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Storability of *Metarhizium anisopliae* 1.15%WP with different moisture content

NV Parjane, GB Kabre, YS Saindane, AM Navale and MR Patil

Abstract
The present investigation on "Storability of *Metarhizium anisopliae* 1.15% WP with different moisture content*"* was carried out at Biocontrol Laboratory, Department of Entomology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri during August 2021 to May 2022. Storability of *Metarhizium anisopliae* was studied using different moisture levels i.e., 8, 12, 16, 20, 24 and 28 per cent to evaluate the shelf life. After every 15 days interval 1 gm sample from each sample was used for serial dilution to test the viability of the product. The CFU count of each sample was recorded after every 15 days interval. The results revealed that highest storage ability of *Metarhizium anisopliae* 1.15% WP was with moisture content of 8 per cent (240 days) and lowest storage ability was of *Metarhizium anisopliae* 1.15% WP with moisture content of 28 per cent (150 days).

Keywords: Storability, *metarhizium anisopliae*, serial dilution, moisture, storage ability

Introduction
Metarhizium anisopliae (Metchnikoff) an entomopathogenic fungus, is an effective alternative control agent against significant agricultural pests (Van Der Geest *et al*., 2000; Liu *et al*., 2002; Hatting *et al*., 2004; Leland *et al*., 2005; Quesada-Moraga *et al*., 2006; Al-Mazaawi *et al*., 2006) [17, 13, 7, 12, 16, 1]. Numerous studies have been conducted on *Metarhizium anisopliae* Sorokin (Ascomycetes: Hypocreales) for the biological control of a variety of insect pests. It has been discovered that *Metarhizium* is efficient against a variety of insect species including Lepidoptera. The fungus *Metarhizium anisopliae* formerly known as *Entomopthora anisopliae* is an entomopathogenic fungus that naturally develops in soils all over the world and infects a variety of insect pests. It is crucial to ascertain the impact of various external conditions on the viability and other integrated pest components due to the growing knowledge of *Metarhizium anisopliae* for employment as a biocontrol agent (Goettel and Hajek, 2003) [6].

Many of the microbial pathogens which shown great effectiveness in the laboratory did not work as well in the field. Poor product stability during storage before application, inadequate active material delivery to the target site and quick material deterioration following application due to environmental variables are common explanations for the aforementioned occurrence (Burges, 1998) [3]. According to Burges, wet storage is preferable for treatments meant for soil while dry storage is optimal for goods applied to foliage. Additionally, he stress the significance of product moisture, storage temperature and carrier medium employed in formulation.

Different scientist has different ideas about how long a mycoinsecticide shelf life should be, although estimates range from 3 to 18 months or even more (Moore *et al*., 1997) [15]. According to the same authors, it is preferable to preserve the viability to cover two cropping seasons and long-term storage is more for the convenience of manufacturers than farmers and should not be permitted to remain an impediment. Temperature and the moisture content of the storage environment are the two most important parameters influencing conidial lifespan (Hong *et al*., 1997) [9].

Hedgecock *et al*. (1995) [8] investigated the effect of moisture content on temperature tolerance and storage of *M. anisopliae var. acridum* in oil formulation and the results showed that viability declined with high temperatures and high moisture contents. High temperature tolerance was greatly improved by drying the conidia with silica gel (McClatchie *et al*., 1994). The optimal moisture content for dried conidia storage was discovered to be 4-5 per cent and a variety of mineral oils proved satisfactory for dried conidia storage. Less moisture content than 4-5 per cent may produce better results, but it is difficult to achieve.

The production of large number of conidia is critical for the commercialization of

mycoinsecticides, but the success of fungal-based bioinsecticides is also dependent on storage conditions. Thus, it becomes very necessary to determine the storability of *Metarhizium anisopliae*. Hence, present research work aims at one of the important parameters of storage of *Metarhizium anisopliae* i.e., Moisture to assess storage ability of *Metarhizium anisopliae* 1.15% WP when prepared with different moisture contents.

Material and Methods

Collection and maintenance of pure culture

The pure culture of *M. anisopliae* IPL/KC/44, Strain No. ITCC 6895 was obtained from the Biocontrol laboratory, Department of Entomology, Post Graduate Institute, MPKV, Rahuri and maintained on PDA slants and petri plates.

Preparation of Potato dextrose broth for mass production of *Metarhizium anisopliae* 1.15% WP

The healthy potatoes were selected and washed thoroughly with tap water and dried at ambient conditions. The epidermis of potatoes was peeled out with the help of hand peeler. Requisite quantity of medium was prepared by cutting potato into about one cubic centimeter sized pieces boiling in heated water in proportion of 200 g pieces in one litre of distilled water. Boiling was continued up to thumb press softening of potato pieces. The potato extract was then strained through the strainer. The volume of the collected supernatant was adjusted to its original volume and fortified with 2% dextrose sugar available from local market. The saline bottles PDB were then kept in autoclave at 121 ºC (15 lbp) for 20 minutes. 80 ml PDB was poured in 500 ml capacity of saline bottle and pinged with cotton and were kept in autoclave at 121ºC (15 lbp) for 20 minutes. After autoclaving the saline bottles along with PDB were allowed to cool down completely and then kept in laminar air flow for 15 to 20 minutes under ultraviolet light before inoculation. Under aseptic condition *M. anisopliae* was inoculated to PDB.

Preparation of *Metarhizium anisopliae* 1.15% WP with different moisture content at ambient temperature

The fungus *M. anisopliae* was inoculated in sterilized potato dextrose broth (PDB) in a saline bottle. The fungus *M. anisopliae* cultured on PDB medium was incubated for 15 days at 25 ºC±2 ºC. 15 days old fully grown fungal mat was harvested and blended with sterilized blender for 5 minutes. The respective fungal formulations were prepared by using grinded fungal mat of the weighing 11.5 gm of the fully grown *M. anisopliae* and was mixed in 1kg of talcum powder. The adjuvant CMC was added @ 5 grams and 1.15% wettable powder formulation was prepared. The samples were prepared at different levels of moisture content i.e., 8, 12, 16, 20, 24 and 28 per cent to evaluate the shelf life. The moisture in the sample were adjusted by adding PDB and moisture content was measured with the help of moisture meter. The samples were stored at ambient temperature at 25- 30 ºC±2 ºC.

