al*., 2009).

MATERIALS AND METHODS

Mass multiplication of talc based formulation

In case of talc based formulation *Trichoderma* isolate was grown in amended (1% chitin, 0.5% and 1% ammonium sulphate) and non amended condition in Czapek dox broth for 5 days. Then 300 ml broth with mycelial mat blended in mixture and mixed with 1 kg of talc and left to dry overnight. After drying stored in polythene bag and kept at room temperature for study for 180 days.

Mass multiplication of pellets (bentonite and wheat flour) based formulation

Trichoderma isolate grown in 50 ml of CDB medium under amended (1% chitin, 0.5% and 1% ammonium sulphate) and non-amended condition. After 5 days broth with mat homogenizes in mixture. Then bentonite 30 g along with 250 ml sterilized distilled water and 1% disodium

alginate added in the mixture and again grinded. Pellets were formed by dropping the blended mixture in 0.5 M hydrated CaCl2 solution by peristaltic pump. Pellets were harvested rinsed in sterilized distilled water and air dried in laminar air flow on blotting paper. These pellets which were 3-4 mm diameter initially shrunk to 1-3 mm after drying and stored in screw capped jars in room temperature for shelf life study. For preparing pellets of wheat flour bentonite was replaced by wheat flour.

Mass multiplication of vermicompost based formulation

The moist vermicompost (50 gm) was kept in polypropylene bag plugged with cotton and sterilized. 6 mm disc of *Trichodrma* isolate grown on amended (1% chitin, 0.5% and 1% ammonium sulphate) and non amended condition in Czapek dox medium put in each bag and incubated at 28 ± 1^0C for 7 days. After initial growth material was mixed thoroughly.

Mass multiplication in sago media

In polypropylene bag, 50 gm sago (treated with 1% yeast peptone sucrose solution) with 2 gm of $CaCO_3$ was mixed thoroughly and plugged with cotton and sterilized. *Trichoderma* isolate was grown with amended (1% chitin, 0.5% and 1% ammonium sulphate) and non-amended condition in synthetic medium. Six mm disc of different treatments were put in each different bags and incubated at 28 ± 1^0C for 7 days. After initial growth *Trichoderma* isolate was mixed thoroughly.

Mass multiplication in dalia media

Fifty gm of dalia mixed with water so that dalia became moist and kept in polypropylene and provided with cotton plug and sterilized. *Trichoderma* isolate was grown with amended (1% chitin, 0.5% and 1% ammonium sulphate) and non-amended condition in synthetic medium. Six mm disc of different treatments were put in each different bags and incubated at 28 ± 1^0C for 7 days. After initial growth *Trichoderma* isolate was mixed thoroughly.

Drying of the formulated product

The entire formulated products prepared were sprayed in thin layer in trays separately and dried in 22^0C for 5 days. The dried product was kept in sealed plastic packets with proper level mentioning *Trichoderma* isolate, treatment, date of preparation and name of the formulation.

Shelf life study of mass multiplied product

Determination of viability of *Trichoderma* isolate in talc based formulation

One gm of talc based formulation was mixed with 9 ml of sterilized distilled water to prepare 10^{-1} dilution. Talc formulation diluted up to 10^{-8} and dilution. One ml of suspension was plated separately with *Trichoderma* Specific Medium and incubated at 28 ± 1^0C for 5 days and cfu was counted. Thus enumeration was continued up to 180 days of storage at an interval of 30 days.

Determination of viability of *Trichoderma* isolate in alginate pellets based formulation

The alginate pellets (0.1 g) were macerated using mortar and pestle with few drops of two phosphate buffer (KH_2PO_4 buffer-dissolve 0.68 g KH_2PO_4 per 100 ml of water and Na_2HPO_4, $7H_20$ buffer- dissolve 0.089 g per 100 ml of water). Then this solution was diluted in sterilized distilled water up to 10^{-7} dilution. One ml from each dilution poured in petriplates with *Trichoderma* Specific Medium and incubated at 28 ± 1°C for five days. Then cfu was counted and enumeration was continued for 180 days at an interval of 30 days.

