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**Research Article**

# Effect of adjuvants on growth and spore attributes of *Metarhizium anisopliae* (Metsch.) Sorokin

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**ABSTRACT:** The pathogenicity of the fungus, *Metarhizium anisopliae*, commonly referred to as the green muscardine fungus, has been demonstrated against numerous insect species inhabiting various habitats. Laboratory experiments were carried out to investigate the impact of naturally derived adjuvants on the growth and spore characteristics of *M. anisopliae.* The natural polysaccharide (guar gum, gum acacia and carboxymethyl cellulose), vegetable oil (groundnut and cottonseed oil) and a synthetic adjuvant were used in the study at three distinct concentrations: 0.05%, 0.1%, and 0.2% v/v or w/v. Among the various adjuvants tested, both gum acacia and guar gum at concentrations of 0.1% and 0.2% were observed to have a positive impact on the growth of the fungus, resulting in enhanced radial mycelial growth, conidiospore production, surface area coverage, and conidiospore germination. Conversely, this fundamental study highlights the detrimental effects of synthetic adjuvants on the growth and spore characteristics of *M. anisopliae*.

**KEYWORDS:** Adjuvants, entomopathogenic fungi, *Metarhizium anisopliae,* spore germination

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## INTRODUCTION

Entomopathogens are regarded as environmentally friendly substitutes for pesticides in managing crop pests, gaining importance in light of the growing focus on environmental conservation and the promotion of sustainable agricultural practices. Due to their eco-friendliness and bio-persistence, these entomopathogens are preferred to kill insects at various life cycle stages (Gul *et al*., 2014). Entomopathogenic fungi play a significant role in sustainable Integrated Pest Management (IPM) for biological plant protection (Sinha *et al*., 2016). The fungus *Metarhizium anisopliae* (Metsch.) Sorokin, also known as the green muscardine fungus is a microbial biopesticide classified under Order: Hypocreales, Family: Clavicipitaceae (Lovett *et al*., 2019). Isolates of the entomopathogenic fungus *M. anisopliae* have been observed to demonstrate pathogenicity against over 200 species of insect pests (Portilla & Torres, 2010). It represents a significant category of bio-agents that form associations with insects residing in diverse habitats, including freshwater, soil surfaces, and aerial locations (Joshi *et al*., 2018). The remarkable characteristics of this microbial agent have generated a great deal of interest. These characteristics include its pathogenicity towards a wide variety of insects, its ease of production using simple substrates, and its favourable compatibility in both soil and shelf life (Patil *et al*., 2012). However, these microbes can lose their viability under unfavourable conditions such as temperature, humidity, and ultraviolet radiation, which affect the lifespan of conidia. (Raypuria *et al*., 2019).

Adding appropriate adjuvants can enhance the shelf-life and performance of formulations, serving as UV protectants, wetting agents, adhesives, or nutrients that support the growth and viability of the fungus (Patil & Jadhav, 2016). The application of adjuvants along with the entomopathogenic fungi can provide protection and stimulate the establishment of the biocontrol agent on the host surface. Utilizing a combination of these applications has the potential to enhance control effectiveness by decreasing the quantity applied, mitigating environmental pollution risks and preventing the build-up of pest resistance (Usha *et al*., 2014). Typically, vegetable oils, naturally occurring polysaccharides like carboxymethyl cellulose and synthetic additives are employed as enhancers to improve the efficacy of bio-formulations under field conditions. However, these additives play a crucial role in promoting growth, spore production, and viability. Seed

pelleting with gum arabic-encapsulated biocontrol bacteria was found effective against clubroot disease (Abdukareem *et al.,* 2023). Yet, fundamental research into the investigation of new adjuvants derived from natural sources and their impact on the growth and spore characteristics of entomopathogenic fungi is limited. Therefore, an attempt was made to elucidate the effect of various adjuvants of natural origin on vegetative growth and spore attributes of the entomopathogenic fungus *M. anisopliae.*

## MATERIALS AND METHODS

### Fungal culture

The pure culture of the entomopathogenic fungus *M. anisopliae* (Isolate AAUBC Ma15) collected from the culture repository of AICRP on Biological Control of Crop Pests, AAU, Anand was used in the study. The fungus was grown and maintained on Potato Dextrose Agar (PDA) in Petri dishes. The conidial suspension was prepared by scrapping the fungal mat using a brush and suspended in distilled water. The prepared suspension was vortexed for 2 min and filtered using a sterile muslin cloth. The conidial concentration was adjusted to $10^8$ conidia/mL using Neubauer's chamber.