Observations recorded

The samples prepared at different levels of moisture content i.e., 8, 12, 16, 20, 24 and 28 per cent to evaluate the shelf life of the fungus *M. anisopliae* were stored at ambient temperature at 25- 30 ºC±2 ºC. After every 15 days interval 1 gm sample from each sample was used for serial dilution (Koch, R. 1883) [11] to test the viability of the product. The CFU count of each sample was recorded for every 15 days interval after 72 hrs of inoculation. Observations on CFU count at serial dilution from 10^6 to 10^9 was recorded.

The experiment was conducted in FCRD with 6 treatments and 4 replications and data was analysed using standard statistical procedures.

Result and Discussion

Storability of *Metarhizium anisopliae* 1.15% WP at Ambient Temperature

The storability of *M. anisopliae* was evaluated at a regular interval of 15 days by using 1 gram sample of *Metarhizium anisopliae* 1.15% WP from the sample prepared with different moisture level *viz.*, 8, 12, 16, 20, 24 and 28 per cent. CFU count was measured at 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, 180, 195, 210, 225, 240, 255 and 270 days after storage kept at room temperature. The CFU count was enumerated by serial dilution technique at 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9} dilution factor after 3 days of inoculation.

The data on CFU count of *M. anisopliae* at 15, 30, 45, 60, 75, 90, 105, 120, 135,150,165,180, 195, 210, 225, 240, 255 and 270 days after storage are presented in Table 1.

CFU Count after 15 Days of Storage

The data revealed that (Table 1) the mean CFU count was significantly superior in treatment containing moisture of 20% (104.25) and it was followed by treatment with moisture content 16% (102.44), 12% (100.06), 8% (96.19), 24% (94.44) and 28% (90.25) respectively.

Among dilution factor the maximum mean CFU count from all the treatments was observed at dilution factor 10^{-6} (110.80) followed by dilution factor 10^{-7} (105.63), 10^{-8} (98.33) and 10^{-9} (76.54), respectively.

The mean CFU count from the dilution factor of 10^{-8} from all the treatments revealed that highest mean CFU count was observed in treatment with moisture content of 20% (106.00) followed by treatment with moisture content 16% (103.00), 12% (99.00), 8% (97.00), 24% (93.00) and 28% (89.00).

CFU Count after 30 Days of Storage

The data revealed that (Table 1) the mean CFU count was significantly superior in treatment containing moisture of 20% (96.50) and it was followed by treatment with moisture 16% (93.50), 12% (87.31), 8% (85.00), 24% (84.13) and 28% (80.44) respectively.

Among dilution factor the maximum mean CFU count from all the treatments was observed at dilution factor 10^{-6} (101.21) followed by dilution factor 10^{-7} (92.50), 10^{-8} (85.67) and 10^{-9} (71.88) respectively.

The mean CFU count from the dilution factor of 10^{-8} from all the treatments revealed that highest mean CFU count was observed in treatment with moisture content of 20% (94.00) followed by treatment with moisture content 16% (90.00), 12% (85.00), 8% (83.00), 24% (82.00) and 28% (80.00).

CFU Count after 45 Days of Storage

The data revealed that (Table 1) the mean CFU count was significantly superior in treatment containing moisture of 20% (92.63) and it was followed by treatment with moisture 16% (90.50), 12% (83.81), 8% (81.25), 24% (72.50) and 28% (65.75) respectively.

Among dilution factor the maximum mean CFU count from all the treatments was observed at dilution factor 10^{-6} (95.50)

followed by dilution factor 10^{-7} (86.88), 10^{-8} (77.00) and 10^{-9} (64.92) respectively.

The mean CFU count from the dilution factor of 10^{-8} from all the treatments revealed that highest mean CFU count was observed in treatment with moisture of 20% (89.00) followed by treatment with moisture content 16% (86.00), 12% (83.00), 8% (79.00), 24% (65.00) and 28% (60.00).

CFU Count after 60 Days of Storage

The data revealed that (Table 1) the mean CFU count was significantly superior in treatment containing moisture of 20% (88.75) and it was followed by treatment with moisture 16% (83.63), 12% (73.75), 8% (69.69), 24% (65.50) and 28% (58.50) respectively.

Among dilution factor the maximum mean CFU count from all the treatments was observed at dilution factor 10^{-6} (91.83) followed by dilution factor 10^{-7} (77.67), 10^{-8} (68.54) and 10^{-9} (55.17) respectively.

The mean CFU count from the dilution factor of 10^{-8} from all the treatments revealed that highest mean CFU count was observed in treatment with moisture content of 20% (87.00) followed by treatment with moisture content 16% (80.50), 12% (68.00), 8% (62.75), 24% (60.00) and 28% (53.00).

Table 1: Storability of *Metarhizium anisopliae* 1.15% WP (CFU count 3 days after inoculation)

