Original Research Article

PERFORMANCE OF *Beauveria bassiana* (Balsamo) Vuillemin STORED AS OIL CULTURES UNDER DIFFERENT TEMPERATURE REGIMES

ABSTRACT:

Oil cultures of *Beauveria bassiana* (Balsamo) Vuilleminwere prepared from the laboratory-maintained isolate by using two vegetable oils*viz.,*Rice Bran oil, Sesamum oil and two mineral oils*viz.,* Liquid Paraffin oil and Heavy grade Mineral Oil. Prepared Oil cultures were stored under three different temperature regimes *viz.,* at room temperature (28 ± 3^{0}C), refrigerated condition (4^{0}C) and freezing temperature (-20^{0}C). These 12 cultures stored under different temperatures along with crude culture and untreated control were evaluated for viability and virulence against third instar *Spodoptera litura*(Fabricius) larva on the day of preparation and upto 180 days under laboratory conditions.Among all oil cultures, Sesamum oil cultures and Liquid Paraffin oil cultures stored at -20^{0}C performed well with highest conidial germination of 87.85% and 83.57% and highest larval mortality of 90.00% and 86.67% on the day of preparation (0 DAS). After 180 DAS of storage, for the above two formulations conidial germination was in the range of 62.87% to 61.43% and larval mortality was60.00%. Comparatively, least performance was observed in rice bran oil formulation stored at room temperature with conidial germination and larval mortality of 77.14% and 80.00% (0 DAS) and 41.43% and 40.00% (180 DAS) respectively.

Usage of oils for the preservation of entomopathogenic fungal based formulations prevent conidial desiccation and improve adhesion and afford protection to fungal conidia from the UV of sunlight improving field performance.

Keywords:

Beauveriabassiana, oil cultures, vegetable oils, mineral oils, different temperature regimes, storage, viability, virulence.

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Comment [mm2]: You need to consider this sentence, because this paragraph is an abstract, not a discussion.

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Only based on literature: Batta, 2003 & Moore et al., 1993).

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1. INTRODUCTION

According to FAO's 2022 report, India consumedover 61,000 tonnes (t) of pesticides in 2020(FAO, 2022). These pesticides in addition to creating pesticide residues, environmental pollution issues and other issues, the widespread use of synthetic pesticides inevitably lead to the development of insecticide resistance, harmful effect on natural enemies, insecticide-induced resurgence of the pests and biodiversity decline (Dong *et al*., 2021; Waqas *et al*., 2021; Guo *et al*., 2020).Considering these limitations, environmentally benign pest management methods have become increasingly popular to rebuild the balance of cropland ecosystems for the sustainable control of crop pests(Yan li *et al.,* 2022). Pest management by biocontrol agents are assuming prominence and have been considered as an important strategy in insect population reduction (Ummidi and Vadlamani, 2014). Among biocontrol agents, microbial pathogens such as bacteria, fungi, viruses, nematodes and protozoans are promising agents for the effective control of insects.

Entomopathogenic fungi are regarded as the most significant among the numerous microbial agents due to their wide host range, eco-friendly nature, effectiveness against target pest *etc.* They are cheaper in long run, show lesser residual effects, and are able to overcome the problem of resistance (Sharma *et al.,* 2023). Entomopathogenic fungi are a specialized group of soil-dwelling microbial organisms that infects and kill insects and other arthropods through cuticle penetration, with traits and modes of action that render them as effective biopesticides that they play a crucial role in insect pest management (Mantzoukas*et al*., 2022). EPF is advantageous in pest control because direct ingestion of fungal propagules is not needed by insects, thus also becoming active against the non-feeding stages of insects (Bateman *et al.,* 1998). They cause insect mortality by nutritional deficiency, destruction of tissues and by release of toxins. The entry of entomopathogenic fungi through the insect cuticle occur by a combination of mechanical pressure and enzymatic degradation (Ramanujam *et al.,* 2014). They have the ability to synthesize extracellular cuticle-degrading enzymes like chitinase, protease and lipase that work together to overcome the chitinous and proteinaceous components of the insect cuticle, penetrating the germ tube inside thus, enabling hyphal access to the haemolymph and colonizing the insects to death(Gebremariam *et al*., 2022; Seema *et al.,* 2013; Beys-da-Silva *et al*., 2014).As, they have a complex mode of action that makes it difficult for pests to develop resistance against them (Dusfour, 2019).

Several species of entomopathogenic fungi have now been industrialized (de Faria and Wraight, 2007).According to a recent report, out of 171 commercial EPF products, majority of them were based on*Beauveria bassiana* (33.9 %) followed by *Metarhizium. anisopliae* (33.9 %), *Isaria fumosorosea* (5.8%) and *B. brongniartii* (4.1%) (Islam *et al.,* 2021). *Beauveria bassiana* is the asexual form (anamorph) of *Cordyceps bassiana* fungus that infects a huge variety of insects, which is used to control crop infestations caused by aphids, thrips and whiteflies (Money, 2016) as well as lepidopteran pests (Singh, 2015). Among the cyclic hexadepsipeptide mycotoxins produced by the different EPF, beauvericin produced by them have shown the most effective larvicidal properties (Wang *et al*., 2012). Even though, effective strains were isolated there was still problem with the development of suitable cost effective formulations which could overcome harsh environmental conditions and ensure biological and chemical stability as well as viability during storage for extended periods (Rachappa *et al*., 2005).

One among the technique is the usage of oils for the preservation of entomopathogenic fungi based formulations. It has been suggested that oil formulations can prevent conidial desiccation and improve adhesion of conidia to the hydrophobic surface of insect cuticle and enabling the opportunistic spread of conidia in to high humidity locations such as the inter-segmental membranes (Inyang *et al*., 2000; Devi and Prasad, 1996; Bateman *et al.,* 1993). In field conditions, higher temperature, lower humidity and Ultraviolet radiation (UV) exposure pose detrimental effectson fungal conidia. This situation warrants shift to the use of oil based formulations which showed good control of insect pests under field condition (Batta, 2003). Oil affords protection to fungal conidia from the UV of sunlight thereby improvement in the field performance (Moore *et al*., 1993).