Determination of viability of *Trichoderma* isolate in vermicompost based formulation

One gm of vermicompost based formulation was mixed with 9 ml of sterilized distilled water to prepare 10^{-1} dilution. Vermicompost ermicompost formulation diluted upto10^{-10} dilution. One ml of suspention were plated separately with TSM and incubated at 28 ± 1^0C for 5 days and cfu was counted. Thus enumeration was continued up to 180 days of storage at an interval of 30 days.

Determination of viability of *Trichoderma* isolate in sago and dalia based formulation

One gm of sago or dalia based formulation was crushed using mortar and pestle with few drops of two phosphate buffer (KH_2PO_4 buffer-dissolve 0.68 g KH_2PO_4 per 100 ml of water and Na_2HPO_4, $7H_20$ buffer-dissolve 0.089 g per 100 ml of water). Then this solution was diluted in sterilized distilled water up to 10^{-12} dilution. 1 ml from each dilution poured in petriplates with *Trichoderma* Specific Medium and incubated at 28 ± 1°C for five days. Then cfu was counted and enumeration was continued for 180 days at an interval of 30 days.

RESULTS

Shelf life of the *Trichoderma* isolate in talc

Trichoderma grown on amended media survived in the talc at a higher population than the non-amended one. At 180 days after preparation the population of the *Trichoderma* was reduced to 65% in non amended condition against 59-61% in amended condition and rate of decline was minimum when *Trichoderma* was grown with 0.5% amonium sulfate. Population level was higher among the treatment when *Trichoderma* grown with 1% chitin during the time under study.

Shelf life of the *Trichoderma* isolate in prills (wheat flour)

At 180 days the population of *Trichoderma* was reduced to 55% in non-amended control, whereas the reduction was only 47-50% in amended ones. The linear regression of the log CFU against time revealed that the trend of population decline was more or less similar in all the treatments and control, however the rate of decline was minimum in 0.5% ammonium sulfate and maximum in control.

Shelf life of the *Trichoderma* isolate in prills (bentonite) formulation

At 180 days the population of *Trichoderma* was reduced to 50% in non-amended control and rate of decline varied between 49-51% in amended ones. The linear regression of the log CFU against time revealed that the trend of population decline was more or less similar in all the treatments and control. The rate of decline however was low in control but, 1% chitin gave the highest number of viable cells after 180 days, which may be due to high initial population.

Shelf life of the *Trichoderma* isolate in vermicompost

Trichodrma grown on amended media survived in the vermicompost substrate at a higher population than the non-amended one. In 180 days the population of *Trichoderma* was reduced to 75% in non-amended control against 53-72% in amended ones. Among the amendments the population reduction was low in 0.5% ammonium sulfate addition. The trend of population decline was similar in all the treatments and control however the rate was minimum in 0.5% ammonium sulfate and 1% chitin.

Shelf life of the *Trichoderma* isolate in dalia

Trichoderma grown on amended media survived in the dalia substrate at a higher population than the non-amended one. At 180 days the population of *Trichoderma* was reduced to 58% in non-amended control against 46-52% in amended ones. The linear regression of the log CFU against time revealed that the trend of population decline was more or less similar in all the treatments and control, however the rate was minimum in 1% ammonium sulfate and maximum in control.

Shelf life of the *Trichoderma* isolate in sago

Trichoderma grown on amended media survived in the sago at a higher population than the non-amended one. At 180 days the population of *Trichoderma* was reduced to 47% in non-amended control against 42% in amended ones. The linear regression of the log CFU against time revealed that the trend of population decline was similar in all the treatments and control but the final population after 180 days was found highest in 1% chitin amended one, may be by virtue of higher onset population.

