

Enhanced Oil-Based Formulation of a Native *Beauveria bassiana* Isolate: Optimisation of Carriers and Adjuvants for Improved Conidial Viability

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Abstract

Background

Environmental factors that negatively affect conidial viability and persistence may reduce the effectiveness of entomopathogenic fungi. A viable method for enhancing the stability and prospective applications of fungal biopesticides is the use of oil-based formulations. The current study evaluated five vegetable oils such as palm, sesame, sunflower, rice bran, and coconut oil in conjunction with four adjuvants: glycerol, Tween-80, polyethylene glycol (PEG), and carboxymethyl cellulose (CMC) to create an efficient oil-based formulation of the native *Beauveria bassiana* VKA 01 isolate.

Result

Conidial viability and fungal development were used to evaluate the oils' compatibility with *B. bassiana* conidia. Next, the efficacy of various oil–adjuvant combinations to sustain viable conidia was evaluated. Compatibility between oils and the fungus was assessed using an inhibition-zone assay, followed by conidial viability testing during storage. Palm oil and sesame oil exhibited no inhibitory effect on fungal growth, while sunflower, rice bran, and coconut oils inhibited conidial development. Palm oil retained significantly higher viable conidia (1.56×10^8 CFU ml⁻¹) than sesame oil (7.3×10^7 CFU ml⁻¹) after 15 days of storage, identifying it as the most suitable carrier. Among adjuvants, glycerol at 3% resulted in the highest conidial retention (2.06×10^8 CFU ml⁻¹) after 30 days, significantly outperforming all other additives. CMC at 1% maintained moderate viability, whereas Tween-80 and PEG reduced CFU counts. The formulation maintained commercially acceptable viability up to five months of storage, after which the viable spore count reduced below the required standard of 1×10^8 CFU ml⁻¹.

Conclusion

These carrier–adjuvant combinations can contribute to the development of cost-effective, environmentally sustainable mycoinsecticide formulations suitable for large-scale production and integrated pest management programmes. These results demonstrate that palm oil, combined with 3% glycerol, provides a stable and compatible formulation for *B. bassiana* VKA 01, offering a promising approach for enhancing the shelf life and reliability of fungal biopesticides.

Background

Entomopathogenic fungi constitute an important component of microbial biocontrol strategies in agriculture. Among them, *Beauveria bassiana* is one of the most widely used agents for suppression of lepidopteran, coleopteran, and hemipteran pests (Steinhaus, 1956; Meyling *et al.*, 2009). Despite their potential, fungal biopesticides often face reduced field efficacy due to environmental constraints such as desiccation, ultraviolet radiation, and temperature fluctuations. Stable and effective formulations are therefore essential to ensure high conidial viability and sustained infectivity.

Oil-based formulations can improve conidial adhesion to insect cuticles, enhance resistance to desiccation, and provide favourable microenvironments for germination. Previous studies have shown that humectants and emulsifiers such as glycerol and Tween-80 can significantly influence the viability and virulence of fungal spores (Easwaramoorthy & Jayaraj, 1977; Kubicek and Druzhinina, 2007). However, the suitability of specific oils and additives depends on the isolate, necessitating empirical optimisation.

The present study aimed to (1) evaluate the compatibility of different vegetable oils as carriers for *B. bassiana* VKA 01, (2) assess the influence of various adjuvants on conidial viability within oil formulations. This optimisation provides a foundation for developing robust fungal biopesticide formulations suitable for field application.

Materials and Methods

Fungal isolate and spore preparation

A native isolate of *Beauveria bassiana* (VKA 01) was cultured on Sabouraud Maltose Agar supplemented with Yeast Extract (SMAY) medium and incubated for two weeks to obtain profuse sporulation. Conidia were harvested following the modified protocol of Pham *et al.* (2010) by suspending them in sterile distilled water containing 0.05 percent Tween-80 and filtering through sterile muslin cloth to remove mycelial debris. The filtrate was centrifuged at 10,000 rpm for 30 min, and the conidial pellet was washed twice with sterile distilled water before being resuspended in either the respective vegetable oil for oil-based formulations or sterile distilled water for aqueous formulations. The conidial concentration was standardised using a haemocytometer before formulation.