While, cryoprotectant preservation at -196^{0}C submerged in liquid nitrogen following freezing at a controlled rate is generally accepted as the optimal storage technique for preserving both cell viability and biological properties *i.e.* infectivity and virulence, it is considerably expensive and time consuming which is not affordable by many laboratories (Humber, 1997; Lastra *et al*., 2002).

Maintenance at optimum temperature for long term preservation of EPF culture is of atmost importance.

Therefore, its necessary for the development of suitable and affordable formulation technologies for the proper maintenance and preservation of different entomopathogenic fungal cultures. There have been relatively fewer studies conducted on optimising the storage conditions for *Beauveriabassiana* oil cultures, which could remain viable for extended periods and be suitable for later retrival. Therefore, the present study was undertaken to evaluate the performance of *B. bassiana* stored as oil cultures under different temperature regimes.

2. MATERIAL AND METHODS

Place and duration of study: Insect Pathology laboratory, Central Instrumentation Laboratory and Insectary, Department of EntomologySri Venkateswara Agricultural College, Tirupati during between September, 2023 and April, 2024**.**

2.1 Preparation of *Beauveria bassiana*oil cultures

For preparation of oil cultures, 4 oils were choosen *viz.,* 2 vegetable oils (Rice bran oil, Sesamum oil) and 2 mineral oils (Heavy grade Mineral oil, Liquid paraffin oil). Saboraud's Dextrose Agar with Yeast Extract Medium (SDAY). was prepared, poured into test tubes, autoclaved at 121°C at 15 Psi for 15 minutes and made as slants. After slants got cooled down, discs or loop of spores and mycelia of laboratory maintained culture of *B.bassiana* were inoculated into slants under aseptic condition and incubated at 25 ± 2°C temperature. Ten to twelve days after inoculation, satisfactory growth and sporulation of *B.bassiana*were obtained in atmost all slants(Fig. 1). Each fungal slant was poured with autoclaved vegetable and mineral oils *viz.,*Rice bran oil, Sesamum oil, Mineral oil and Liquid paraffin oil respectively until the entire fungi grown in slant media got submerged with oil(Fig. 2).

Fig. 1. Slant culture of *B. bassiana*

Fig. 2.*B. bassiana* oil cultures

2.2 Storage of *Beauveria bassiana*oil cultures at different temperature regimes

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The prepared oil cultures of *B. bassiana* with Rice bran oil, Sesamum oil, Mineral oil and Liquid paraffin oil were maintained at room temperature (28 ± 3°C), refrigerated condition (4^0C) in Refrigerator at Insect Pathology laboratory and freezing temperature (-20^0C) in Deep Freezer at Central instrumentation Lab of the college.

A total of treatments 14 treatments (including control) of *B. bassiana* oil cultures were prepared.

T_1:Mineral oil culture of *B. bassiana* stored at Room temperature (28 ± 3°C)
T_2:Mineral oil culture of *B. bassiana* stored at Refrigerator temperature (4^0C)
T_3:Mineral oil culture of *B. bassiana* stored at freezing temperature (-20^0C)
T_4:Liquid paraffin oil culture of *B. bassiana* stored at Room temperature (28 ± 3°C)
T_5:Liquid paraffin oil culture of *B. bassiana* stored at Refrigerator temperature (4^0C)
T_6:Liquid paraffin oil culture of *B. bassiana* stored at freezing temperature (-20^0C)
T_7:Rice bran oil culture of *B. bassiana* stored at Room temperature (28 ± 3°C)
T_8:Rice bran oil culture of *B. bassiana* stored at Refrigerator temperature (4^0C)
T_9:Rice bran oil culture of *B. bassiana* stored at freezing temperature (-20^0C)
T_{10}:Sesamum oil culture of *B. bassiana* stored at Room temperature (28 ± 3°C)
T_{11}:Sesamum oil culture of *B. bassiana* stored at Refrigerator temperature (4^0C)
T_{12}:Sesamum oil culture of *B. bassiana* stored at freezing temperature (-20^0C)
T_{13}:Unformulated culture of *B. bassiana* (Not preserved in oil)
T_{14}:Untreated control

2.3 Evaluation of *B. bassiana* oil cultures stored under different temperature regimes

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The viability of *B. bassiana*conidia and its efficacy was studied on the day of preparation (0 DAS) and at monthly intervals upto 180 days (DAS).

2.3.1 Assessment of viability of oil cultures

Serial dilutions of 1 x 10^5 spores ml^{-1} of all oil cultures were employed for viability experiments. Spore count was taken with the help of haemocytometer. Two to three drops of spore suspension were placed in the cavity slide. The cavity slide was prepared by arranging moistened cotton in petriplates and incubated in the incubator at 22^0C. After 24 hrs, the spore suspension was observed under microscope for counting total number of spores and number of germinated spores. The germination percentage of *Beauveria bassiana* conidia at different treatments were calculated.

2.3.2 Assessment of virulence of oil cultures against third instar *Spodoptera litura* larvae

From each treatment, 0.5 ml of oil culture was poured into 100 ml of distilled water and added with 100 µl of Triton- X 100 (0.1 %) into conical flasks to make spray suspension. Triton- X 100 (0.1 %) as a wetting agent for uniform dispersion of spores with oil.Spore suspensions of 1×10^7 spores per ml were standardized after assessing the number of spores in the suspension with an improved Neubauer haemocytometer. Spray suspensions were sprayed on castor leaves with atomizer and air dried. Freshly moulted third instar larvae were allowed to feed on them. The treatments were replicated thriceto confirm the reproducibility of the results. The bioassays were conductedatroom temperature under laboratory conditions. From next day, larvae were fed with fresh castor leaves. Daily observations on post treatment changes in larvae and larval mortality were recorded.

2.4 Analysis of the Data

All the recorded observations *i.e.* conidial viability and larval mortality were converted to percentage values. The percentage values were converted to angular transformed values. The observations were statistically analysed by using SPSS v 16 software. Data were subjected to analysis of variance (ANOVA) at P<0.01 level of significance. Means were compared by Duncan's Multiple Range Test (DMRT)(Saheb *et al.,* 2021).

