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Shelf-life assessment through CFU counts of liquid formulations of *Metarhizium anisopliae* at ambient temperature and cold storage

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Abstract
The results on assessment of self-life of nine liquid formulations of entomopathogenic fungus, *Metarhizium anisopliae*, revealed that the liquid formulations *viz*., Soybean oil (0.25%) + Tween-20 (0.01%) + *M. a*, Safflower oil (0.25%) + Tween-20 (0.01%) + *M. a*, Sunflower oil (0.25%) + Tween-20 (0.01%)+ *M. a*, Sesamum oil (0.25%) + Tween-20 (0.01%) + *M. a*, Peanut oil (0.25%) + Tween-20 (0.01%) + *M. a* and Coconut oil (0.25%) + Tween-20 (0.01%)+ *M. a* were viable up to 105 days. At cold storage (10 ºC) the liquid formulations based on Soybean oil (0.25%) + Tween-20 (0.01%) + *M. a*, Safflower oil (0.25%) + Tween-20 (0.01%) + *M. a*, Sunflower oil (0.25%) + Tween-20 (0.01%) + *M. a*, Sesamum oil (0.25%) + Tween-20 (0.01%) + *M. a*, Peanut oil (0.25%) + Tween-20 (0.01%)+ *M. a*, Palm oil (0.25%) + Tween-20 (0.01%) + *M. a*, were viable up to 120 days. CMC (0.5%) + Tween-20 (0.01%) + *M. a* and Coconut oil (0.25%) + Tween-20 (0.01%) + *M. a*, based liquid formulations were found to be durable up to 105 days.

Keywords: Storability, formulation, CMC, Oil, *Metarhizium anisopliae*, CFU, Liquid, Tween-20 etc

Introduction
The development of biological control agents is a major criterion for sustainable agriculture. While the formulation may improve product stability and viability, it may also reduce the uniformity of many biological control agents in the field. Biocontrol products for diseases (Biofungicides), weeds (bioherbicides) and insect (bioinsecticides) are developed. Many biocontrol agents contain dried milk, powdered casein, gelatin, saponins, oils and soaps. It is crucial in the case of microbial insecticides that the material used does not hinder infections from establishing themselves by causing premature growth or germination. As formulation agents, spreaders and wetting agents are also used. Wetting agents have included dry milk, powdered casein, gelatin, saponins, oils and soaps. The commercial development of a fungal biological control agent depends on its production and formulation, which must provide a cost-benefit to the end user, have a reasonable shelf-life (Remain viable and infective during storage) and deliver consistent insect control under field circumstances (Shah and Pell, 2003). In fact, many microbial pathogens that had performed well in the lab did not do as well in the field. The major causes are poor product stability during storage before application, inadequacy of active material reaching the target site and quick degradation of material after application owing to climatic factors. Dry storage is the greatest option for products that will be applied to foliage, while wet storage is optimal for product intended for soil application (Burges, 1998) [5]. The prepared product (Bio-Blast) of *M. anisopliae* has a two-year shelf life at room temperature (Rath *et al.*, 1995) [10]. In light of this, the current research is aimed at determining the feasibility of oil based liquid formulations of *Metarhizium anisopliae* (Metschnikoff) Sorokin for its storability at ambient temperature and cold storage condition.

Material and Methods
Fungal Culture
The Pure culture of *Metarhizium anisopliae* IPL/KC/44, Strain No- ITCC 6895 was obtained from the Biocontrol laboratory, Department of Agriculture Entomology, Post Graduate Institute, MPKV, Rahuri. Laboratory studies with 9 liquid formulations having three replications in completely randomized design were carried out in the Ecofriendly Pest and Disease Management Laboratory, Department of Agriculture Entomology, MPKV, Rahuri during 2020 to 2021.