Carrier materials and additives

Five sterilised vegetable oils-sunflower, rice bran, coconut, sesame, and palm—served as candidate carriers. Additives included glycerol, Tween-80, PEG, and CMC for liquid formulations (Table 1). CMC was UV-sterilised; all other additives were autoclaved.

Table 1

Additives evaluated for the development of oil-based formulations of *Beauveria bassiana* VKA 01 and including functional roles

Sl. No.	Additives	Utility
1	Glycerol	Nutrient carrier, humectant, plasticizer, emulsifier, osmotic protectant, adhesive
2	Tween 80	Emulsifier, stabilizer
3	Poly Ethylene Glycol (PEG)	Emulsifier, stabilizer
4	Carboxy Methyl Cellulose (CMC)	Thickener, binder, sticker, viscosity modifier, stabilizer, water retention agent, lubricant

Oil compatibility assay

The inhibition-zone technique (Brown and Kothari, 1975) was used to assess compatibility. PDA plates were inoculated with conidia, and oil-soaked sterile filter discs were placed at the centre. Plates were incubated at room temperature for 7 days, and growth inhibition was visually evaluated.

Viability of spores stored in selected oils

Palm and sesame oils (the two non-inhibitory oils) were used to store conidia for 15 days at room temperature at an initial concentration of 2.45×10^8 CFU ml⁻¹. Serial dilution and plating (Miles et al., 1938) quantified viable conidia.

Evaluation of additives in oil-based formulations

Adjuvants were mixed into palm oil at 0.5%, 1%, and 2% (Tween-80, PEG, CMC) or 1%, 3%, and 5% (glycerol). Spore suspensions (2.5×10^8 CFU ml⁻¹) were stored for 30 days before CFU enumeration.

Shelf life evaluation

Shelf life studies of the most promising oil-based formulations of *B. bassiana* VKA 01 strain identified in the previous study were carried out under ambient conditions. Ten milliliters each of the oil-based formulation of *B. bassiana* VKA 01 strain containing 1.33×10^9 cfu ml⁻¹ was taken and stored in a 15 ml autoclavable glass bottle with an aluminium lid. Oil containing spores alone was used as a control. Samples were drawn from formulations at 30-day intervals up to 12 months and evaluated for viability (cfu m⁻¹) of the entomopathogen as described in previous sections. The data were subjected to statistical analysis. Similarly, 10 ml of the aqueous formulation of *B. bassiana* VKA 01 strain containing 1.66×10^9 spores ml⁻¹ was taken and stored in a 15 ml autoclavable glass bottle. *B. bassiana* VKA 01 strain alone in sterile distilled water was maintained as a control. Samples were drawn from formulations at 30-day intervals up to 12 months and evaluated for viability (cfu m⁻¹) of the entomopathogen as already described.

Results

Compatibility of oils with *Beauveria bassiana*

Marked differences were observed among the vegetable oils evaluated for compatibility with *Beauveria bassiana* VKA 01 (Fig. 1). Sunflower and rice bran oils caused strong inhibition of mycelial growth, as evidenced by pronounced inhibition zones, indicating poor compatibility with the fungal isolate. Coconut oil produced moderate inhibition, suggesting partial suitability but potential limitations as a carrier material.

In contrast, sesame and palm oils exhibited no detectable inhibition of fungal growth, confirming their compatibility with *B. bassiana*. Quantitative assessment of conidial viability further differentiated these two oils (Table 2). After 15 days of storage, palm oil retained a significantly higher conidial population (1.56×10^8 CFU ml⁻¹) compared with sesame oil (7.3×10^7 CFU ml⁻¹). The superior performance of palm oil highlights its enhanced capacity to preserve conidial viability over time and supports its selection as the most suitable carrier for subsequent formulation studies.