DISCUSSION

After going through the data of population of *Trichoderma* isolate in different substrates with different amendment for 180 days, the data were analyzed to reveal the effect of the substrates irrespective of amendments (Table 1) and effect of amendments on storage life irrespective of substrates (Table 2) and their interaction (Table 3) irrespective of time.

The data from the Table 1 indicates that sago and dalia proved to be a good substrate for storage of the bioagent under study in comparison to the conventional substrate i.e., talc. Higher population of the *Trichoderma* in the mentioned substrates may have its reason in their initial population which was in tune of 10^{12}. On the other hand among the amendments added in the growth media 1% chitin may be an effective supplement to the growth media for increasing its efficacy (Table 2) as well its survival in storage under room temperature. From the interaction table (Table 5) between different substrate and different amendments selected for the shelf life study a combination of sago and 1% chitin seems to be an apt choice for efficient multiplication and storage of *Trichoderma* isolate.

Data from Table 1 was put to linear regression to find out the rate of decline in population with respect to time in substrates irrespective of any amendments. The rate of decline in population was found low for alginate prills both in bentonite and wheat, thus the formulation can be used if the initial population level can be enhanced. Rate of decline was higher in organic substrates as vermicompost, sago and dalia with highest rate in dalia may be due to depletion in nutrient level. There is indication that initial population buildup and retention of the same during storage may be a function of the substrate used and amendment may only enhance the bio-efficacy of the stored bio-agent. However, the rate of decline was observed lower in 0.5% ammonium sulfate and 1% chitin amendment than the control as derived from the Figure 2 and Table 5 but, as there is an advantage of higher initial population in chitin amended substrates the final population remains higher than the other amended ones.

Economic analysis

The economic analysis for production cost of formulated product (Table 6) indicated that alginate prills, both in bentonite and wheat flour may not be a choice for commercial production as its material cost is very high in comparison to other substrates used for formulation due to the cost of alginate required to immobilize the organism in prills. Other formulations and substrates were comparable in material cost. The higher cost of material required for production of formulated product where chitin was used

for efficacy enhancement was due to higher cost of chitin, although the population level in chitin amended substrates were always a few folds higher than other formulations. Thus, the cost for equivalent population in other amendments may be much higher than what it is calculated. Among the organic substrates the cost of formulation in sago was highest and vermicompost was lowest. The cost of sago formulation was high because of its high base cost. Considering the economics of production and role of amendments in increasing the population as well as shelf life 1% chitin in dalia may be a good substrate for growth and subsequent formulation.