Factors	Mean CFU count of *M. anisopliae* days after finished product preparation (at room temperature)																	
Factor A (T)	**15 DAS**	**30 DAS**	**45 DAS**	**60 DAS**	**75 DAS**	**90 DAS**	**105 DAS**	**120 DAS**	**135 DAS**	**150 DAS**	**165 DAS**	**180 DAS**	**195 DAS**	**210 DAS**	**225 DAS**	**240 DAS**	**255 DAS**	**270 DAS**
T_1: 8%	96.19 (9.81)*	85.00 (9.23)	81.25 (9.03)	69.69 (8.35)	59.63 (7.71)	51.75 (7.19)	40.75 (6.32)	35.25 (5.89)	28.75 (5.35)	25.75 (5.06)	23.00 (4.77)	21.00 (4.53)	15.25 (3.85)	10.50 (3.19)	7.19 (2.71)	3.81 (1.93)	1.19 (1.19)	0.00 (0.71)
T_2: 12%	100.06 (10.01)	87.31 (9.36)	83.81 (9.17)	73.75 (8.60)	62.94 (7.93)	55.50 (7.45)	43.25 (6.52)	38.50 (6.15)	31.50 (5.59)	28.00 (5.27)	20.75 (4.51)	15.50 (3.82)	12.50 (3.45)	8.50 (2.80)	4.50 (2.05)	1.25 (1.26))	0.00 (0.71)	0.00 (0.71)
T_3: 16%	102.44 (10.12)	93.50 (9.67)	90.50 (9.51)	83.63 (9.13)	68.63 (8.28)	58.69 (7.66)	45.75 (6.71)	42.19 (6.44)	36.50 (6.00)	24.63 (4.94)	17.75 (4.15)	13.13 (3.52)	8.75 (2.90)	3.75 (1.87)	1.50 (1.25)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
T_4: 20%	104.25 (10.21)	96.50 (9.83)	92.63 (9.62)	88.75 (9.41)	79.75 (8.92)	63.13 (7.94)	50.25 (7.02)	41.25 (6.27)	30.75 (5.43)	18.75 (4.25)	11.00 (3.28)	5.25 (2.14)	1.25 (1.19)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
T_5: 24%	94.44 (9.72)	84.13 (9.18)	72.50 (8.52)	65.50 (8.09)	56.56 (7.46)	41.50 (6.43)	30.88 (5.52)	24.69 (4.91)	17.75 (4.17)	10.94 (3.20)	4.19 (1.98)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
T_6: 28%	90.25 (9.51)	80.44 (8.97)	65.75 (8.10)	58.50 (7.60)	48.00 (6.87)	34.38 (5.83)	28.19 (5.29)	20.00 (4.43)	11.75 (3.21)	5.25 (2.14)	1.75 (1.21)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
S.E	0.03	0.04	0.02	0.03	0.03	0.03	0.04	0.03	0.03	0.03	0.03	0.01	0.01	0.01	0.01	0.01	0.01	NS
C. D 5%	0.08	0.10	0.06	0.07	0.08	0.07	0.10	0.09	0.09	0.08	0.08	0.03	0.02	0.02	0.02	0.02	0.03	-
Factor B Dilution																		
D_1: 10^{-6}	110.80 (10.57)	101.21 (10.08)	95.50 (9.78)	91.83 (9.59)	79.58 (8.94)	63.46 (7.97)	55.92 (7.48)	48.83 (6.97)	39.33 (6.26)	29.83 (5.41)	22.04 (4.58)	16.17 (3.50)	11.33 (2.92)	7.67 (2.32)	4.17 (1.82)	1.67 (1.24)	0.50 (0.90)	0.00 (0.71)
D_2: 10^{-7}	105.63 (10.30)	92.50 (9.64)	86.88 (9.34)	77.67 (8.83)	66.83 (8.18)	54.50 (7.37)	44.42 (6.66)	39.25 (6.25)	28.83 (5.36)	20.79 (4.51)	14.00 (3.46)	10.33 (2.85)	7.17 (2.33)	4.17 (1.81)	2.67 (1.50)	1.00 (1.07)	0.29 (0.84)	0.00 (0.71)
D_3: 10^{-8}	98.33 (9.94)	85.67 (9.28)	77.00 (8.78)	68.54 (8.28)	60.75 (7.78)	50.42 (7.10)	38.50 (6.21)	31.00 (5.55)	23.96 (4.78)	15.83 (3.84)	10.92 (3.09)	7.42 (2.41)	4.83 (1.94)	2.50 (1.50)	1.50 (1.21)	0.71 (1.00)	0.00 (0.71)	0.00 (0.71)
D_4: 10^{-9}	76.54 (8.77)	71.88 (8.50)	64.92 (8.07)	55.17 (7.42)	43.17 (6.55)	34.92 (5.89)	20.54 (4.56)	15.50 (3.96)	12.54 (3.44)	9.08 (2.82)	5.33 (2.14)	2.67 (1.53)	1.83 (1.35)	0.83 (1.03)	0.46 (0.89)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
S.E	0.02	0.03	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01	NS
C. D 5%	0.07	0.08	0.05	0.06	0.06	0.06	0.09	0.08	0.07	0.06	0.07	0.02	0.02	0.01	0.02	0.01	0.02	-