Table 2. Effect of selected vegetable oils on viability (CFU $\times 10^8$ ml⁻¹) of *Beauveria bassiana* VKA 01 strain spores

Sl. No.	Treatments	<i>B. bassiana</i> VKA 01 strain (cfu)
1	Palm oil + <i>B. bassiana</i> VKA spore	1.56 x 10 ⁸
2	Sesame oil + <i>B. bassiana</i> VKA spore	7.3 x 10 ⁷

Effect of adjuvants on conidial viability

Adjuvants exerted pronounced and differential effects on conidial survival in palm-oil-based formulations. Glycerol consistently enhanced conidial viability at all concentrations tested, confirming its role as a highly effective formulation additive. The maximum conidial density (2.06 × 10⁸ CFU ml⁻¹) was recorded in the formulation containing 3% glycerol, which was significantly higher than all other treatments (Table 3).

Glycerol at 1% and 5% concentrations also supported relatively high CFU values; however, viability at these concentrations was significantly lower than that observed at 3%, indicating the presence of an optimal concentration range. These results suggest that moderate glycerol levels provide an optimal balance between moisture retention and metabolic activity of the conidia.

Table 3
Effect of adjuvants and their concentrations on viability (CFU × 10⁸ ml⁻¹) of *Beauveria bassiana* VKA 01 in palm-oil formulations stored for 30 days.

S. No.	Treatment	<i>B. bassiana</i> cfu (x10 ⁸ ml ⁻¹)
1	Palm oil + <i>B. bassiana</i> VKA 01 strain spore + Tween 80 0.5%	0.23 ^{fg}
2	Palm oil + <i>B. bassiana</i> VKA 01 strain spore + Tween 80 1%	0.35 ^e
3	Palm oil + <i>B. bassiana</i> VKA 01 strain spore + Tween 80 2%	0.28 ^{efg}
4	Palm oil + <i>B. bassiana</i> VKA 01 strain spore + glycerol 1%	1.04 ^c
5	Palm oil + <i>B. bassiana</i> VKA 01 strain spore + glycerol 3%	2.06 ^a
6	Palm oil + <i>B. bassiana</i> VKA isolate spore + glycerol 5%	1.74 ^b
7	Palm oil + <i>B. bassiana</i> VKA 01 strain spore + PEG 0.5%	0.34 ^{ef}
8	Palm oil + <i>B. bassiana</i> VKA 01 strain spore + PEG 1%	0.33 ^{ef}
9	Palm oil + <i>B. bassiana</i> VKA 01 strain spore + PEG 2%	0.31 ^{ef}
10	Palm oil + <i>B. bassiana</i> VKA 01 strain spore + CMC 0.5%	0.28 ^{efg}
11	Palm oil + <i>B. bassiana</i> VKA 01 strain spore + CMC 1%	0.74 ^d
12	Palm oil + <i>B. bassiana</i> VKA 01 strain spore + CMC 2%	0.37 ^e
13	Palm oil + <i>B. bassiana</i> VKA 01 strain spore (Control)	0.18 ^{fg}

Mean values in each column followed by a common letter are not significantly different by DMRT (p = 0.05)

Carboxymethyl cellulose (CMC) at 1% concentration maintained moderate conidial viability, indicating its potential as a secondary stabilising agent. In contrast, polyethylene glycol (PEG) and Tween-80 significantly reduced CFU counts at all concentrations tested, with values comparable to the control treatment lacking adjuvants. These additives, therefore, demonstrated limited suitability for oil-based formulations of *B. bassiana* VKA 01.

Evaluation of shelf life of *Beauveria bassiana* VKA 01 strain formulations

The results of the study on shelf life of different formulations of *B. bassiana* VKA 01 strain stored under ambient conditions for up to 12 months are presented in Table 4.

The oil-based formulation of *Beauveria bassiana* exhibited excellent spore viability throughout the storage period, particularly when supplemented with adjuvants. One month after storage, the oil-based formulation with adjuvants exhibited the highest spore viability, 11.2 × 10⁸ CFU ml⁻¹, indicating minimal loss of conidial viability during the initial storage period.

After two months of storage, the oil formulation with adjuvants retained a high viable spore count of 9.3×10^8 CFU ml⁻¹, remaining well above the minimum prescribed standard of 1×10^8 CFU ml⁻¹ for microbial biopesticides. This demonstrated the effectiveness of the adjuvants in maintaining conidial stability during storage. A gradual decline in viability was observed with increasing storage duration. At three months after storage, the oil formulation with adjuvants recorded 8.1×10^8 CFU ml⁻¹, which remained well within the acceptable viability limit, indicating good shelf stability.