### Adjuvants

The natural polysaccharide (Guar gum, Gum acacia and Carboxymethyl Cellulose (CMC); Himedia, Mumbai, India), vegetable oil (Groundnut and Cottonseed oil; Arcogul, Gujarat, India, and a synthetic adjuvant (Non-ionic, silicone-based spreader containing polydimethylsiloxane as a key ingredient (57%); Agreo solutions, Mumbai, India) were used in the study. Before use, all the adjuvants were sterilized at 160°C for 2 h in a hot air oven. The details of adjuvants and their attributes are presented in Table 1. In laboratory assays, these adjuvants were evaluated at three distinct concentrations: 0.05%, 0.1%, and 0.2% v/v or w/v. This selection was based on the typical recommendation to use adjuvants at a concentration of 0.1% when applying formulations in field conditions.

### Effect of different adjuvants on *M. anisopliae* mycelial growth, conidiospore production and surface area

The effect of adjuvants on mycelial growth was assessed by following the protocol of Mwamburi *et al*. (2015). Petri dishes containing 20 mL PDA were amended with adjuvants at three varying concentrations (0.05%, 0.1%, and 0.2% v/v or w/v). Each dish received inoculation with a 5 mm diameter mycelial-agar plug obtained from the edge of a 6-day-old culture. Petri dishes were then placed in incubation at 26±2°C for 12 days. On the 12$^{th}$ day of incubation, radial growth was measured as the average perpendicular growth minus the diameter of the mycelial agar plug (5 mm).

To assess the effect of adjuvants on the spore production, after 12 days of incubation, the spores of individual plates will be harvested using a sterile conical flask containing 20 ml 0.05% Tween 80 solution. The central ten discs (5 mm) from each test will be drawn and placed in the tween solution. The conical flasks will be vortexed for 30 seconds to extract the spores from the disc. The extracted spores will be quantified using a Neubauer haemocytometer (Akbar *et al*., 2012).

Surface area coverage was assessed following the methodology outlined by Patil and Jadhav (2016), with some modifications. The adjuvants, at the concentrations previously specified, were incorporated into 100 ml of Potato Dextrose Broth (PDB) in 250 ml capacity conical flasks. Each flask was then inoculated with 1 ml of spore suspension ($10^8$ conidia/mL) and placed in incubation at 26±2°C for 12 days. On the 12$^{th}$ day of incubation, surface area coverage was determined following the procedure described by Ritika *et al*. (2019).

### Effect of different adjuvants on *M. anisopliae* conidiospore germination

The agar slide technique outlined by Dhar *et al*. (2016) was followed to assess the effect of adjuvants on conidiospore germination efficiency. The three glass slides were positioned

**Table 1.** List of various adjuvants along with their highlighted properties

| Sl. No. | Adjuvants | Property | References |
|---|---|---|---|
| 1 | Gum acacia | Adhesive | Rahman and Dutta (2023) |
| 2 | Guar gum | Thickening agent | Tahmouzi *et al*. (2023) |
| 3 | Carboxymethyl cellulose (CMC) | Sticker, stabilizer, thickener | Petlamul *et al*. (2017) |
| 4 | Groundnut oil | Adhesive, encoater and nutrient | Jyothi *et al*. (2014) |
| 5 | Cottonseed oil | Adhesive | He *et al*. (2014) |
| 6 | Synthetic adjuvant (Non-ionic, silicone-based spreader containing polydimethylsiloxane) | Penetration, and adhesion, reduce the tension of spray droplets | Bhuiyan *et al*. (2024) |

inside a Petri dish that was lined with blotting paper and subsequently subjected to sterilization. With a micropipette, a fine layer of liquefied PDA medium was uniformly applied onto each of the glass slides. Following this, for the slides coated with the media, 100 µl of conidial suspension obtained from Petri plates amended with adjuvants as outlined in the section describing the effect of different adjuvants on *M. anisopliae* mycelial growth, conidiospore production and surface area**,** was evenly distributed. The inoculated slides were positioned back in Petri dishes and the blotting paper lining of the dish was moistened using sterilized water. The Petri dishes were incubated at 26±2°C. At 24 and 48 h after incubation, the slides were examined under a phase-contrast microscope (40X magnification) to document the count of conidial germination. Approximately 300 conidia were assessed per replicate in each treatment, and the percentage of conidiospore germination was then calculated.