1.15% w/v liquid formulations of *Metarhizium anisopliae*

The Fungal liquid formulations 1.15 % w/v were prepared by using with T_1- CMC (0.5%) + Tween-20 (0.01%)+ *M. a*, T_2- Coconut oil (0.25%) + Tween-20 (0.01%)+ *M. a*, T_3- Peanut oil (0.25%) + Tween-20 (0.01%)+ *M. a*, T_4- Safflower oil (0.25%) + Tween-20 (0.01%)+ *M. a*, T_5- Soybean oil (0.25%) + Tween-20 (0.01%)+ *M. a*, T_6- Sesamum oil (0.25%) + Tween-20 (0.01%)+ *M. a*, T_7- Palm oil (0.25%) + Tween-20 (0.01%)+ *M. a*, T_8- Sunflower oil (0.25%) + Tween-20 (0.01%)+ *M. a*, T_9- Sterile distilled water+ Tween-20 (0.01%)+ *M. a* (Control). The fungus *Metarhizium anisopliae* cultured on potato dextrose broth medium, incubated for 15 days at 25±2 ^{0}C. The 15 days old fully grown fungal mat was harvested in plastic container and blended with sterilized blender for 5 minutes. The respective fungal formulations were prepared by using grinded fungal mat of weight 11.5 grams mixing in 1 liter of sterilized distilled water and the suspension was vortexed for five minute to dissociate conidial clumps and filtered through one layer of cheese cloth to remove remaining clumps and mycelial debris and also known quantity of adjuvants were added along with fix quantity of Tween-20 (0.01%) (1ml/liter/suspension). The adjuvant CMC was used @ 5 grams and different oils @ 2.5 ml per liter of formulation. Likewise, all nine liquid formulations of strength 1.15% w/v were prepared and stored in sterilized plastic bottles of 1000 ml capacity.

Assessment of CFU count of liquid formulations of *Metarhizium anisopliae*

The furnished liquid formulations of 1.15 % w/v were prepared and 1000 ml of each liquid formulation were stored in freeze at 10 ºC and at ambient temperature in room to evaluate the shelf life of different liquids. The samples were stored at ambient temperature at 25-30 ºC ± 2 ºC and at cold storage (10 ºC). The CFU count of fresh prepared liquid formulation was taken on PDA media. 1 ml sample from each liquid formulation was used for serial dilution to test the viability of the product. The CFU count was recorded at 3 days after inoculation with the help of digital colony counter. The observations of CFU (10^8) count were taken at 15 days intervals. The experiment was conducted in CRD with 3 replication and 9 treatments and data were analyzed using standard statistical procedures.

Results and Discussion

A serial dilution technique (10^8 dilution factor) was used to determine the colony forming units at regular intervals of 15 days from the day of finished product preparation to evaluate the shelf life of different liquid formulations of *M. anisopliae*. One ml suspension from the stock (10^8) of different liquid formulations of *M. anisopliae* was taken and inoculated on PDA media to assess the colony forming units. The colony forming units were observed at 3rd day after inoculation and counted using a digital colony counter. The initial colony forming units were assessed at 1 day after preparation of the product. The shelf life of *M. anisopliae* liquid formulations was examined at 15, 30, 45, 60, 75, 90, 105, 120, and 135 days after storage at ambient and cold storage temperatures (10 ºC) as described in table 1, 2 and 3. After 135 days after storage the CFU count at ambient temperature and the product stored at 10 ^{0}C was zero i.e. no viable spores hence stopped further assessment.

Assessment of CFU count of different liquid formulations of *M. anisopliae*

Initial cfu count after finish product preparation

After preparing finished product, CFU counts were taken on next day. The data demonstrated that the mean CFU count in samples held at ambient and cold storage temperatures was non-significant

CFU count after 15 days of storage

The data in table 1 exhibited that the mean CFU count of Sunflower oil (0.25%) + Tween-20 (0.01%)+ *M. a* was significantly superior (93.33×10^8 cfu/ml) over distilled water based liquid formulation, However at par with Safflower oil (0.25%) + Tween-20 (0.01%)+ *M. a*, (90.33×10^8 cfu/ml), Palm oil (0.25%) + Tween-20 (0.01%)+ *M. a*, (88×10^8 cfu/ml), Peanut oil (0.25%) + Tween-20 (0.01%)+ *M. a*, (86×10^8 cfu/ml), CMC (0.5%) + Tween-20 (0.01%)+ *M. a*, (84×10^8 cfu/ml) and Sesamum oil (0.25%) + Tween-20 (0.01%)+ *M. a*, (83.67×10^8 cfu/ml), at ambient temperature.