The conidial germination was expressed as per cent conidial viability by using the formula;

$$\text{Per cent conidia viability} = \frac{\text{Number of conidia germinated}}{\text{Total conidia}} \times 100$$

The larval mortality was expressed as per cent larval mortality by using the formula;

$$\text{Per cent larval mortality} = \frac{\text{Number of larvae dead due to infection}}{\text{Total number of larvae treated}} \times 100$$

3. RESULTS AND DISCUSSION

A total of twelve types of oil cultures were prepared by using four oils and these cultures were maintained at three different temperatures. Additionally, an unformulated crude suspension of *Beauveria bassiana* and untreated control were maintained. The post treatment larval changes and the results of viability and virulence were presented below.

After releasing the larvae onto treated leaves, it was observed that there was a noticeable reduction in their tendency to feed, particularly on leaves treated with sesame oil.When the larvae were fed with fresh leaves the day after treatment, they exhibited a reduced inclination to feed. The room temperature (28 ± 3°C) was highly conducive for mycosis development. Freshly moulted 3rd instar larvae were significantly moresusceptible to EPF treatments compared to late 3rd and 4th instar *S. litura* larvae.After 3-4 days of treatment with *B. bassiana,* theinfected larvae exhibitedsluggishmovement andconsumedlessfoodmaterial.Thelarvalintegumentgot shrunken and larval bodies had become exceedingly smooth. Majority of larvae were dead within 1 week of inoculation with fungal spores. There was sparse growth of fungal mycelium on the surface of larval integument and whitish mycelial growth of fungus was conspicuous from 4th day after inoculation. The entire larval body was covered with puffy whitish mycelia and whitish fungal spores was produced on to larval surface.As the time progressed, the puffiness of the fungus on the larval body reduced gradually. The larval body eventually turned into a hard and mummified cadaver. The stiffness of the cadaver after death might be due to excessive fungal growth inside larval body.Larval mortality occurred more rapidly within 5-6 days with oil formulations, compared to 8-10 days with unformulated crude suspensions. The results were in agreement with Ummidi and Vadlamani (2014) who conducted bioassay studies of oil formulations of *B. bassiana* against *S.litura.* Based on compatibility studies,almond oil, olive oil, gingelly oil, castor oil based formulations were chosen for bioassay. He reported that all the four formulations displayed higher mortalities of the target pest compared to unformulated conidia, with *B. bassiana* showed higher mortality with gingelly oil and almond oil.

In the present study it was observed that, the infected larvae progressed to next instar and pupated earlier compared to untreated larvae *i.e.* significant decrease in larval period was observed due to infection in comparison with untreated control. Some affected larvae metamorphised to malformed pupae and adult.These results corroborate with the findings of Torrado-Leon *et al.* (2006) who documented the interference in the moulting process of *Bemisia tabacci*(Gennadius) nymphs when treated with *B. bassiana.* More than 30% of the imagos emerged from treated nymphs were unable to detach completely from the exuvium.

It was noticed that on the day of preparation, the highest mean per cent conidial viability of 87.85% was recorded in Sesamum oil cultures (T_{10}, T_{11}, T_{12}), followed by Liquid paraffin oil cultures (T_4, T_5, T_6) and Rice bran oil cultures (T_7, T_8, T_9) which recorded 83.57% and 80.71% respectively (Fig. 3). Comparatively lower conidial viability of 77.14% was observed in oil cultures formulated using Mineral oil (T_1, T_2, T_3). The unformulated culture (T_{13}) recorded a conidial viability of 63.57% (Table. 1). The data regarding the mean percent larval mortality indicated that the maximum larval mortality of 90.00% was recorded in Sesamum oil cultures (T_{10}, T_{11}, T_{12}), followed by Liquid paraffin oil cultures (T_4, T_5, T_6) and Rice bran oil cultures (T_7, T_8, T_9) which recorded 86.67% and 83.33% respectively (Fig. 4). Comparatively lowerlarval mortality of 80.00% was observed in oil cultures formulated using Mineral oil (T_1, T_2, T_3). The unformulated culture (T_{13}) recorded a larval mortality of 63.33%. There was no larval mortality recorded in untreated control T_{14}. (Table. 2)

In the investigations after thirty days of storage, the highest mean per cent conidial viability of 82.14% was recorded with Sesamum oil cultures stored at -20^{0}C (T_{12}). The treatment T_{11} (80.71%) was on par with T_6 (79.28%). The treatments T_9 (76.42%), T_8 (75.71%) and T_3 & T_5 (74.28%) were on par with each other. These were followed by the treatments T_2 and T_4 & T_{10} (70.71%). Comparatively lowerconidial viability of 67.85% was observed in oil cultures formulated using Mineral oil (T_1) and Rice Bran oil at room temperature (T_7). The unformulated culture (T_{13}) recorded a conidial viability of 61.43% (Table. 1). The observations of larval mortalities of *Beauveria bassiana* oil cultures after being stored for 30 days revealed that the highest per cent larval mortality of 86.67% was observed in T_{12} followed by T_6 (83.33%) and T_9 (80.00%). The next superior treatmentsT_3, T_8& T_{11} (76.67%) and T_5 (73.33%) were on par with each other. These were followed by the treatments T_2, T_4& T_{10} (70.00%) and T_1& T_7 (66.67%). Comparatively lowermortality of 60.00% was observed in T_{13}. The per cent larval mortality in untreated control was 0.00% (Table. 2).

Among the treatments after sixty days of storage, the treatment (T_{12})Sesamum oil cultures stored at -20^{0}C was recorded with the highest mean per cent conidial viability of 79.28%. T_{11} (76.42%) was on par with T_9 (75.71%). The next better treatment was T_6 (74.28%). The treatments T_3& T_5 (70.71%), T_8 (69.28%) and T_4 (67.85%) were on par with each other. These were followed by the treatments T_2&T_{10} (65.71%), T_7 (64.28%) and T_1 (61.43%) were on par with each other. The unformulated culture (T_{13}) recorded a conidial viability of 58.57% (Table. 1). After 60 days of storage, highest mean per cent larval mortality of 83.33% was observed in T_{12} followed by T_{11} (80.00%), T_6& T_9 (76.67), T_3, T_5 & T_8 (70.00%) and T_2, T_4& T_{10} (66.67%). The treatments T_7 (63.33%) and T_1 (60.00%) were on par with each other. Comparatively lower mortality of 56.67% was observed in T_{13}. There was no larval mortality recorded in untreated control T_{14} (Table. 2).