Factors	Mean CFU count of *M. anisopliae* days after finished product preparation (at room temperature)																	
	15 DAS	**30 DAS**	**45 DAS**	**60 DAS**	**75 DAS**	**90 DAS**	**105 DAS**	**120 DAS**	**135 DAS**	**150 DAS**	**165 DAS**	**180 DAS**	**195 DAS**	**210 DAS**	**225 DAS**	**240 DAS**	**255 DAS**	**270 DAS**
Interaction																		
T_1D_1	112.00 (10.61)	96.00 (9.82)	92.00 (9.62)	87.00 (9.35)	79.00 (8.92)	64.00 (8.03)	59.00 (7.71)	49.00 (7.04)	39.00 (6.28)	38.00 (6.20)	35.00 (5.96)	33.00 (5.79)	26.00 (5.15)	20.00 (4.53)	11.00 (3.39)	7.00 (2.74)	3.00 (1.87)	0.00 (0.71)
T_1D_2	103.75 (10.21)	90.00 (9.51)	87.00 (9.35)	72.00 (8.51)	61.00 (7.84)	58.00 (7.65)	44.00 (6.67)	39.00 (6.28)	31.00 (5.61)	27.00 (5.24)	25.00 (5.05)	24.00 (4.95)	17.00 (4.18)	11.00 (3.39)	9.00 (3.08)	5.00 (2.35)	1.75 (1.49)	0.00 (0.71)
T_1D_3	97.00 (9.87)	83.00 (9.14)	79.00 (8.92)	62.75 (7.95)	57.25 (7.60)	48.00 (6.96)	40.00 (6.36)	35.00 (5.96)	28.00 (5.34)	23.00 (4.85)	20.00 (4.53)	18.00 (4.30)	12.00 (3.54)	7.00 (2.74)	6.00 (2.55)	3.25 (1.93)	0.00 (0.71)	0.00 (0.71)
T_1D_4	76.00 (8.75)	71.00 (8.46)	67.00 (8.22)	57.00 (7.58)	47.00 (6.89)	37.00 (6.12)	20.00 (4.52)	18.00 (4.40)	17.00 (4.18)	15.00 (3.94)	12.00 (3.53)	9.00 (3.08)	6.00 (2.55)	4.00 (2.12)	2.75 (1.80)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
T_2D_1	114.00 (10.70)	98.25 (9.94)	94.00 (9.72)	89.00 (9.46)	80.00 (8.97)	66.00 (8.15)	61.00 (7.84)	54.00 (7.38)	44.00 (6.67)	40.00 (6.36)	32.00 (5.70)	28.00 (5.34)	21.00 (4.64)	17.00 (4.18)	9.00 (3.08)	3.00 (1.87)	0.00 (0.71)	0.00 (0.71)
T_2D_2	108.00 (10.42)	92.00 (9.62)	89.25 (9.47)	76.00 (8.75)	65.00 (8.09)	61.00 (7.84)	48.00 (6.96)	44.00 (6.67)	33.00 (5.79)	29.00 (5.43)	23.00 (4.85)	18.00 (4.30)	16.00 (4.06)	10.00 (3.24)	6.00 (2.55)	1.00 (1.22)	0.00 (0.71)	0.00 (0.71)
T_2D_3	99.00 (9.97)	85.00 (9.25)	83.00 (9.14)	68.00 (8.28)	57.75 (7.63)	54.50 (7.42)	42.00 (6.52)	37.00 (6.12)	31.00 (5.61)	27.00 (5.24)	19.00 (4.42)	12.50 (3.60)	10.00 (3.24)	6.00 (2.55)	3.00 (1.87)	1.00 (1.22)	0.00 (0.71)	0.00 (0.71)
T_2D_4	79.25 (8.93)	74.00 (8.63)	69.00 (8.34)	62.00 (7.91)	49.00 (7.03)	4.050 (6.40)	22.00 (4.74)	19.00 (4.42)	18.00 (4.30)	16.00 (4.06)	9.00 (3.08)	4.00 (2.12)	3.00 (1.87)	1.00 (1.22)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
T_3D_1	115.50 (10.77)	112.00 (10.61)	110.00 (10.51)	108.00 (10.42)	84.50 (9.22)	70.75 (8.44)	64.00 (8.03)	57.00 (7.58)	51.00 (7.18)	38.00 (6.20)	29.00 (5.43)	24.00 (4.95)	17.00 (4.18)	9.00 (3.08)	5.00 (2.35)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
T_3D_2	111.25 (10.57)	95.00 (9.77)	94.00 (9.72)	83.00 (9.14)	66.00 (8.15)	63.00 (7.97)	51.00 (7.18)	48.75 (7.02)	37.00 (6.12)	25.00 (5.05)	21.00 (4.64)	13.00 (3.67)	9.00 (3.08)	4.00 (2.12)	1.00 (1.22)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)

T_3D_3	103.00 (10.17)	90.00 (9.51)	86.00 (9.30)	80.50 (9.00)	73.00 (8.57)	59.00 (7.71)	44.00 (6.67)	42.00 (6.52)	39.00 (6.28)	21.00 (4.63)	14.00 (3.81)	12.00 (3.54)	7.00 (2.74)	2.00 (1.58)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
T_3D_4	80.00 (8.97)	77.00 (8.80)	72.00 (8.51)	63.00 (7.97)	51.00 (7.18)	42.00 (6.52)	24.00 (4.94)	21.00 (4.64)	19.00 (4.42)	14.50 (3.87)	7.00 (2.74)	3.00 (1.87)	2.00 (1.58)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
T_4D_1	117.00 (10.84)	115.00 (10.75)	112.00 (10.61)	110.00 (10.51)	93.00 (9.67)	78.50 (8.89)	68.00 (8.28)	64.00 (8.03)	49.00 (7.03)	31.00 (5.61)	20.00 (4.53)	12.00 (3.54)	4.00 (2.12)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
T_4D_2	113.00 (10.65)	98.00 (10.75)	95.00 (9.77)	93.00 (9.67)	89.00 (9.46)	67.00 (8.22)	59.00 (7.71)	52.00 (7.25)	36.00 (6.04)	23.00 (4.85)	11.00 (3.39)	7.00 (2.74)	1.00 (1.22)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
T_4D_3	106.00 (10.32)	94.00 (9.72)	89.00 (9.46)	87.00 (9.35)	82.00 (9.08)	63.00 (7.97)	48.00 (6.96)	34.00 (5.87)	27.00 (5.24)	14.00 (3.81)	9.00 (3.08)	2.00 (1.58)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)

Factors	**Mean CFU count of *M. anisopliae* days after finished product preparation (at room temperature)**																	
	15 DAS	**30 DAS**	**45 DAS**	**60 DAS**	**75 DAS**	**90 DAS**	**105 DAS**	**120 DAS**	**135 DAS**	**150 DAS**	**165 DAS**	**180 DAS**	**195 DAS**	**210 DAS**	**225 DAS**	**240 DAS**	**255 DAS**	**270 DAS**
T_4D_4	81.00 (9.03)	79.00 (8.92)	74.50 (8.66)	65.00 (8.09)	55.00 (9.45)	44.00 (6.67)	26.00 (5.15)	15.00 (3.94)	11.00 (3.39)	7.00 (2.74)	4.00 (2.11)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
T_5D_1	108.00 (10.42)	94.00 (9.72)	85.00 (9.25)	80.00 (8.97)	72.00 (8.51)	53.00 (7.31)	44.00 (6.67)	38.00 (6.20)	29.00 (5.43)	20.00 (4.53)	9.25 (3.12)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
T_5D_2	100.75 (10.06)	91.00 (9.57)	82.00 (9.08)	73.00 (8.57)	67.00 (8.22)	40.00 (6.36)	33.50 (5.83)	28.75 (5.41)	21.00 (4.64)	13.75 (3.77)	4.00 (2.11)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
T_5D_3	93.00 (9.67)	82.00 (9.08)	65.00 (8.09)	60.00 (7.78)	51.50 (7.21)	46.00 (6.82)	30.00 (5.52)	21.00 (4.63)	11.75 (3.50)	8.00 (2.91)	3.50 (1.98)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
T_5D_4	72.00 (8.51)	69.50 (8.37)	58.00 (7.65)	49.00 (7.03)	30.00 (7.45)	27.00 (5.24)	16.00 (4.06)	11.00 (3.38)	9.25 (3.11)	2.00 (1.58)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
T_6D_1	101.00 (10.07)	92.00 (9.62)	80.00 (8.97)	77.00 (8.80)	69.00 (8.34)	48.50 (7.00)	39.50 (6.32)	31.00 (5.61)	24.00 (4.95)	12.00 (3.54)	7.00 (2.74)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
T_6D_2	97.00 (9.87)	89.00 (9.46)	74.00 (8.63)	69.00 (8.34)	53.00 (7.31)	38.00 (6.20)	31.00 (5.61)	23.00 (4.85)	15.00 (3.94)	7.00 (2.74)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
T_6D_3	89.00 (9.46)	80.00 (8.97)	60.00 (7.78)	53.00 (7.31)	43.00 (6.59)	32.00 (5.70)	27.00 (5.24)	17.00 (4.18)	7.00 (2.74)	2.00 (1.58)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
T_6D_4	71.00 (8.46)	60.75 (7.84)	49.00 (7.04)	35.00 (5.96)	27.00 (5.24)	19.00 (4.41)	15.25 (3.97)	9.00 (3.08)	1.00 (1.22)	0.00 (0.07)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
S.E	0.06	0.07	0.05	0.05	0.06	0.05	0.07	0.07	0.06	0.05	0.06	0.02	0.02	0.01	0.01	0.01	0.02	NS
CD 5%	0.17	0.21	0.13	0.15	0.16	0.15	0.21	0.19	0.18	0.15	0.17	0.06	0.05	0.03	0.04	0.04	0.05	-