At cold storage the data on mean CFU count of Soybean oil (0.25%) + Tween-20 (0.01%)+ *M. a*, and Sunflower oil (0.25%) + Tween-20 (0.01%)+ *M. a* (93.67×10^8 cfu/ml) was found significantly superior over distilled water based liquid formulation (87.33×10^8 cfu/ml) and was at par with Peanut oil (0.25%) + Tween-20 (0.01%)+ *M. a*, (93.67×10^8 cfu/ml), Palm oil (0.25%) + Tween-20 (0.01%)+ *M. a*, (92.33×10^8 cfu/ml), Safflower oil (0.25%) + Tween-20 (0.01%)+ *M. a*, (91.67×10^8 cfu/ml), Sesamum oil (0.25%) + Tween-20 (0.01%)+ *M. a*, (88.67×10^8 cfu/ml), CMC (0.5%) + Tween-20 (0.01%)+ *M. a*, (85.67×10^8 cfu/ml) and Coconut oil (0.25%) + Tween-20 (0.01%)+ *M. a*, (84.00×10^8 cfu/ml). The results clearly indicate that up to 15 days after finished product was prepared all the liquid formulations were superior.

CFU count after 30 days of storage

The data evidenced that the mean CFU count of Soybean oil (0.25%) + Tween-20 (0.01%)+ *M. a*, (92.33×10^8 cfu/ml) was found to be significantly superior over other treatments except Safflower oil (0.25%) + Tween-20 (0.01%)+ *M. a*, (87.67×10^8 cfu/ml) and Sunflower oil (0.25%) + Tween-20 (0.01%)+ *M. a* (87.67×10^8 cfu/ml) which were found at par with each other. The next effective treatment was Peanut oil (0.25%) + Tween-20 (0.01%) + *M. a*, with CFU count 80.67×10^8 cfu/ml at ambient temperature. The lowest number of CFU was recorded in distilled water based liquid formulation (44.33×10^8 cfu/ml), at ambient temperature.

At cold storage the data on mean CFU count revealed that Soybean oil (0.25%) + Tween-20 (0.01%)+ *M. a*, was found to be significantly superior over all the treatments with highest CFU count 96.33×10^8 cfu/ml except Safflower oil (0.25%) + Tween-20 (0.01%)+ *M. a*, (91×10^8 cfu/ml), and was at par with each other. The next best treatment was Sunflower oil (0.25%) + Tween-20 (0.01%) + *M. a* with CFU count 88×10^8 cfu/ml. The lowest number of CFU was recorded in Coconut oil (0.25%) + Tween-20 (0.01%) + *M. a*, (54×10^8 cfu/ml).

Table 1: CFU assessment of different liquid formulations of *M. anisopliae* at ambient temperature and cold storage (10°C) (From 0 to 30 days)