It was observed that among all the treatments after ninety days of storage, the treatment $T_{12}$was recorded with the highest mean per cent conidial viability of 75.71% and it was on par with T_{11} (74.28%) and T_9 (73.57%). The next superior treatment was T_6 (70.71%). The treatments T_3& T_5 (65.00%) was on par with T_8 (65.71%). T_{10} (63.57%), T_2 (62.87%) and T_4 (61.43%) were on par with each other. These were followed by the treatments, T_7& T_1 (57.87%). The unformulated culture (T_{13}) recorded a conidial viability of 51.43% (Table. 1).The results of mean per cent larval mortality after ninety days of storage of oil cultures revealed that the highest per cent larval mortality was recorded in T_{12} (76.67%), followed by T_9& T_{11} (73.33%). The treatments T_6 (70.00%) and T_3 & T_5 (66.67%) were on par with each other. These treatments were followed by T_8 & T_{10} (63.33%) and T_2& T_4 (60.00%) which were on par with each other. Comparatively lower per cent larval mortalities were observed in T_1& T_7 (56.67%) and T_{13} (50.00%). The per cent larval mortality in untreated control was 0.00% (Table. 2)

The data on observation of mean per cent conidial viability after 120 DAS among all the treatments, T_{12} was recorded with the highest mean per cent conidial viability of 74.28%. T_{11} (67.85%) was on par with T_9 (69.28%). The next superior treatment T_6 (65.71%), T_3 (64.28%) and T_5 (61.43%) were on par with each other. The treatments T_2 & T_8 (58.57%), T_{10} (57.87%) and T_4& T_7 (54.29%) were statistically indifferent from each other. These were followed by the treatment T_1 (51.43%). The unformulated culture (T_{13}) recorded a conidial viability of 45.71% (Table. 1). The observations after 120 days of storage of *B. bassiana* oil cultures revealed that maximum of 73.33% mean per cent larval mortality was recorded in T_{12} followed by T_9 (70.00%) and T_6 & T_{11} (66.67%). T_3 (63.33%) was on par with T_5 (60.00%). These treatments were followed by T_2, T_8 and T_{10} (56.67%), T_4& T_7 (53.33%) followed by T_1 (50.00%) and T_{13} (43.33%). There was no mortality recorded in untreated control T_{14} (Table. 2).

Table 1: Viability of *Beauveria bassiana* conidia stored under different temperature regimes after formulating as oil cultures (2023-2024)

S. No.	Treatment	Mean Per cent Conidia germination						
		0 DAS	**30 DAS**	**60 DAS**	**90 DAS**	**120 DAS**	**150 DAS**	**180 DAS**
1	T_1	77.14^{c} (61.44)	67.85^{cd} (55.46)	61.43^{de} (51.61)	57.87^{d} (49.53)	51.43^{cd} (45.82)	44.29^{de} (41.72)	41.43^{f} (40.07)
2	T_2	77.14^{c} (61.44)	70.71^{bcd} (57.23)	65.71^{cde} (54.16)	62.87^{cd} (52.46)	58.57^{bcd} (49.93)	51.43^{bcde} (45.82)	48.57^{def} (44.18)
3	T_3	77.14^{c} (61.44)	74.28^{abc} (59.53)	70.71^{bcd} (57.23)	65.00^{bc} (53.73)	64.28^{abc} (53.30)	58.57^{abcd} (49.93)	54.29^{bcd} (47.46)
4	T_4	83.57^{b} (66.09)	70.71^{bcd} (57.23)	67.85^{bcd} (55.46)	61.43^{cd} (51.61)	54.29^{bcd} (47.46)	49.29^{bcde} (44.59)	45.71^{def} (42.54)

5	T_5	83.57^{b} (66.09)	74.28^{abc} (59.53)	70.71^{bcd} (57.23)	65.00^{bc} (53.73)	61.43^{abc} (51.61)	58.57^{abcd} (49.93)	51.43^{cde} (45.82)
6	T_6	83.57^{b} (66.09)	79.28^{ab} (62.92)	74.28^{abc} (59.53)	70.71^{ab} (57.23)	65.71^{abc} (54.16)	63.75^{abc} (52.98)	61.43^{ab} (51.61)
7	T_7	80.71^{bc} (63.95)	67.85^{cd} (55.46)	64.28^{de} (53.30)	57.87^{d} (49.53)	54.29^{bcd} (47.46)	48.57^{cde} (44.18)	44.29^{ef} (41.72)
8	T_8	80.71^{bc} (63.95)	75.71^{abc} (60.47)	69.28^{bcd} (56.34)	65.71^{bc} (54.16)	58.57^{bcd} (49.93)	55.71^{abcd} (48.28)	51.43^{cde} (45.82)
9	T_9	80.71^{bc} (63.95)	76.42^{abc} (60.95)	75.71^{ab} (60.47)	73.57^{a} (59.06)	69.28^{ab} (56.34)	64.28^{ab} (53.30)	58.57^{abc} (49.93)
10	T_{10}	87.85^{a} (69.60)	70.71^{bcd} (57.23)	65.17^{de} (53.83)	63.57^{cd} (52.87)	57.87^{bcd} (49.53)	49.29^{bcde} (44.59)	47.85^{def} (43.77)
11	T_{11}	87.85^{a} (69.60)	80.71^{ab} (63.95)	76.42^{ab} (60.95)	74.28^{a} (59.53)	67.85^{ab} (55.46)	62.87^{abc} (52.46)	54.29^{bcd} (47.46)
12	T_{12}	87.85^{a} (69.60)	82.14^{a} (65.00)	79.28^{a} (62.92)	75.71^{a} (60.47)	74.28^{a} (59.53)	67.85^{a} (55.46)	62.87^{a} (52.46)
13	T_{13}	63.57^{d} (52.87)	61.43^{d} (51.61)	58.57^{e} (49.93)	51.43^{e} (45.82)	45.71^{d} (42.54)	39.29^{e} (38.82)	32.85^{g} (34.97)
14	T_{14}	0.00^{e} (0.00)	0.00^{e} (0.00)	0.00^{f} (0.00)	0.00^{f} (0.00)	0.00^{f} (0.00)	0.00^{f} (0.00)	0.00^{h} (0.00)
	General mean	75.10	68.01	64.24	60.36	55.97	50.98	46.79
	F	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
	Sig.	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	SE(m)±	**0.72**	**0.37**	**0.61**	**0.59**	**0.57**	**0.56**	**0.56**
	C.D.	**2.10**	**1.07**	**1.78**	**1.72**	**1.67**	**1.64**	**1.63**
	C.V.	**2.09**	**1.16**	**2.02**	**2.05**	**2.10**	**2.20**	**2.31**