**Statistical analysis**

The obtained data was subjected to one-way ANOVA using SPSS v21.0. The means were compared by Duncan's Multiple Range Test (DMRT) (p=0.05).

## RESULTS AND DISCUSSION

**Radial mycelial growth**

All the different adjuvants at three different concentrations showed significant effects on the radial mycelial growth of *M. anisopliae*. Maximum radial growth was observed in media supplemented with gum acacia @ 0.2%, 0.1% and guar gum @ 0.2% with 74.63±1.65 mm, 72.97±0.49 mm and 72.50±0.08 mm, respectively (Table 2). These were found statistically equivalent to each other. An increase in the concentrations of these adjuvants resulted in more radial growth of the fungus. Among the oil adjuvants, groundnut oil was more efficient than cottonseed oil in improving the radial growth of *M. anisopliae*. All adjuvants enhanced the radial growth of *M. anisopliae* as compared to control (without adjuvants). Whereas in the case of media supplemented with synthetic adjuvant, there was a significant inhibition in the radial mycelial growth. An increase in the concentration of synthetic adjuvant resulted in a significant reduction in the radial mycelial growth of *M. anisopliae*. Kumar *et al*. (2008) recorded that groundnut oil led to radial growth of 25 mm of *M. anisopliae*.

**Conidiospore/spore production**

The conidiospore production by *M. anisopliae* grown on media containing different concentrations of adjuvants had significant differences. The highest spore yield was recorded in gum acacia at both 0.2% ($4.2x10^8$ conidia/ml) and 0.1% ($3.2x10^8$ conidia/ml), as well as guar gum @ 0.2% ($3.3x10^8$ conidia/ml) and 0.1% ($2.8x10^8$ conidia/ml), however, there was no substantial difference observed between them, indicating that they were statistically equivalent. The oil adjuvants exhibited differing levels of conidia production. The highest counts were observed with groundnut oil, both at 0.2% ($2.4x10^8$ conidia/ml) and 0.1% ($1.8x10^8$ conidia/ml) concentrations. Conversely, in the case of cottonseed oil, the highest spore yield ($1.9x10^8$ conidia/ml) was recorded at 0.2%, followed by 0.1% ($1.6x10^8$ conidia/ml) (Table 2). However, there were no significant differences between these yields, indicating they were comparable. The synthetic adjuvant exhibited the lowest conidiospore production. As the concentration of the synthetic adjuvant increased, there was a notable and statistically significant decrease observed in the conidiospore production by *M. anisopliae*.

Band *et al*. (2023) recorded a mean spore count of $7.65x10^8$ spores/ml when *M. anisopliae* was grown on media containing CMC and $9.30x10^8$ spores/ml when grown in groundnut oil. Kumar *et al*. (2008) recorded a mean spore yield of $1.44x10^7$ spores/ml when *M. anisopliae* was grown in media containing groundnut oil. Chin *et al*. (2022) observed that CMC and gum acacia applied to seeds, successfully retain high percentages of spores on the seed surface (78.54-84.93%), a reflection of high coating efficiency. 1.5% w:v carboxymethylcellulose and 25.0 % w:v gum Arabic indicated better compatibility with *T. asperellum* as more viable spores were entrapped on seed surfaces compared to control. Prakash *et al*. (2015) observed that spores of *M. anisopliae* formulated in groundnut oil caused a mortality of 76.66% against *Spodoptera litura*. Cottonseed oil formulated with *Metarhizium flavoviridae* against desert locust (*Schistocerca gregaria*) showed a significant increase in the infectivity of insects, even at low humidity and high temperatures. (Bateman *et al*., 1993).