Four months after storage, the viable spore count declined to 4.1×10^8 CFU ml⁻¹. Despite this reduction, the formulation continued to maintain viability considerably above the legally prescribed minimum standard, suggesting that the oil-based carrier effectively protected the conidia from deterioration.

At five months after storage, the oil formulation with adjuvants retained 1.1×10^8 CFU ml⁻¹, which was still marginally above the prescribed standard of 1×10^8 CFU ml⁻¹, demonstrating that the formulation remained commercially acceptable up to five months of storage.

Table 4
Effect of storage of *Beauveria bassiana* VKA 01 strain formulations on viability of spores

S. No.	Oil formulation	Initial count (ml ⁻¹)	Monthly cfu count ml ⁻¹									
			1	2	3	4	5	6	7	8	9	10`
1	With adjuvants	1.3x10 ⁹	11.2x10 ⁸ (9.03) ^a	9.3x10 ⁸ (8.97) ^a	8.1x10 ⁸ (8.92) ^a	4.1x10 ⁸ (8.61) ^a	1.1x10 ⁸ (8.03) ^a	4.1x10 ⁷ (7.6) ^b	6.25x10 ⁵ (5.74) ^b	4.5x10 ⁴ (4.66) ^b	3.2x10 ³ (3.50) ^b	0.00 (0.0) ^b
2	without adjuvants	1.3x10 ⁹	6.8x10 ⁸ (8.8) ^b	9.3x10 ⁷ (7.9) ^d	2.9x10 ⁷ (7.5) ^d	2.4x10 ⁴ (4.4) ^d	3.1x10 ² (2.5) ^d	0.00 (0.0) ^e	0.00 (0.0) ^e	0.00 (0.0) ^e	0.00 (0.0) ^c	0.00 (0.0) ^b

Beyond five months, the viability of the oil-based formulation declined below the prescribed standard. Thus, the oil-based formulation enriched with adjuvants maintained adequate conidial viability for up to five months under storage conditions, highlighting its potential as an effective formulation for extending the shelf life of *B. bassiana* conidia.

Discussion

The present study emphasised that carrier selection is a critical determinant of formulation success for *Beauveria bassiana*. Palm oil emerged as the most compatible and effective carrier for isolate VKA 01, supporting both uninhibited mycelial growth and improved conidial survival during storage. This observation agrees with earlier reports indicating that saturated vegetable oils provide a stable physicochemical environment that protects fungal conidia from oxidative stress and moisture loss (Ekwenye, 2006). In contrast, sunflower, rice bran, and coconut oils inhibited fungal growth, underscoring that carrier oils are not universally suitable across fungal species or isolates.

The superior performance of palm oil may be attributed to its fatty acid composition and oxidative stability, which likely reduce lipid peroxidation and maintain membrane integrity of conidia. These properties are particularly advantageous for maintaining viability during storage and under fluctuating environmental conditions.

Among the adjuvants evaluated, glycerol was identified as the most effective additive for enhancing conidial stability (Fig. 2). Its optimal performance at 3% concentration aligns with previous studies describing glycerol as a humectant that improves water retention, stabilises cell membranes, and enhances resistance to desiccation stress (Jones and Burges, 1998; Kubicek and Druzhinina, 2007). The decline in CFU observed at 5% glycerol suggests that excessive viscosity may impede oxygen diffusion or restrict metabolic activity, thereby reducing conidial viability.

CMC provided moderate benefits, likely due to its role as a viscosity modifier that helps stabilise conidia in suspension without inducing physiological stress. In contrast, the negative effects of Tween-80 and PEG on conidial viability differ from some previous reports that describe their utility as emulsifiers. These discrepancies may reflect isolate-specific sensitivities, interactions with palm oil, or adverse effects on conidial membrane integrity, emphasising the need for isolate-specific formulation optimisation.