DAS: Days After Storage**,** Values are the means of three replications,
Values in parentheses are angular transformed values
Means in a column followed by same superscript are not significantly different according to DMRT atP≤ 0.05.
SE(m)± = Standard error of mean**C.V.** = Coefficient of variation
C.D. = Critical Difference at 1% level of significance

T_1: Mineral oil culture of *B. bassiana* stored at Room temperature (28 ± 3°C)**,** T_2:Mineral oil culture of *B. bassiana* stored at Refrigerator temperature (4^{0}C)**,** T_3:Mineral oil culture of *B. bassiana* stored at freezing temperature (-20^{0}C)**,** T_4:Liquid paraffin oil culture of *B. bassiana* stored at Room temperature (28 ± 3°C)**,** T_5:Liquid paraffin oil culture of *B. bassiana* stored at Refrigerator temperature (4^{0}C)**,** T_6:Liquid paraffin oil culture of *B. bassiana* stored at freezing temperature (-20^{0}C)**,** T_7:Rice bran oil culture of *B. bassiana* stored at Room temperature (28 ± 3°C)**,** T_8:Rice bran oil culture of *B. bassiana* stored at Refrigerator temperature (4^{0}C)**,** T_9:Rice bran oil culture of *B. bassiana* stored at freezing temperature (-20^{0}C)**,** T_{10}:Sesamum oil culture of *B. bassiana* stored at Room temperature (28 ± 3°C)**,** T_{11}:Sesamum oil culture of *B. bassiana* stored at Refrigerator temperature (4^{0}C)**,** T_{12}:Sesamum oil culture of *B. bassiana* stored at freezing temperature (-20^{0}C)**,** T_{13}:Unformulated culture of *B. bassiana* (Not preserved in oil)**,** T_{14}:Untreated control.

Table 2: Virulence of *Beauveria bassiana* conidia stored under different temperature regimes after formulating as oil cultures against third instar larva of *Spodoptera litura* under laboratory conditions (2023-2024)

S. No	Treatment	Mean per cent larval Mortality of third instar *Spodoptera litura* larvae						
		0 DAS	**30 DAS**	**60 DAS**	**90 DAS**	**120 DAS**	**150 DAS**	**180 DAS**
1	T_1	80.00^{a} (63.44)	66.67^{de} (54.74)	60.00^{de} (50.77)	56.67^{cd} (48.33)	50.00^{de} (45.00)	43.33^{cd} (41.17)	40.00^{cd} (39.23)
2	T_2	80.00^{a} (63.44)	70.00^{cde} (56.79)	66.67^{cde} (54.74)	60.00^{bcd} (50.77)	56.67^{bcde} (48.83)	50.00^{abcd} (45.00)	46.67^{abc} (43.09)
3	T_3	80.00^{a}	76.67^{bcd}	70.00^{bcd}	66.67^{abc}	63.33^{abcd}	56.67^{abc}	53.33^{abc}

		(63.44)	(61.12)	(56.79)	(54.74)	(52.73)	(48.83)	(46.91)
4	T_4	86.67^{a} (68.59)	70.00^{cde} (56.79)	66.67^{cde} (54.74)	60.00^{bcd} (50.77)	53.33^{cde} (46.91)	50.00^{abcd} (45.00)	43.33^{bcd} (41.17)
5	T_5	86.67^{a} (68.59)	73.33^{bcd} (58.91)	70.00^{bcd} (56.79)	66.67^{abc} (54.74)	60.00^{abcd} (50.77)	56.67^{abc} (48.83)	50.00^{abc} (45.00)
6	T_6	86.67^{a} (68.59)	83.33^{ab} (65.90)	76.67^{abc} (61.12)	70.00^{abc} (56.79)	66.67^{abc} (54.74)	63.33^{ab} (52.73)	60.00^{a} (50.77)
7	T_7	83.33^{a} (65.90)	66.67^{de} (54.74)	63.33^{de} (52.73)	56.67^{cd} (48.83)	53.33^{cde} (46.91)	46.67^{bcd} (43.09)	43.33^{bcd} (41.17)
8	T_8	83.33^{a} (65.90)	76.67^{bcd} (61.12)	70.00^{bcd} (56.79)	63.33^{bcd} (52.73)	56.67^{bcde} (48.83)	53.33^{abcd} (46.91)	50.00^{abc} (45.00)
9	T_9	83.33^{a} (65.90)	80.00^{abc} (63.44)	76.67^{abc} (61.12)	73.33^{ab} (58.91)	70.00^{ab} (56.79)	63.33^{ab} (52.73)	56.67^{ab} (48.83)
10	T_{10}	90.00^{a} (71.57)	70.00^{cde} (56.79)	66.67^{cde} (54.74)	63.33^{bcd} (52.73)	56.67^{bcde} (48.83)	50.00^{abcd} (45.00)	46.67^{abc} (43.09)
11	T_{11}	90.00^{a} (71.57)	76.67^{bcd} (61.12)	80.00^{ab} (63.44)	73.33^{ab} (58.91)	66.67^{abc} (54.74)	60.00^{abc} (50.77)	53.33^{abc} (46.91)
12	T_{12}	90.00^{a} (71.57)	86.67^{a} (68.59)	83.33^{a} (65.90)	76.67^{a} (61.12)	73.33^{a} (58.91)	66.67^{a} (54.74)	60.00^{a} (50.77)
13	T_{13}	63.33^{b} (52.73)	60.00^{e} (50.77)	56.67^{e} (48.83)	50.00^{d} (45.00)	43.33^{e} (41.17)	36.63^{d} (37.25)	30.00^{d} (33.21)
14	T_{14}	0.00^{c} (0.00)	0.00^{f} (0.00)	0.00^{f} (0.00)	0.00^{e} (0.00)	0.00^{f} (0.00)	0.00^{e} (0.00)	0.00^{e} (0.00)
	General mean	77.38	68.33	64.76	59.76	55.00	49.76	45.24
	F	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
	Sig.	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	SE(m)±	**1.85**	**1.74**	**1.62**	**1.65**	**1.52**	**1.43**	**1.36**
	C.D.	**5.40**	**5.06**	**4.73**	**4.81**	**4.43**	**4.16**	**3.96**
	C.V.	**5.21**	**5.47**	**5.33**	**5.76**	**5.63**	**5.65**	**5.73**