Tr. No.	Treatment	CFU count of *M. anisopliae* @ 10^8/ml after 3rd day after inoculation					
		0 days		15 days		30 days	
		Ambient Temp.	Cold storage	Ambient Temp.	Cold storage	Ambient Temp.	Cold storage
T_1	CMC (0.5%) + Tween-20 (0.01%)+ *M. a*	93.00 (9.67)*	94.33 (9.74)	84.00 (9.19)	85.67 (9.28)	72.67 (8.55)	78.33 (8.88)
T_2	Coconut oil (0.25%) + Tween-20 (0.01%)+ *M. a*	93.33 (9.69)	94.00 (9.72)	79.00 (8.90)	84.00 (9.19)	47.00 (6.88)	54.00 (7.38)
T_3	Peanut oil (0.25%) + Tween-20 (0.01%)+ *M. a*	93.67 (9.70)	94.00 (9.72)	86.00 (9.30)	93.67 (9.70)	80.67 (9.01)	85.33 (9.26)
T_4	Safflower oil (0.25%) + Tween-20 (0.01%)+ *M. a*	90.00 (9.51)	90.00 (9.51)	90.33 (9.53)	91.67 (9.60)	87.67 (9.39)	91.00 (9.56)
T_5	Soybean oil (0.25%) + Tween-20 (0.01%)+ *M. a*	95.67 (9.81)	95.33 (9.79)	93.00 (9.67)	93.67 (9.70)	92.33 (9.63)	96.33 (9.84)
T_6	Sesamum oil (0.25%) + Tween-20 (0.01%)+ *M. a*	92.33 (9.63)	92.67 (9.65)	83.67 (9.17)	88.67 (9.44)	62.33 (7.93)	70.67 (8.44)
T_7	Palm oil (0.25%) + Tween-20 (0.01%)+ *M. a*	93.00 (9.67)	93.33 (9.69)	88.00 (9.41)	92.33 (9.63)	69.00 (8.34)	75.33 (8.71)
T_8	Sunflower oil (0.25%) + Tween-20 (0.01%) + *M. a*	93.67 (9.70)	94.33 (9.74)	93.33 (9.69)	93.67 (9.70)	87.67 (9.39)	88.00 (9.41)
T_9	Sterile distilled water+ Tween-20 (0.01%) + *M. a* (Control)	94.33 (9.74)	94.67 (9.76)	78.33 (8.87)	87.33 (9.37)	44.33 (6.69)	77.67 (8.84)
	SE±	0.07	0.07	0.18	0.14	0.13	0.13
	CD at 5%	NS	NS	0.54	0.42	0.40	0.38

*Figures in parenthesis are √x+0.5 transformed values; 0.001% Tween- 20 was used as an emulsifier in each liquid formulation.

CFU count after 45 days of storage

The data revealed that the mean CFU count of Soybean oil (0.25%) + Tween-20 (0.01%)+ *M. a*, (89.33×10^8 cfu/ml) was found to be significantly superior over all the treatments except Safflower oil (0.25%) + Tween-20 (0.01%)+ *M. a*, (86×10^8 cfu/ml) and was at par with each other. The next best treatment was Sunflower oil (0.25%) + Tween-20 (0.01%) + *M. a* with CFU count 77.33×10^8 cfu/ml. The lowest number of CFU was recorded in distilled water based liquid formulation (26.67×10^8 cfu/ml), at ambient temperature.

At cold storage the data on mean CFU count revealed that Soybean oil (0.25%) + Tween-20 (0.01%)+ *M. a*, was found to be significantly superior over all the treatments with highest CFU count 94.00×10^8 cfu/ml. The next best treatment was Safflower oil (0.25%) + Tween-20 (0.01%) + *M. a*, with CFU count 88×10^8 cfu/ml. The lowest number of CFU was recorded in Coconut oil (0.25%) + Tween-20 (0.01%) + *M. a*, (42.67×10^8 cfu/ml). The results clearly indicates that at 45 days after storage the CFU count was more in cold storage than ambient temperature in all the liquid formulations (table 2).

CFU count after 60 days of storage

The data revealed that the mean CFU count of Soybean oil (0.25%) + Tween-20 (0.01%)+ *M. a*, (87.67×10^8 cfu/ml) was found to be significantly superior over all the treatments except Safflower oil (0.25%) + Tween-20 (0.01%)+ *M. a*, (82×10^8 cfu/ml) which was at par with each other. The next best treatment was Sunflower oil (0.25%) + Tween-20 (0.01%)+ *M. a* with CFU count 72.00×10^8 cfu/ml. The lowest number of CFU was recorded in distilled water based liquid formulation (2.67×10^8 cfu/ml) at ambient temperature.

At cold storage the data on mean CFU count revealed that Soybean oil (0.25%) + Tween-20 (0.01%) + *M. a*, was found to be significantly superior over all the treatments with highest CFU count 91.67×10^8 cfu/ml. The next best treatment was Safflower oil (0.25%) + Tween-20 (0.01%) + *M. a*, with CFU count 83×10^8 cfu/ml. The lowest number of CFU was recorded in distilled water based liquid formulation 12.33×10^8 cfu/ml (table 2).