Overall, the results highlight the importance of integrated carrier–adjuvant optimisation in developing effective formulations of entomopathogenic fungi. The palm oil–glycerol (3%) formulation identified in this study represents a promising strategy for improving the shelf stability and practical applicability of *B. bassiana* in biological pest management programmes.

The shelf-life evaluation of the oil-based formulation of *Beauveria bassiana* was carried out by assessing the viable spore count at 30-day intervals for up to ten months of storage (Fig. 3). One month after storage, the oil-based formulation supplemented with adjuvants recorded the

highest viable spore count of 11.2×10^8 CFU ml⁻¹, indicating excellent conidial stability during the initial storage period. The formulation maintained high viability after two months of storage, recording 9.3×10^8 CFU ml⁻¹, which was well above the legally prescribed minimum standard of 1×10^8 CFU ml⁻¹ for microbial biopesticides.

A gradual decline in spore viability was observed with increasing storage duration. At three months after storage, the oil-based formulation with adjuvants retained 8.1×10^8 CFU ml⁻¹, demonstrating that the formulation continued to preserve conidial viability effectively. Four months after storage, the viable spore count decreased to 2.4×10^8 CFU ml⁻¹, but still remained above the prescribed minimum standard.

The formulation maintained commercially acceptable viability up to five months of storage, after which the viable spore count declined below the required standard of 1×10^8 CFU ml⁻¹. From six months onwards, the formulation failed to maintain the prescribed level of viable spores, and no acceptable viability was observed beyond this period.

The superior shelf life of the oil-based formulation may be attributed to the protective effect of the oil carrier and adjuvants, which reduce moisture loss, improve conidial adhesion, and protect spores from oxidative and environmental stresses during storage. Several earlier studies support these findings. Oil-based formulations have been reported to enhance the storage stability and viability of entomopathogenic fungi by providing a protective microenvironment around the conidia. Studies by Burges (1998), and Jackson et al. (2010) have demonstrated that oil formulations improve conidial longevity and maintain infectivity during prolonged storage. Similarly, Inglis et al. (2001) reported that oil-based formulations protect fungal propagules from desiccation and other environmental stresses, thereby extending shelf life. The present findings corroborate these reports and demonstrate that the optimized oil-based formulation with adjuvants is capable of maintaining satisfactory conidial viability for up to five months under storage conditions.

Conclusion

The optimized palm oil–glycerol formulation offers a promising strategy for improving the storage stability of the native *Beauveria bassiana* isolate VKA 01 while maintaining high conidial viability. By enhancing formulation quality with readily available and economical ingredients, this study contributes to the development of robust, eco-friendly mycoinsecticides for sustainable pest management. The formulation maintained commercially acceptable viability up to five months of storage, after which the viable spore count declined below the required standard of 1×10^8 CFU ml⁻¹. From six months onwards, the formulation failed to maintain the prescribed level of viable spores, and no acceptable viability was observed beyond this period. Further validation under field conditions will facilitate its large-scale adoption and commercial application.

Declarations

Consent for publication

Not applicable

Competing interests

The authors declare no competing interests.

Author Contribution

N.A.N.: original draft, methodology, investigation. M.S.: supervision, review, and editing

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Data Availability

All data supporting the findings of this study are available within the paper and its Supplementary Information