Note: * Figures in parentheses are √ X + 0.5 transformed values

CFU Count after 75 Days of Storage

The data revealed that (Table 1) the mean CFU count was significantly superior in treatment containing moisture of 20% (79.75) and it was followed by treatment with moisture content 16% (68.63), 12% (62.94), 8% (59.63), 24% (56.56) and 28% (48.00) respectively.

Among dilution factor the maximum mean CFU count from all the treatments was observed at dilution factor 10^{-6} (79.58) followed by dilution factor 10^{-7} (66.83), 10^{-8} (60.75) and 10^{9} (43.17) respectively.

The mean CFU count from the dilution factor of 10^{-8} from all the treatments revealed that highest mean CFU count was observed in treatment with moisture content of 20% (82.00) followed by treatment with moisture content 16% (73.00), 12% (57.75), 8% (57.25), 24% (51.50) and 28% (43.00).

CFU Count after 90 Days of Storage

The data revealed that (Table 1) the mean CFU count was significantly superior in treatment containing moisture of 20% (63.13) and it was followed by treatment with moisture content 16% (58.69), 12% (55.50), 8% (51.75), 24% (41.50) and 28% (34.38) respectively.

Among dilution factor the maximum mean CFU count from all the treatments was observed at dilution factor 10^{-6} (63.46) followed by dilution factor 10^{-7} (54.50), 10^{-8} (50.42) and 10^{-9} (34.92) respectively.

The mean CFU count from the dilution factor of 10^{-8} from all the treatments revealed that highest mean CFU count was observed in treatment with moisture content of 20% (63.00) followed by treatment with moisture content 16% (59.00), 12% (54.50), 8% (48.00), 24% (46.00) and 28% (32.00).

CFU Count after 105 Days of Storage

The data revealed that (Table 1) the mean CFU count was significantly superior in treatment containing moisture of 20% (50.25) and it was followed by treatment with moisture content 16% (45.75), 12% (43.25), 8% (40.75), 24% (30.88) and 28% (28.19) respectively.

Among dilution factor the maximum mean CFU count from all the treatments was observed at dilution factor 10^{-6} (55.92) followed by dilution factor 10^{-7}, (44.42), 10^{-8} (38.50) and 10^{-9} (20.54) respectively.

The mean CFU count from the dilution factor of 10^{-8} from all the treatments revealed that highest mean CFU count was observed in treatment with moisture content of 20% (48.00) followed by treatment with moisture content 16% (44.00), 12% (42.00), 8% (40.00), 24% (30.00) and 28% (27.00).

CFU Count after 120 Days of Storage

The data revealed that (Table 1) the mean CFU count was significantly superior in treatment containing moisture of 16% (42.19) and it was followed by treatment with moisture content 20% (41.25), 12% (38.50), 8% (35.25), 24% (24.69) and 28% (20.00) respectively.

Among dilution factor the maximum mean CFU count from all the treatments was observed at dilution factor 10^{-6} (48.83) followed by dilution factor 10^{-7}, (39.25), 10^{-8} (31.00) and 10^{-9} (15.50), respectively.

The mean CFU count from the dilution factor of 10^{-8} from all the treatments revealed that highest mean CFU count was

observed in treatment with moisture content of 16% (42.00) followed by treatment with moisture content 12% (37.00), 8% (35.00), 20% (34.00), 24% (21.00) and 28% (17.00).
After 120 days of storage the CFU of the treatment with moisture content 16% was greater than 20% moisture level indicates that after 120 days of storage treatment with 16% moisture can maintain high CFU than treatment with moisture content of 20%.

CFU Count after 135 Days of Storage
The data revealed that (Table 1) the mean CFU count was significantly superior in treatment containing moisture of 16% (36.50) and it was followed by treatment with moisture content 12% (31.50), 20% (30.75), 8% (28.75), 24% (17.75) and 28% (11.75) respectively.
Among dilution factor the maximum mean CFU count from all the treatments was observed at dilution factor 10^{-6} (39.33) followed by dilution factor 10^{-7} (28.83), 10^{-8} (23.96) and 10^{-9} (12.54) respectively.
The mean CFU count from the dilution factor of 10^{-8} from all the treatments revealed that highest mean CFU count was observed in treatment with moisture content of 16% (39.00) followed by treatment with moisture content 12% (31.00), 8% (28.00), 20% (27.00) 24% (11.75) and 28% (7.00).