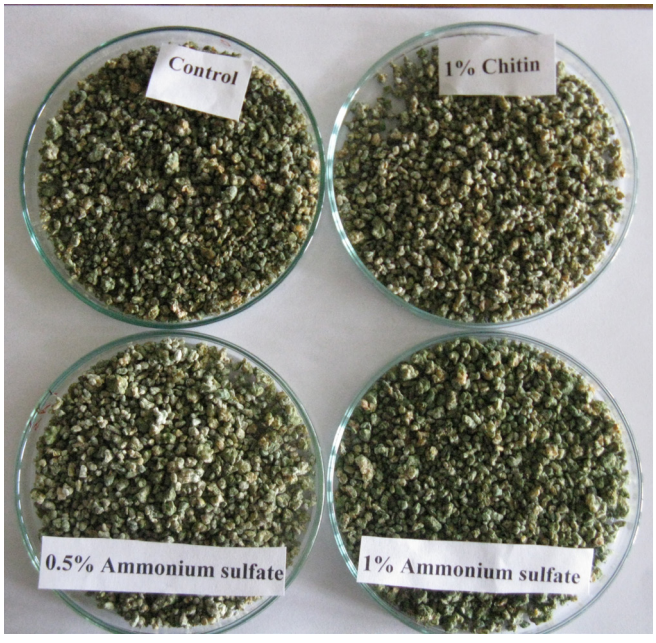

Plate 1. Sago based formulation

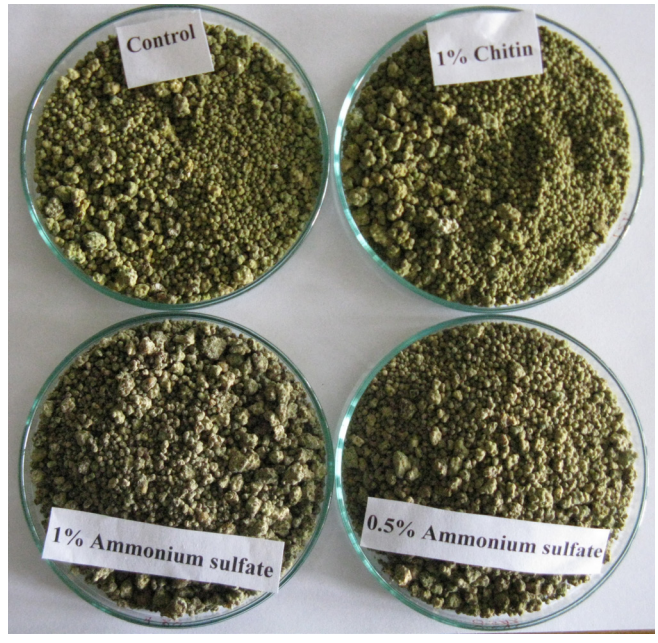

Plate 2. Dalia based formulation

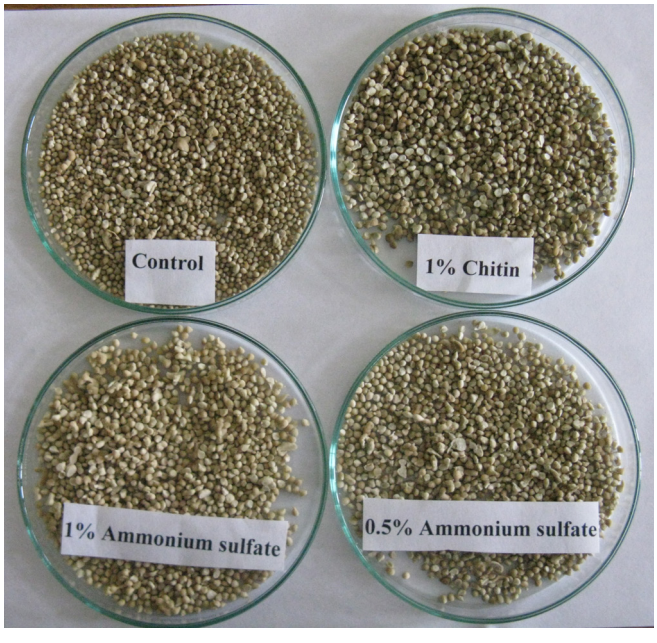

Plate 3. Prills (Wheat)

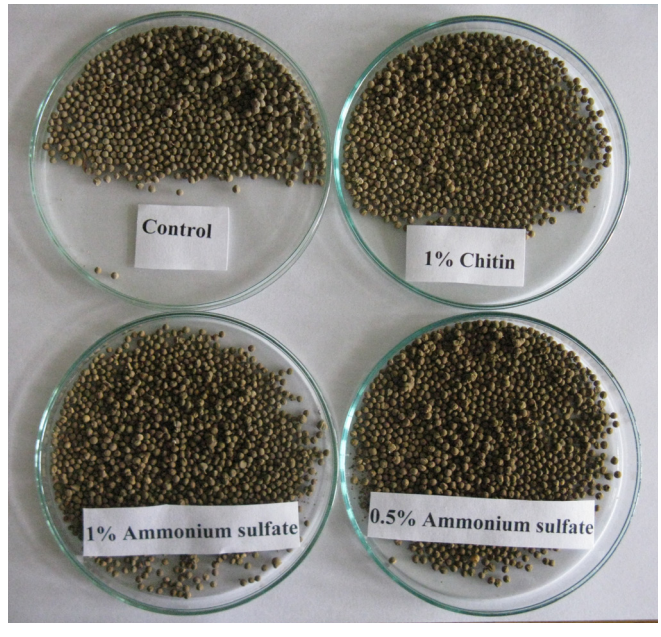

Plate 4. Prills (Bentonite)