CFU count after 75 days of storage

The data revealed that the mean CFU count of Soybean oil (0.25%) + Tween-20 (0.01%) + *M. a*, (75×10^8 cfu/ml) was found to be significantly superior over all the treatments. The next best treatment was Sunflower oil (0.25%) + Tween-20 (0.01%) + *M. a* with CFU count 60×10^8 cfu/ml. The lowest number of CFU was recorded in Coconut oil (0.25%) + Tween-20 (0.01%) + *M. a*, 23.33×10^8 cfu/ml. Colony forming units were not observed in distilled water based liquid formulation at ambient temperature, indicating storage life of liquid based formulations without oil at ambient temperature was two and half months only.

At cold storage the data on mean CFU count revealed that Soybean oil (0.25%) + Tween-20 (0.01%)+ *M. a*, was found to be significantly superior over all the treatments with highest CFU count 88×10^8 cfu/ml. The next best treatment was Safflower oil (0.25%) + Tween-20 (0.01%) + *M. a*, with CFU count 79.67×10^8 cfu/ml. The lowest number of CFU was recorded in distilled water based liquid formulation 3.33×10^8 cfu/ml (table 2).

Table 2: CFU assessment of different liquid formulations of *M. anisopliae* at ambient temperature and cold storage (10ºC) (From 45 to 75 days)

Tr. No.	Treatment	CFU count of *M. anisopliae* @ 10^8/ml after 3rd day after inoculation					
		45 days		60 days		75 days	
		Ambient Temp.	Cold storage	Ambient Temp.	Cold storage	Ambient Temp.	Cold storage
T_1	CMC (0.5%) + Tween-20 (0.01%)+ *M. a*	58.00 (7.65)	64.00 (8.03)	44.33 (6.69)	50.00 (7.11)	35.33 (5.98)*	40.00 (6.36)
T_2	Coconut oil (0.25%) + Tween-20 (0.01%)+ *M. a*	36.33 (6.07)	42.67 (6.57)	27.00 (5.24)	33.00 (5.78)	23.33 (4.88)	30.67 (5.58)
T_3	Peanut oil (0.25%) + Tween-20 (0.01%)+ *M. a*	70.33 (8.42)	79.00 (8.92)	69.00 (8.34)	77.00 (8.80)	56.33 (7.54)	70.00 (8.40)
T_4	Safflower oil (0.25%) + Tween-20 (0.01%)+ *M. a*	86.00 (9.30)	88.00 (9.41)	82.00 (9.08)	83.00 (9.14)	39.67 (6.33)	79.67 (8.95)
T_5	Soybean oil (0.25%) + Tween-20 (0.01%)+ *M. a*	89.33 (9.48)	94.00 (9.72)	87.67 (9.39)	91.67 (9.60)	75.00 (8.69)	88.00 (9.41)
T_6	Sesamum oil (0.25%) + Tween-20 (0.01%)+ *M. a*	49.33 (7.06)	53.33 (7.34)	41.00 (6.44)	47.00 (6.89)	31.33 (5.64)	37.67 (6.18)
T_7	Palm oil (0.25%) + Tween-20 (0.01%)+ *M. a*	54.00 (7.38)	57.67 (7.63)	33.00 (5.79)	39.33 (6.31)	25.67 (5.11)	32.33 (5.73)
T_8	Sunflower oil (0.25%) + Tween-20 (0.01%)+ *M. a*	77.33 (8.82)	79.67 (8.95)	72.00 (8.51)	77.67 (8.84)	60.00 (7.78)	72.67 (8.55)
T_9	Sterile distilled water+ Tween-20 (0.01%)+ *M. a* (Control)	26.67 (5.21)	48.67 (7.01)	2.67 (1.74)	12.33 (3.57)	0.00 (0.71)	3.33 (1.95)
	SE±	0.09	0.07	0.13	0.10	0.10	0.07
	CD at 5%	0.28	0.22	0.38	0.30	0.30	0.22

*Figures in parenthesis are √x+0.5 transformed values; 0.001% Tween- 20 was used as an emulsifier in each liquid formulation.