DAS: Days After Storage**,** Values are the means of three replications,
Values in parentheses are angular transformed values
Means in a coloumn followed by same superscript are not significantly different according to DMRT at P≤ 0.05.
SE(m)± = Standard error of mean**C.V.** = Coefficient of variation**C.D.** = Critical Difference at 1% level of significance

T_1: Mineral oil culture of *B. bassiana* stored at Room temperature (28 ± 3°C)**,** T_2:Mineral oil culture of *B. bassiana* stored at Refrigerator temperature (4^{0}C)**,** T_3:Mineral oil culture of *B. bassiana* stored at freezing temperature (-20^{0}C)**,** T_4:Liquid paraffin oil culture of *B. bassiana* stored at Room temperature (28 ± 3°C)**,** T_5:Liquid paraffin oil culture of *B. bassiana* stored at Refrigerator temperature(4^{0}C)**,** T_6:Liquid paraffin oil culture of *B. bassiana* stored at freezing temperature (-20^{0}C)**,** T_7:Rice bran oil culture of *B. bassiana* stored at Room temperature (28 ± 3°C)**,** T_8:Rice bran oil culture of *B. bassiana* stored at Refrigerator temperature (4^{0}C)**,** T_9:Rice bran oil culture of *B. bassiana* stored at freezing temperature (-20^{0}C)**,** T_{10}:Sesamum oil culture of *B. bassiana* stored at Room temperature (28 ± 3°C)**,** T_{11}:Sesamum oil culture of *B. bassiana* stored at Refrigerator temperature (4^{0}C)**,** T_{12}:Sesamum oil culture of *B. bassiana* stored at freezing temperature (-20^{0}C)**,** T_{13}:Unformulated culture of *B. bassiana* (Not preserved in oil)**,** T_{14}:Untreated control.

Fig.3. Viability of *Beauveria bassiana* conidia stored under different temperature regimes after formulating as oil cultures (2023-2024)