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Figures

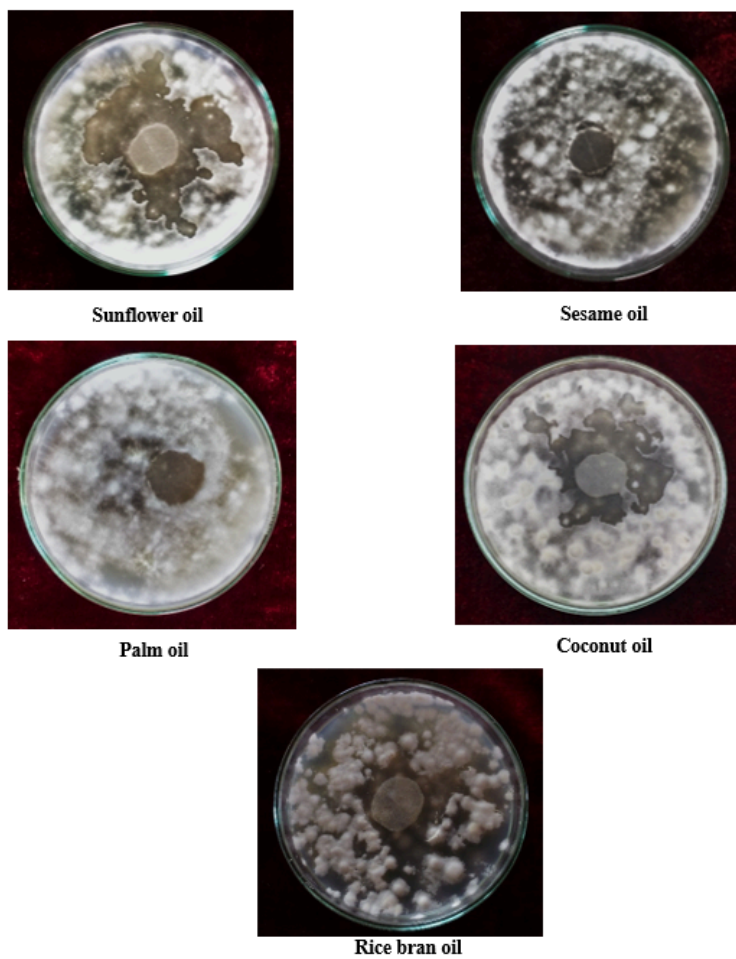


Figure 1

Inhibition of mycelial growth of *Beauveria bassiana* VKA 01 by different vegetable oils