CFU count after 90 days of storage

The data revealed that the mean CFU count of Soybean oil (0.25%) + Tween-20 (0.01%)+ *M. a*, (25.67×10^8 cfu/ml) was found to be significantly superior over all the treatments except Safflower oil (0.25%) + Tween-20 (0.01%)+ *M. a*, (23.67×10^8 cfu/ml) which was at par with each other. The next best treatment was Peanut oil (0.25%) + Tween-20 (0.01%) + *M. a*, with CFU count 20×10^8 cfu/ml. The lowest number of CFU was recorded in Palm oil (0.25%) + Tween-20 (0.01%) + *M. a*, (5.67×10^8 cfu/ml) at ambient temperature. Colony forming units were not observed in distilled water based liquid formulation at ambient temperature.

At cold storage the data on mean CFU count revealed that Soybean oil (0.25%) + Tween-20 (0.01%)+ *M. a*, was found to be significantly superior over all the treatments with highest CFU count 43.33×10^8 cfu/ml. The next best treatment was Sunflower oil (0.25%) + Tween-20 (0.01%) + *M. a* with CFU count 37×10^8 cfu/ml. The lowest number of CFU was recorded in Palm oil (0.25%) + Tween-20 (0.01%) + *M. a*, 12.67×10^8 cfu/ml. In a liquid formulation based on distilled water, no colony forming units were found (table 3). The results showed clear indication that after 3 months of storage of liquid based formulation lost its viability at cold temperature i.e. 10ºC also.

CFU count after 105 days of storage

The data revealed that the mean CFU count of Soybean oil (0.25%) + Tween-20 (0.01%)+ *M. a*, (6.33×10^8 cfu/ml) was found to be significantly superior over all the treatments except Safflower oil (0.25%) + Tween-20 (0.01%)+ *M. a*, (6×10^8 cfu/ml) which was at par with each other. The next best treatment was Sunflower oil (0.25%) + Tween-20 (0.01%) + *M. a* with CFU count 4.67×10^8 cfu/ml. No colony forming units were observed in distilled water, CMC and Palm oil (0.25%) + Tween-20 (0.01%) + *M. a*, at ambient temperature, indicated that product lost its viability.

At cold storage the data on mean CFU count revealed that Soybean oil (0.25%) + Tween-20 (0.01%)+ *M. a*, was found to be significantly superior over all the treatments with highest CFU count 28.67×10^8 cfu/ml. The next best treatment was Sunflower oil (0.25%) + Tween-20 (0.01%) + *M. a* with CFU count 23.67×10^8 cfu/ml. The lowest number of CFU was recorded in Coconut oil (0.25%) + Tween-20 (0.01%)+ *M. a*, 3×10^8 cfu/ml. No colony forming units was observed in distilled water based liquid formulation (table 3).

CFU count after 120 days of storage

The data revealed that the mean CFU count was found to be non-significant in samples keep at ambient temperature. No colony forming units was observed in all liquid formulations of *M. anisopliae*. The results are given clear indication that at ambient temperature all the oil based liquid formulations also lost their viability.

At cold storage the data on mean CFU count revealed that Soybean oil (0.25%) + Tween-20 (0.01%)+ *M. a*, (5.67×10^8 cfu/ml) was found to be significantly superior over all the treatments except Sunflower oil (0.25%) + Tween-20 (0.01%)+ *M. a* (5.33×10^8 cfu/ml), Sesamum oil (0.25%) + Tween-20 (0.01%)+ *M. a*, (5×10^8 cfu/ml) and Peanut oil (0.25%) + Tween-20 (0.01%)+ *M. a*, (5×10^8 cfu/ml) which were at par with each other. The next best treatment was Safflower oil (0.25%) + Tween-20 (0.01%) + *M. a*, with CFU count 4.67×10^8 cfu/ml. The lowest number of CFU was recorded in Palm oil (0.25%) + Tween-20 (0.01%) + *M. a*, (3.67×10^8 cfu/ml). Colony forming units was not observed in CMC, distilled water and Coconut oil (0.25%) + Tween-20 (0.01%) + *M. a*, (table 2).

CFU count after 135 days of storage

Colony forming units was not observed in all liquid formulations of *M. anisopliae*. The data revealed that the mean CFU count was found to be non-significant in samples keep at ambient and cold storage temperature. All the liquid formulations prepared lost their viability after 4 and half

months of cold storage at 10 ºC.