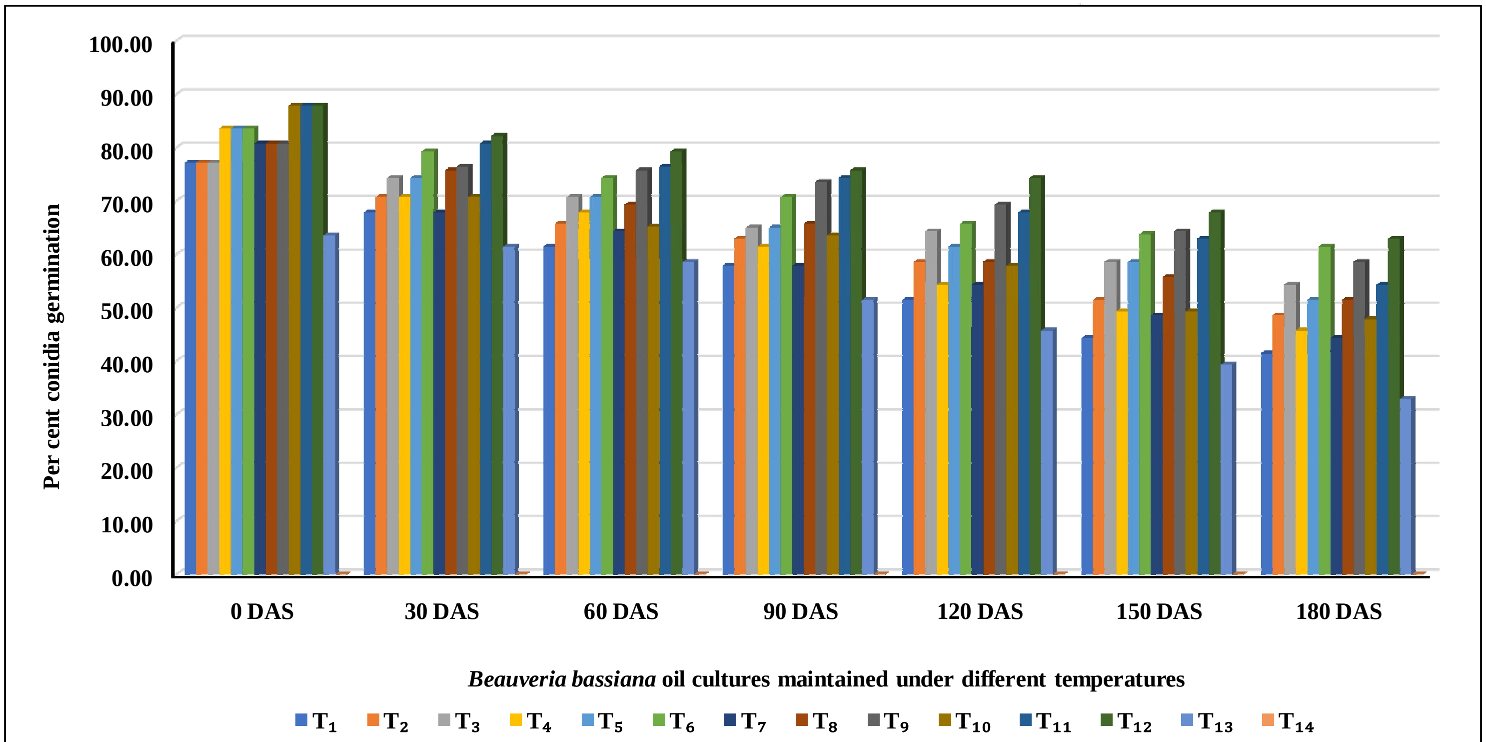
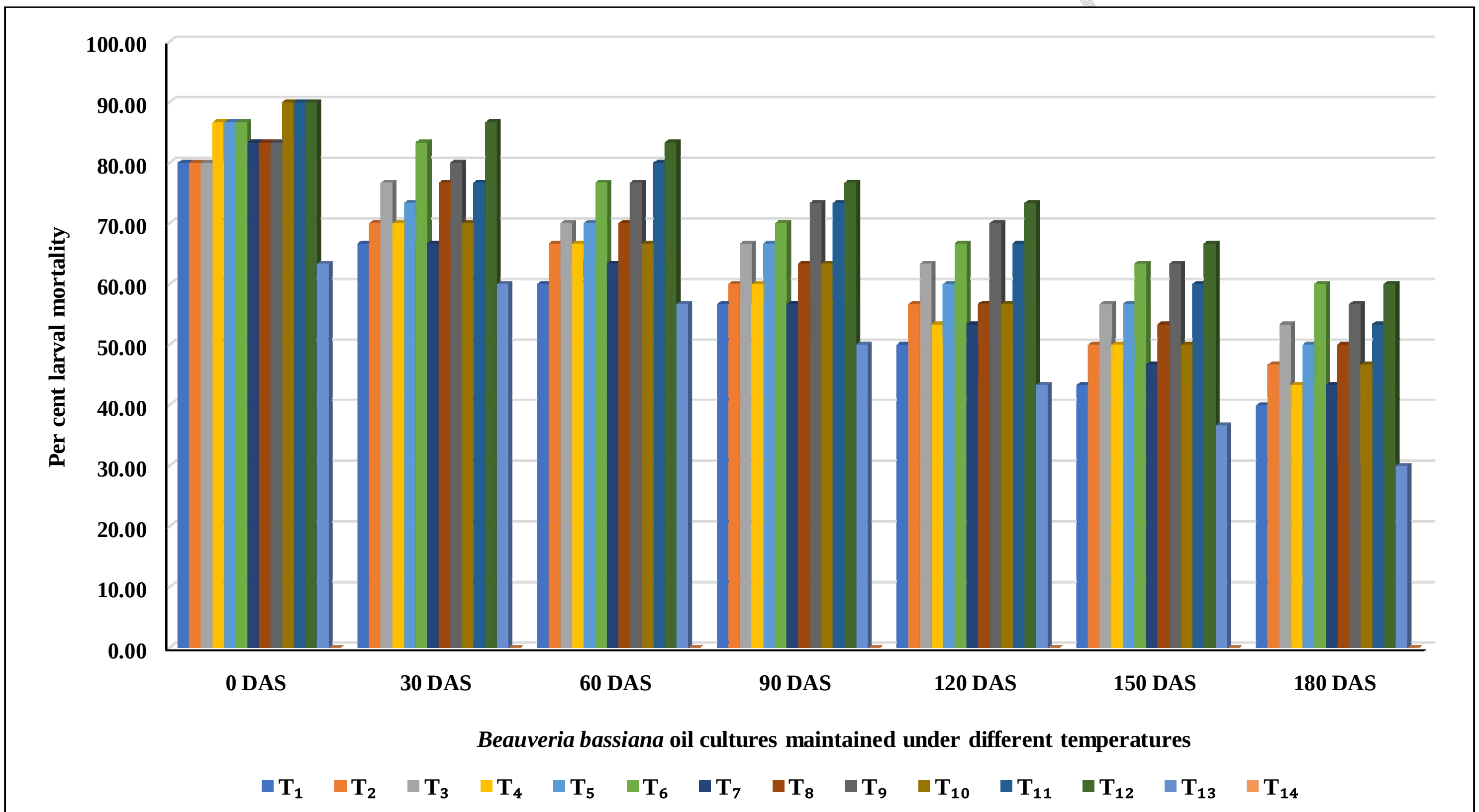


Fig.4. Virulence of *Beauveria bassiana* conidia stored under different temperature regimes after formulating as oil cultures against third instar larva of *Spodoptera litura* under laboratory conditions (2023-2024)



The results after 150 days of storage of different oil cultures of *Beauveria bassiana* revealed that among the treatments T_{12} was recorded with the highest mean per cent conidial viability of 67.85%, followed by T_9 (64.28%). T_6 (63.75%) was on par with T_{11} (62.87%). The treatment T_3 & T_5 (58.57%) on par withT_8 (55.71%). The treatments T_2 (51.43%) and T_{10}& T_4 (49.29%) were statistically indifferent. These were followed by the treatments T_7 (48.57%) and T_1 (44.29%). The unformulated culture (T_{13}) recorded a conidial viability of 39.29% (Table. 1). Storage of oil cultures for 150 days in the laboratory showed that highest mean per cent larval mortality of 66.67% was recorded in T_{12} followed by T_6& T_9 (63.33%). T_{11} (60.00%) and T_3 & T_5 (56.67%) were on par with each other. The treatments T_8 (53.33%) and T_2, T_4 & T_{10} (50.00%) were significantly indifferent with each other. These were followed by T_7 (46.67%), T_1 (43.33%) and T_{13} (36.63%). The per cent larval mortality in untreated control was 0.00%. (Table. 2).