Table 1. Shelf life of *Trichoderma* isolate in different substrates irrespective of any amendment

Days after preparation	Substrate					
	Talc	Vermi-compost	Sago	Dalia	Prills (Wheat flour)	Prills (Bentonite)
30	8.695	9.802	12.332	12.693	7.499	7.752
60	7.376	7.884	12.105	12.140	7.471	7.305
90	6.237	5.792	10.874	11.707	6.587	6.753
120	5.470	5.487	8.732	8.839	5.110	5.537
150	4.464	4.468	8.413	6.676	4.513	4.447
180	3.340	2.792	6.915	5.810	3.732	3.772
Mean	5.930 D	6.038 C	9.895 A	9.644 B	5.819 E	5.927 D
$LSD_{0.05}$	0.084					
SEM ±	0.030					

Table 2. Shelf life of *Trichoderma* isolate in case of different amendment irrespective of any substrates

Days after preparation	Treatment			
	Control	1% Chitin	0.5% Amonium Sulfate	1% Amonium sulfate
30	9.551	10.026	9.706	9.901
60	8.658	9.362	8.977	9.191
90	7.526	8.288	8.073	8.080
120	6.260	6.812	6.504	6.541
150	5.183	5.747	5.556	5.502
180	3.968	4.663	4.530	4.413
Mean	**6.858**	**7.483**	**7.224**	**5.960**
$LSD_{0.05}$	**0.069**			
SEM ±	**0.025**			

Table 3. Interaction of substrate and amendment on storage life of *Trichoderma* isolate

Treatments	Substrate for storage of *Tricoderma* isolates in formulation						Mean
	Talc	Vermi-compost	Sago	Dalia	Flour-Prills	Bentonite Prills	
Contol	5.537	5.738	9.395	9.261	5.436	5.779	**6.858 C**
1% Chitin	6.406	6.235	10.209	9.896	6.072	6.080	**7.483 A**
0.5% Amonium Sulfate	5.843	6.073	9.932	9.746	5.859	5.891	**7.224 B**
1% Amonium sulfate	5.935	6.104	10.046	9.675	5.907	5.960	**7.271 B**
Mean	**5.930 D**	**6.038 C**	**9.895 A**	**9.644 B**	**5.819 E**	**5.927 D**	
	Treatment		**Substrate**			**Interaction**	
$LSD_{0.05}$	**0.069**		**0.084**			**0.170**	
SEM±	**0.025**		**0.030**			**0.061**	

Table 4. Linear regression of the population in storage under the influence of substrates irrespective of amendments

Sl. No.	Substrates	Equation	R^2
1.	Talc	-0.0346x + 9.5581	0.9948
2.	Vermicompost	-0.0434x + 10.598	0.9602
3.	Sago	-0.0384x + 13.925	0.9584
4.	Dalia	-0.0511x + 15.012	0.9367
5.	Alginate Prills (Bentonite)	-0.0283x + 8.8967	0.9763
6.	Alginate Prills (Wheat)	-0.0278x + 8.7373	0.9578

Table 5. Linear regression of the population in storage under the influence of amendments irrespective of substrates

Sl. No.	Amendment	Equation	R^2
1.	Control	-0.0377x + 10.818	0.998
2.	1% Chitin	-0.0373x + 11.397	0.9918
3.	0.5% Ammonium Sulfate	-0.0359x + 10.996	0.9905
4.	1% Ammonium sulfate	-0.0381x + 11.276	0.9923

Table 6. Economic analysis of cost of materials required to produce 1 kg of formulated biocontrol product UBT18 in different substrates with and without amendments