CFU Count after 150 Days of Storage
The data revealed that (Table 1) the mean CFU count was significantly superior in treatment containing moisture of 12% (28.00) and it was followed by treatment with moisture content 8% (25.75), 16% (24.63), 20% (18.75), 24% (10.94) and 28% (5.25) respectively.
Among dilution factor the maximum mean CFU count from all the treatments was observed at dilution factor 10^{-6} (29.83) followed by dilution factor 10^{-7} (20.79), 10^{-8} (15.83) and 10^{-9} (9.08) respectively.
The mean CFU count from the dilution factor of 10^{-8} from all the treatments revealed that highest mean CFU count was observed in treatment with moisture content of 12% (27.00) followed by treatment with moisture content 8% (23.00), 16% (21.00), 20% (14.00), 24% (8.00) and 28% (2.00).
After 150 days of storage the CFU of the treatment with moisture content 12% was greater than 16% moisture level indicates that after 150 days of storage treatment with 12% moisture can maintain high CFU than treatment with moisture content of 16%.

CFU Count after 165 Days of Storage
The data revealed that (Table 1) the mean CFU count was significantly superior in treatment containing moisture of 8% (23.00) and it was followed by treatment with moisture content 12% (20.75), 16% (17.75), 20% (11.00), 24% (4.19) and 28% (1.75) respectively.
Among dilution factor the maximum mean CFU count from all the treatments was observed at dilution factor 10^{-6} (22.04) followed by dilution factor 10^{-7} (14.00), 10^{-8} (10.92) and 10^{-9} (5.33) respectively.
The mean CFU count from the dilution factor of 10^{-8} from all the treatments revealed that highest mean CFU count was observed in treatment with moisture content of 8% (20.00) followed by treatment with moisture content 12% (19.00), 16% (14.00), 20% (9.00), 24% (3.50) and 28% (0.00).
After 165 days of storage the CFU of the treatment with moisture content 8% was greater than 12% moisture level indicates that after 150 days of storage treatment with 8% moisture can maintain high CFU than treatment with moisture content of 12%.
The treatment with moisture content 28% after 165 days of storage at a dilution factor of 10^{-8} did not showed any colony forming units indicating that *M. anisopliae* 1.15% WP with moisture content of 28% can be stored up to 150 days only.

CFU Count after 180 Days of Storage
The data revealed that (Table 1) the mean CFU count was significantly superior in treatment containing moisture of 8% (21.00) and it was followed by treatment with moisture content 12% (15.50), 16% (13.13), 20% (5.25), 24% (0.00) and 28% (0.00) respectively.
Among dilution factor the maximum mean CFU count from all the treatments was observed at dilution factor 10^{-6} (16.17) followed by dilution factor 10^{-7} (10.33), 10^{-8} (7.42) and 10^{-9} (2.67) respectively.
The mean CFU count from the dilution factor of 10^{-8} from all the treatments revealed that highest mean CFU count was observed in treatment with moisture content of 8% (18.00) followed by treatment with moisture content 12% (12.50), 16% (12.00), 20% (2.00), 24% (0.00) and 28% (0.00).
The treatment with moisture content 24% after 180 days of storage at a dilution factor of 10^{8} did not showed any colony forming units indicating that *M. anisopliae* 1.15% WP with moisture content of 28% can be stored up to 165 days only.

CFU Count after 195 Days of Storage
The data revealed that (Table 1) the mean CFU count was significantly superior in treatment containing moisture of 8% (15.25) and it was followed by treatment with moisture content 12% (12.50), 16% (8.75), 20% (1.25), 24% (0.00) and 28% (0.00) respectively.
Among dilution factor the maximum mean CFU count from all the treatments was observed at dilution factor 10^{-6} (11.33) followed by dilution factor 10^{-7} (7.17), 10^{-8} (4.83) and 10^{-9} (1.83) respectively.
The mean CFU count from the dilution factor of 10^{-8} from all the treatments revealed that highest mean CFU count was observed in treatment with moisture content of 8% (12.00) followed by treatment with moisture content 12% (10.00), 16% (7.00), 20% (0.00), 24% (0.00) and 28% (0.00).
The treatment with moisture content 20% after 195 days of storage at a dilution factor of 10^{8} did not showed any colony forming units indicating that *M. anisopliae* 1.15% WP with moisture content of 20% can be stored up to 180 days only.

CFU Count after 210 Days of Storage
The data revealed that (Table 1) the mean CFU count was significantly superior in treatment containing moisture of 8% (10.50) and it was followed by treatment with moisture content 12% (8.50), 16% (3.75), 20% (0.00), 24% (0.00) and 28% (0.00) respectively.
Among dilution factor the maximum mean CFU count from all the treatments was observed at dilution factor 10^{-6} (7.67) followed by dilution factor 10^{-7} (4.17), 10^{-8} (2.50) and 10^{-9} (0.83) respectively.
The mean CFU count from the dilution factor of 10^{-8} from all the treatments revealed that highest mean CFU count was observed in treatment with moisture content of 8% (7.00) followed by treatment with moisture content 12% (6.00), 16% (2.00), 20% (0.00), 24% (0.00) and 28% (0.00).

CFU Count after 225 Days of Storage

The data revealed that (Table 1) the mean CFU count was significantly superior in treatment containing moisture of 8% (7.19) and it was followed by treatment with moisture content 12% (4.50), 16% (1.50), 20% (0.00), 24% (0.00) and 28% (0.00) respectively.

Among dilution factor the maximum mean CFU count from all the treatments was observed at dilution factor 10^{-6} (4.17) followed by dilution factor 10^{-7} (2.67), 10^{-8} (1.50) and 10^{-9} (0.46) respectively.

The mean CFU count from the dilution factor of 10^{-8} from all the treatments revealed that highest mean CFU count was observed in treatment with moisture content of 8% (6.00) followed by treatment with moisture content 12% (3.00), 16% (0.00), 20% (0.00), 24% (0.00) and 28% (0.00).

The treatment with moisture content 16% after 225 days of storage at a dilution factor of 10^{8} did not showed any colony forming units indicating that *M. anisopliae* 1.15% WP with moisture content of 16% can be stored up to 210 days only.

CFU Count after 240 Days of Storage

The data revealed that (Table 1) the mean CFU count was significantly superior in treatment containing moisture of 8% (3.81) and it was followed by treatment with moisture content 12% (1.25), 16% (0.00), 20% (0.00), 24% (0.00) and 28% (0.00) respectively.