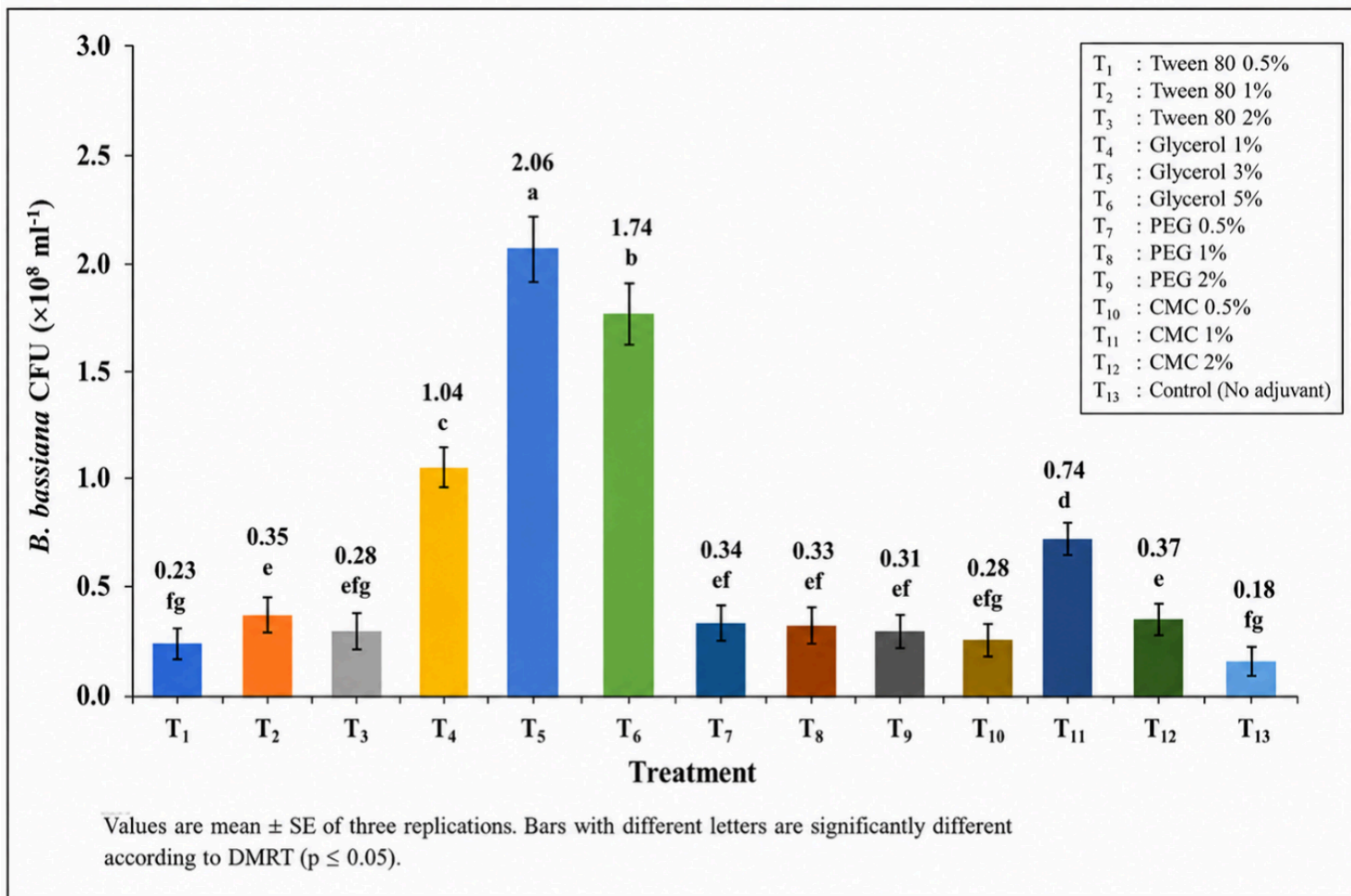


Figure 2

Effect of adjuvants on conidial viability of *Beauveria bassiana* VKA 01 in palm oil-based formulation (CFU (10^8 ML $^{-1}$)) after 30 days of storage

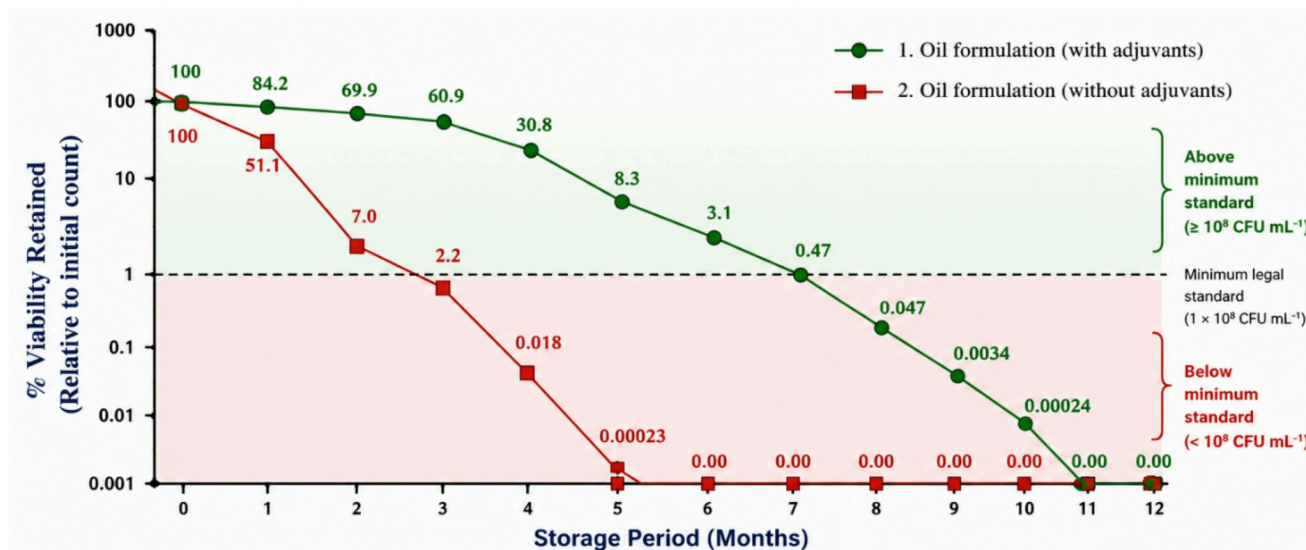


Figure 3

Survival curve of *Beauveria bassiana* VKA in oil formulations with adjuvants and without adjuvants for a period of 12 months of storage