The overall results on storability studies on different liquid formulation of *M. anisopliae* at ambient storage temperature clearly indicated that soybean, safflower, safflower, sesamum, peanut and Coconut oil (0.25%) + Tween-20 (0.01%)+ *M. a*, s were viable up to three and half months from the day of finished product preparation with better colony forming units. The shelf life of distilled water based liquid formulation was two months while shelf life of palm oil and CMC (0.5%) + Tween-20 (0.01%) + *M. a*, were three months. After the 105th day of storage, no colony forming units were found in any of the liquid formulations indicating that they lost their viability.

At cold storage (10ºC) the overall results on storability studies of different liquid formulations revealed that soybean, sunflower, peanut, safflower, sesamum and Palm oil (0.25%) + Tween-20 (0.01%)+ *M. a*, s were viable up to 120th days i.e. 4 months from the day of finished product preparation. CMC and Coconut oil (0.25%) + Tween-20 (0.01%) + *M. a*, were found to be durable up to 105th days. Distilled water based liquid formulation was durable up to 75th days at 10^{0}C. After the 120th day of storage, no colony forming units were found in any of the liquid formulations indicating that they lost their viability.

Table 3: CFU assessment of different liquid formulations of *M. anisopliae* at ambient temperature and cold storage (10^{0}C) (From 90 to 120 days)

Tr. No.	Treatment	CFU count of *M. anisopliae* @ 10^{8}/ml after 3rd day after inoculation					
		90 days		105 days		120 days	
		Ambient Temp.	Cold storage	Ambient Temp.	Cold storage	Ambient Temp.	Cold storage
T_1	CMC (0.5%) + Tween-20 (0.01%)+ *M. a*	9.00 (3.08)	19.00 (4.41)	0.00 (0.71)	8.67 (3.03)	0.00 (0.78)	0.00 (0.78)
T_2	Coconut oil (0.25%) + Tween-20 (0.01%)+ *M. a*	7.00 (2.73)	13.33 (3.72)	1.00 (1.22)	3.00 (1.87)	0.00 (0.78)	0.00 (0.78)
T_3	Peanut oil (0.25%) + Tween-20 (0.01%)+ *M. a*	20.00 (4.53)	31.33 (5.64)	4.33 (2.20)	18.67 (4.38)	0.00 (0.78)	5.00 (2.35)
T_4	Safflower oil (0.25%) + Tween-20 (0.01%)+ *M. a*	23.67 (4.92)	30.67 (5.58)	6.00 (2.55)	14.33 (3.85)	0.00 (0.78)	4.67 (2.27)
T_5	Soybean oil (0.25%) + Tween-20 (0.01%)+ *M. a*	25.67 (5.11)	43.33 (6.62)	6.33 (2.61)	28.67 (5.40)	0.00 (0.78)	5.67 (2.48)
T_6	Sesamum oil (0.25%) + Tween-20 (0.01%)+ *M. a*	8.33 (2.96)	17.33 (4.22)	1.00 (1.22)	7.67 (2.86)	0.00 (0.78)	5.00 (2.34)
T_7	Palm oil (0.25%) + Tween-20 (0.01%)+ *M. a*	5.67 (2.48)	12.67 (3.63)	0.00 (0.71)	4.00 (2.12)	0.00 (0.78)	3.67 (2.03)
T_8	Sunflower oil (0.25%) + Tween-20 (0.01%)+ *M. a*	19.33 (4.45)	37.00 (6.12)	4.67 (2.27)	23.67 (4.92)	0.00 (0.78)	5.33 (2.41)
T_9	Sterile distilled water+ Tween-20 (0.01%)+ *M. a* (Control)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.78)	0.00 (0.78)
	SE±	0.08	0.06	0.04	0.04	0.00	0.05
	CD at 5%	0.25	0.18	0.12	0.12	NS	0.15

*Figures in parenthesis are √x+0.5 transformed values; 0.001% Tween- 20 was used as an emulsifier in each liquid formulation.