Among dilution factor the maximum mean CFU count from all the treatments was observed at dilution factor 10^{-6} (1.67) followed by dilution factor 10^{-7} (1.00), 10^{-8} (0.71) and 10^{-9} (0.00) respectively.

The mean CFU count from the dilution factor of 10^{-8} from all the treatments revealed that highest mean CFU count was observed in treatment with moisture content of 8% (3.25.) followed by treatment with moisture content 12% (1.00), 16% (0.00), 20% (0.00), 24% (0.00) and 28% (0.00).

CFU Count after 255 Days of Storage

The data revealed that (Table 1) the mean CFU count was significantly superior in treatment containing moisture of 8% (1.19) and it was followed by treatment with moisture content 12% (0.00), 16% (0.00), 20% (0.00), 24% (0.00) and 28% (0.00) respectively.

Among dilution factor the maximum mean CFU count from all the treatments was observed at dilution factor 10^{-6} (0.50) followed by dilution factor 10^{-7} (0.29), 10^{-8} (0.00) and 10^{-9} (0.00) respectively.

The mean CFU count from the dilution factor of 10^{-8} from all the treatments revealed that highest mean CFU count was observed in treatment with moisture content of 8% (0.00) followed by treatment with moisture content 12% (0.00), 16% (0.00), 20% (0.00), 24% (0.00) and 28% (0.00).

The treatment with moisture content 8% and 12% moisture after 255 days of storage at a dilution factor of 10^{-8} did not showed any colony forming units indicating that *M. anisopliae* 1.15% WP with moisture content of 8% and 12% can be stored up to 210 days only.

However, *M. anisopliae* 1.15% WP with moisture content of 8% shows CFU at a dilution factor 10^{-6} (3.00) but, *M. anisopliae* 1.15% WP with moisture content of 12% doesn't show any CFU at any dilution factor.

CFU Count after 270 Days of Storage

Data from (Table 1) the mean CFU of *M. anisopliae* 1.15% WP after 270 days of storage didn't show any colony forming units at any dilution factor.

The current findings are in accordance with the results of Diedhiou *et al*., (2014) [5] who reported that dry spore formulations of *Metarhizium anisopliae* remained virulent for longer periods of time however, in the current study treatment with 8% moisture content showed higher storability than *M. anisopliae* stored with 20% moisture content.

The current findings are similar to the results of Jackson *et al*., (1995) [10] who found that storing fungus following air drying resulted in survival for 7 months. In the current study fungus can be preserved for 8 months by air drying it before storage at 8% moisture.

The current results corroborate with those of Hong *et al*., (1997) who found a negative logarithmic relationship between the moisture content of *M. anisopliae var. acridium* aerial conidia and their ability to survive thermal stress at 50 ºC. The moisture content and survival of *M. anisopliae* have a negative logarithmic relationship in current investigation also.

The current findings are in line with the results of Couch and Ignoffo (1981) [4] who recommended that formulations must be stable for at least one year. *M. anisopliae* was effectively stored for 8 months in the current investigation.

The current findings are not in agreement with results of Bell and Hamalle (1974) [2] who stated that *M. anisopliae* may be preserved for up to 3 years without measurable loss of viability. *M. anisopliae* was only viable for 8 months in our investigation.

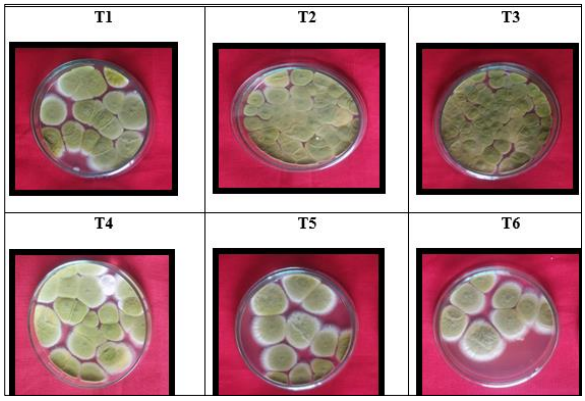

Plate 1: Storability study of *Metarhizium anisopliae* 1.15% WP at 120 days after storage (8%, 12%, 16%, 20%, 24% and 28% respectively)

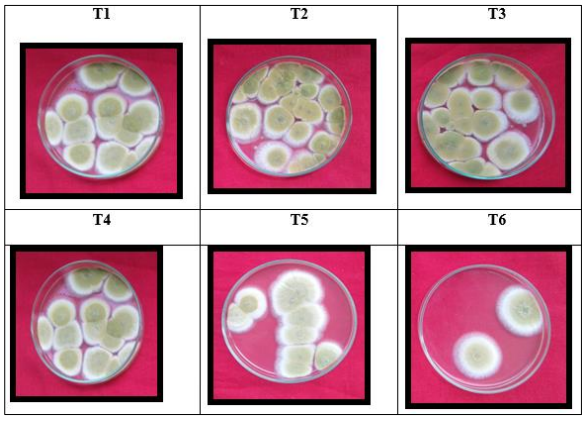

Plate 2: Storability study of *Metarhizium anisopliae* 1.15% WP at 150 days after storage (8%, 12%, 16%, 20%, 24% and 28% respectively)

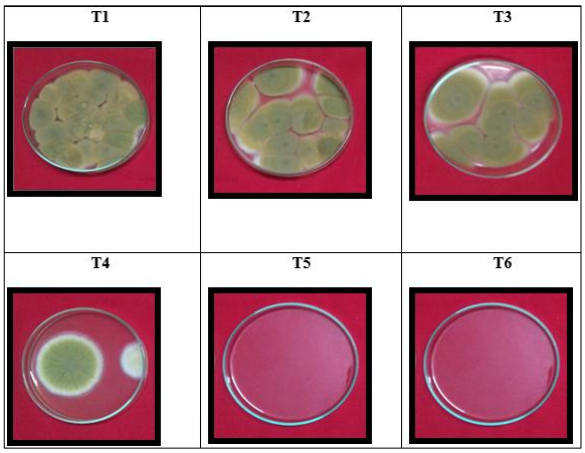

Plate 3: Storability study of *Metarhizium anisopliae* 1.15% WP after 180 days of storage (8%, 12%, 16%, 20%, 24% and 28% respectively)