Items	Substrates Cost per kilogram					
	Talc	**Bentonite**	**Wheat Flour**	**Vermicompost**	**Dalia**	**Sago**
Substrate	20	650	150	10	15	20
Medium	2.5	2	2	-	-	10
Base Cost	22.5	652	152	10	15	30
Cost of amendments per kg production						
1% chitin	5.58	3.72	3.72	18.6	18.6	18.6
0.5% Ammonium sulfate	0.7	0.4	0.4	2.1	2.1	2.1
1% Ammonium sulfate	1.4	0.8	0.8	4.2	4.2	4.2
Cost of materials with amendments for production of 1kg of produce						
1% chitin	28.08	655.72	155.72	28.6	33.6	48.6
0.5% Ammonium sulfate	23.2	652.4	152.4	12.1	17.1	32.1
1% Ammonium sulfate	23.9	652.8	152.8	14.2	19.2	34.2

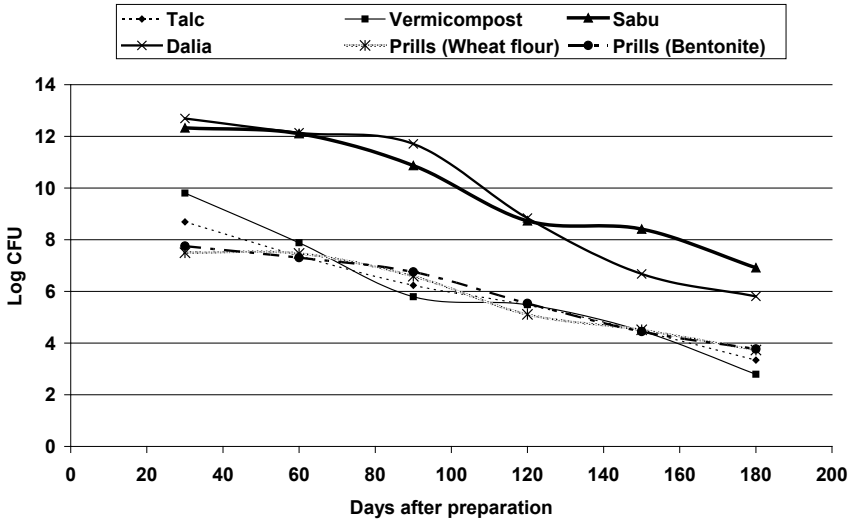

Fig. 1. Decline in population in the substrates during the storage in room temperature irrespective of any amendments.

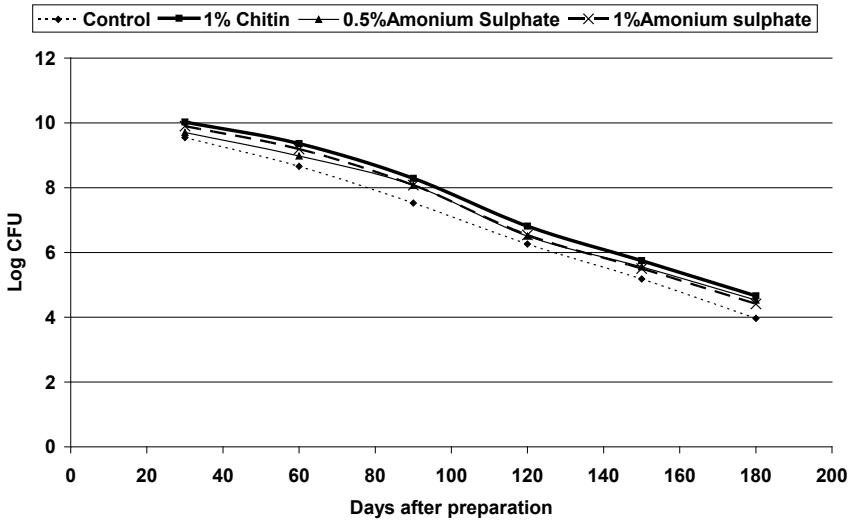

Fig. 2. Decline in population under the influence of amendments during the storage in room temperature irrespective of any substrates.

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