The current finding on storability of different liquid formulation (1×10^{8} conidia/ml) of *M. anisopliae* are in agreement with Patil and Jadhav (2017) who observed that the viability of CMC (0.5%) + Tween-20 (0.01%)+ *M. a*, of *M. anisopliae* was more and yield maximum colony forming units (18.33×10^{8} conidia/ml). Bharani and Namasivayam (2016) who observed that among the fungal oil formulation based sunflower oil, groundnut oil, neem oil, castor seed oil, diesel and water, Sunflower oil (0.25%) + Tween-20 (0.01%) + *M. a* had the maximum number of fungal colonies, indicating 12.0×10^{8}, 43.2×10^{8} and 44.1×10^{8} cfu/ml fungal count at 10, 20 and 30 days after post treatment at ambient temperature. Batta (2003) who observed that the invert emulsion (water-in-oil formulation) of conidia of the entomopathogenic fungus *Metarhizium anisopliae* were formulated with a coconut and soybean oil preparation providing the most stable emulsion layer (93 per cent) and lowest viscosity (27 cps). The fungal conidia remained viable in the formulation for 30.8 months with a 50% reduction (half-life) in conidial viability after 4.6 months at 20±1 ^{0}C. In present findings the liquid formulations lost its viability in 4 and half months. Bhanu Prakash *et al.* (2015) [3] who studied seven oil formulations and two powdered formulations of *M. anisopliae* for shelf life at 4 °C, revealed 60 per cent viability of conidial formulation with gingelly oil, stored for six months. The viability of conidia recoded a decline at room temperature and relative stability at -30 ºC. The present findings were carried out at 10 ºC temperature and ambient temperature. Daoust *et al.* (1983) [6] who reported that the inoculums of *M. anisopliae* formulated in oils increased the efficacy of pathogen and prolonged viability of conidia are in agreement with present results. Ibrahim *et al.* (2015) [8] found that conidial viability of the oil employed in the formulations steadily reduced over time at varying rates. Conidia viability was higher at 4 ºC than at ambient storage temperature and constant 28 °C for all liquid formulations tested. Dry conidia and conidia formulated and stored in different oils at 4 ^{0}C for 16 weeks maintained viability above 80% Olive oil and recycled vegetable oil had deleterious impact on conidial survival at 28 ºC after 12 weeks of storage and after 16 weeks of storage at room temperature. Hazarika *et al.* (2017) [7] found that oil based formulation of *M. anisopliae* **(**Org-Metajal) can be stored up to 30 months in deep freeze conditions, 22 months in freeze temperature and 18 months in room temperature. The present findings were carried out at 10ºC temperature and ambient temperature. The current

findings are not in agreement with Alves *et al.* (2002) [1] who reported that oil-based liquid formulations of *M. anisopliae* stored at 10 ºC were viable up to 40 weeks, did not resulted in any significant difference in viability and they remained above 91% viability of conidia in medium-term storage.

Conclusion

Shelf life of liquid formulations based on Soybean oil (0.25%) + Tween-20 (0.01%)+ *M. a*, Safflower oil (0.25%) + Tween-20 (0.01%)+ *M. a*, Sunflower oil (0.25%) + Tween-20 (0.01%)+ *M. a*, Sesamum oil (0.25%) + Tween-20 (0.01%)+ *M. a*, Peanut oil (0.25%) + Tween-20 (0.01%)+ *M. a*, Palm oil (0.25%) + Tween-20 (0.01%)+ *M. a*, were viable up to 120 days in cold storage, while at ambient temperature the liquid formulations based on Soybean oil (0.25%) + Tween-20 (0.01%)+ *M. a*, Safflower oil (0.25%) + Tween-20 (0.01%)+ *M. a*, Sunflower oil (0.25%) + Tween-20 (0.01%)+ *M. a*, Sesamum oil (0.25%) + Tween-20 (0.01%)+ *M. a*, Peanut oil (0.25%) + Tween-20 (0.01%)+ *M. a* and Coconut oil (0.25%) + Tween-20 (0.01%)+ *M. a* were viable up to three and half months. At cold storage, highest viable count was observed in Soybean oil (0.25%) + Tween-20 (0.01%) + *M. a* based liquid formulation.

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