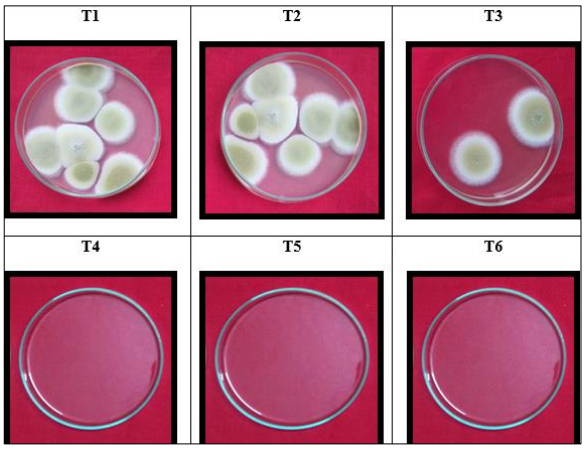

Plate 4: Storability study of *Metarhizium anisopliae* 1.15% WP after 210 days of storage (8%, 12%, 16%, 20%, 24% and 28% respectively)

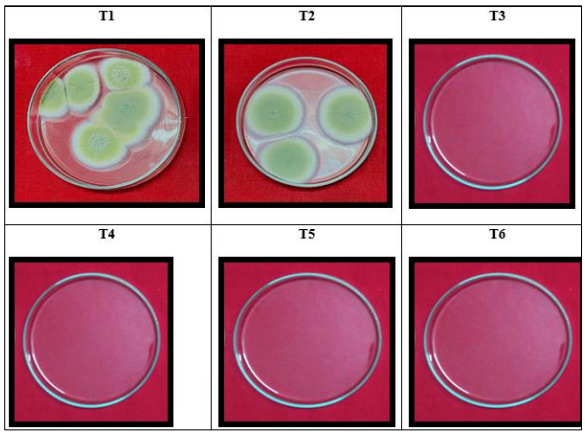

Plate 5: Storability study of *Metarhizium anisopliae* 1.15% WP after 225 days of storage (8%, 12%, 16%, 20%, 24% and 28% respectively)

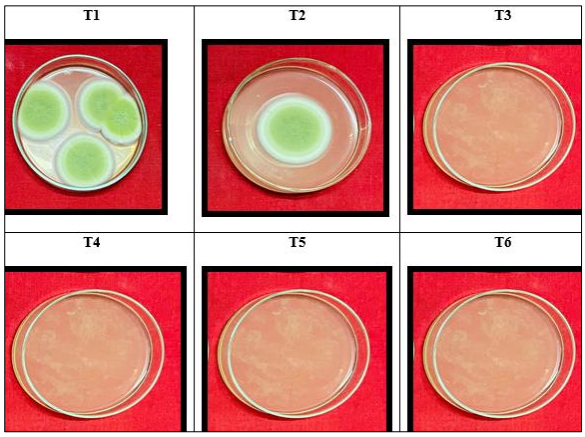

Plate 6: Storability study of Metarhizium anisopliae 1.15% WP after 240 days of storage (8%, 12%, 16%, 20%, 24% and 28% respectively

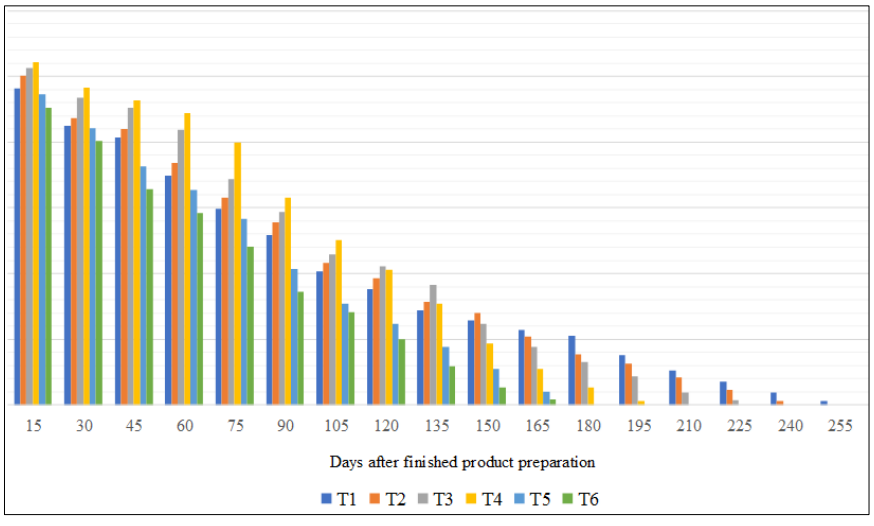

Fig 1: Storability of *Metarhizium anisopliae* 1.15% WP with different moisture levels at room temperature after 3 days of inoculation

Conclusion

Study on storability of *M. anisopliae* with different moisture content at ambient temperature indicates that the CFU count of *M. anisopliae* 1.15% initially was in increasing order from moisture content 8, 12, 16 and 20 per cent respectively and from moisture content 24 and 28 per cent CFU started decreasing. The sequence of CFU was same up to 105 days after finished product preparation. But CFU after 120 days after finished product preparation, 20 per cent moisture level which was highest up to 105 days declined and moisture level of 16 per cent was with highest CFU count which were followed by 20, 12, 8, 24 and 28 per cent. Later, at 150 days

12 per cent moisture content recorded highest CFU followed by 8, 16, 20, 24 and 28 per cent respectively. After 165 days onward highest CFU Count was observed in 8 per cent moisture level and it reported highest storage life of 255 days. Storability of *Metarhizium anisopliae* with different moisture content in decreasing order is 28% 150 days > 24% 165 days > 20% 180 days > 16% 210 days > 12% 240 days and 8% 240 days.

The results are giving clear indication that if *M. anisopliae* 1.15% WP finished product has to be stored for more than six months should be prepared with 8% moisture content. If the product is to be used earlier less than 4 to 5 months duration even more moisture content 20 to 24 per cent is also desirable as the highest CFU count is obtained in present investigation.

Storability study revealed that highest storage ability was with *Metarhizium anisopliae* 1.15% WP with a moisture content of 8 per cent and the lowest storage ability was of *Metarhizium anisopliae* with a moisture content of 28 per cent.

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