The results of the percent viability after 180 days of storage clearly shows that, the highest mean per cent conidial viability of 62.87% was recorded with T_{12} followed by T_6 (61.43%), T_9 (58.57%), T_3 & T_{11} (54.29%) and T_5& T_8 (51.43%). The treatments T_2 (48.57%), T_{10} (47.85%) and T_4 (45.71%) were on par with each other. These were followed by the treatments T_7 (44.29%) and T_1(41.43%). The unformulated culture (T_{13}) recorded a conidial viability of 32.85%(Table. 1)**.** The data after 180 days of storage of *Beauveria bassiana* oil cultures clearly revealed that highest mean larval mortality of 60.00%was recorded with T_{12} & T_6 followed by T_9 (56.67%). The treatments T_3& T_{11} (53.33%), T_5&T_8 (50.00%) and T_2& T_{10} (46.67%) were on par with each other. These were followed by the treatments T_4& T_7 (43.33%) followed by T_1 (40.00%) and T_{13} (30.00%). There was no larval mortality recorded in untreated control T_{14}(Table. 2).

Comparatively higher mean per cent conidial viabilities of *Beauveria bassiana* oil cultures were observed in the treatments T_{12}, T_6, T_9 and T_{11}, from the day of preparation to 180 days after storage. The viability varied as follows: T_{12} (87.85% to 62.87%), T_6 (83.57 to 61.43%), T_9 (80.71 to 58.57%) and T_{11} (87.85 to 54.29%). Comparatively lowerper cent conidial viability were recorded in T_1 (77.41 to 41.43%) and T_{13} (63.57 to 32.85%). The highest mean per cent larval mortalities were observed in Treatments T_{12}, T_6, T_9 and T_{11} when treated against third instar *S. litura* larvae at monthly intervals. The mean per cent larval mortalities ranged as follows:T_{12} (90.00% to 60.00%), T_6 (86.67 to 60.00%), T_9 (83.33 to 56.67%) and T_{11} (90.00 to 53.33%). Comparatively lowerper cent larval mortalities were recorded in T_1 (80.00 to 40.00%) and T_{13} (63.33 to 30.00%).

The pathogenicity levels correlated with conidial viabilities, showing a gradual decrease in both viability and virulence over the 180-day storage period.

From the present study, it can be concluded that the performance of *Beauveria bassiana* was best with oil formulations compared to unformulated crude cultures. Among the oils used, Sesamum oil formulations were the most effective followed by Liquid paraffin oil, Rice bran oil and mineral oil.

Considering the storage conditions, the formulations stored atfreezing temperature(-20^{0}C)performed better to those stored at Refrigerated temperature (4^{0}C) and Room temperature (28 ± 3°C).

Sesamum oil cultures had been found out to be best among all oil cultures, this may be due to Sesquiterpene present in it which have insecticidal properties that serve as a synergist for pyrethroid insecticides.(Wei *et al.,* 2022, Baker *et al.,* 2018 and Sirato-Yasumoto *et al.,* 2001).

The performance of oil cultures were best at lower temperatures comparedto room temperature, which may be due to reduced metabolism and growth at lower temperatures thereby maintaining viability for an extended period for extended period in such lower temperatures. Murugasridevi*et al*. (2021) prepared oil-based formulationby dissolving 1 g of pure conidia (10^{10} conidia g^{-1}) of *B. bassiana* (Bb 112) in 100 ml of paraffin oil, along with adjuvants like polyethylene glycol and tween 80 to enhance the efficacy of the formulation and stored it under different storage conditions *viz*. ambient temperature (28 ± 2^{0}C) and refrigerated condition (4^{0}C). Shelf-life assessment showed that the formulation stored under ambient temperature and refrigerated condition recorded 587.00 and 590.77 × 10^8 CFU ml^{-1}, respectively on the day of preparation and 167.66 and 216.66 × 10^8 CFU ml^{-1}, respectively after 24 weeks.

The results were in agreement with Lakshmidevi *et al*. (2020) prepared 13 types of plant oil-based bio-formulation of *Beauveria bassiana*(TBb8 strain) using corn, soyabean, castor, rice bran, mustard, mahuva, pinnai, neem, groundnut, palm, coconut and gingelly oils with different combinations and tested its efficacy against the lepidopteran fruit borer *Helicoverpa armigera* (Hubner). Among 13 oils, corn oil was found superior by maintaining a population of 7.0 x 10^9 cfu/ml after 210 days of storage. Oil formulation of TBb8 isolate (1x10^8 spore/ml) treated *H. armigera*showed larval mortality of 70.0%, pupation period of 13.7 days, pupal malformation of 17.9% and adult malformation was recorded as 16.2%.

Sandhu*et al.* (1993) evaluated the effects of temperature on conidial viability and virulence of *B. bassiana* against third instar*Helicoverpa armigera*(Hubner) larvae over a 24-month period. He stored unformulated conidia of *B. bassiana* at five different temperatures (0^0, 10^0, 20^0, 30^0 and 40^{0}C). Based on the experimental results they reported that conidia survived longest at lower temperatures (0–20^{0}C). At higher temperatures, (30–40^{0}C) conidia did not survive.

Oliveira *et al.* (2011) evaluated the effect of preservation of *B. bassiana* isolates for one yearon growth, production and viability of spores as well as macro- and micro-morphology by using 3 storage methods *viz*. freezing at -20^{0}C in an aqueous glycerol solution; freeze-drying (lyophilized) and maintaining at ambient temperature with continual sub-culturing in PDA medium. They reported that glycerol-freeze method at -20^{0}C is the most suitable method for long-term preservation of fungal species.

4. CONCLUSIONS

Comment [mm7]: The conclusion does not answer the aim of this research

The results indicated that *B. bassiana* could be a potent biocontrol agent for managing lepidopteran pests. The combinations of oils and controlled storage temperature presents a promising strategy in enhancing the effectiveness of *B. bassiana* compared to unformulated crude culture. This approach not only enhances the practical application but also supports sustainable pest management practices by providing a reliable and environmentally friendly alternative to chemical pesticides. Further research should be conducted in this line at field level to identify the most effective oil type and concentrations suiting different environment conditions and pest targets, as well as optimizing the application methods to ensure uniform coverage and maximum reach to pests. Additionally, research on compatibility between entomopathogenic fungal oil cultures and other biocontrol agents or agricultural practices will improve its effectiveness and reliability.

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