**Surface area**

The growth of *M. anisopliae* in the media supplemented with different concentrations of various adjuvants was recorded as the surface area covered. The observations on surface area were taken at 12 days after inoculation. The surface covered by *M. anisopliae* ranged from 28.02±0.61% to 98.08±0.50%. The surface area covered by all the treatments was statistically significant over control (without adjuvants) except for the media supplemented with synthetic adjuvant. The surface area was exhibited by media augmented with groundnut oil @ 0.2%, cottonseed oil @ 0.2%, gum acacia @ 0.2% and 0.1%, guar gum @ 0.2% covering significantly highest surface area of 98.08±0.5%, 98.08±0.5%, 98.03±0.29%, 95.13±0.51%, and 95.01±0.19%, respectively, but these were at statistically at par with each other. Whereas, *M. anisopliae* with synthetic adjuvant @ 0.2% recorded the lowest surface area (28.02±0.61%). So, from these results, we can conclude that the natural adjuvants

with a higher concentration supported the maximum growth of *M. anisopliae* (Table 2).

Gum acacia is a high molecular weight polysaccharide, which may have enhanced the growth of fungus to cover more surface area. Similarly, guar gum is a galactomannan polysaccharide containing galactose and mannose, which helps in promoting the vegetative growth of fungus. Raypuria *et al*. (2019) researched the interaction between various adjuvants and found that CMC caused the least inhibition of surface area. Moreover, a higher CMC concentration of 0.5% promoted the greatest extent of surface growth. The enhanced fungal growth at elevated CMC levels was linked to the potential for *M. anisopliae* to secrete cellulolytic enzymes in the presence of CMC. These enzymes play a crucial role in the natural breakdown of materials into carbon, a vital process for the proliferation of microorganisms, as outlined by Betty *et al*. (2013). Out of the two oil adjuvants, groundnut oil was better than cottonseed oil in providing better surface area coverage.

**Conidial germination**

The mean conidial germination percentage of *M. anisopliae* grown in different concentrations of adjuvants at 24 and 48 h after incubation is presented in Table 3. Significant variations in conidial germination were observed among treatments employing different adjuvants (Figures 1 and 2). Conidial germination exhibited more pronounced differences at 24 h of incubation compared to 48 h. The various adjuvants significantly affected the conidial germination of *M. anisopliae* as compared to the control (without adjuvant), except for the *M. anisopliae* grown on media incorporated with synthetic adjuvant. *M. anisopliae* grown on media containing gum acacia and guar gum, showed the highest conidial germination values after 24 h of incubation. CMC, groundnut oil and cottonseed oil

**Table 3.** The effect of adjuvants on conidial germination of *M. anisopliae*

| Treatment | Concentration (%) | Germination (%) | |
|---|---|---|---|
| | | 24 h | 48 h |
| Gum acacia | 0.05 | 94.86±1.54*$^{abc}$ | 100±0.00*$^{a}$ |
| | 0.1 | 97.63±1.53$^{a}$ | 100±0.00$^{a}$ |
| | 0.2 | 98.14±1.29$^{a}$ | 100±0.00$^{a}$ |
| Guar gum | 0.05 | 91.93±1.78$^{abcd}$ | 100±0.00$^{a}$ |
| | 0.1 | 96.03±2.02$^{a}$ | 100±0.00$^{a}$ |
| | 0.2 | 97.70±1.28$^{a}$ | 100±0.00$^{a}$ |
| CMC | 0.05 | 88.39±1.22$^{bcde}$ | 98.66±1.86$^{a}$ |
| | 0.1 | 94.07±1.33$^{abc}$ | 99.98±0.05$^{a}$ |
| | 0.2 | 95.08±0.51$^{ab}$ | 100±0.00$^{a}$ |
| Groundnut oil | 0.05 | 88.11±1.63$^{cde}$ | 99.42±1.14$^{a}$ |
| | 0.1 | 92.45±1.29$^{abcd}$ | 99.52±0.74$^{a}$ |
| | 0.2 | 94.35±0.88$^{abc}$ | 99.75±0.41$^{a}$ |
| Cottonseed oil | 0.05 | 77.93±1.65$^{g}$ | 98.78±0.33$^{a}$ |
| | 0.1 | 80.65±1.53$^{fg}$ | 99.56±0.61$^{a}$ |
| | 0.2 | 85.88±0.79$^{def}$ | 99.86±0.23$^{a}$ |
| Synthetic adjuvant | 0.05 | 37.76±1.37$^{h}$ | 63.40±0.83$^{b}$ |
| | 0.1 | 33.22±1.81$^{hi}$ | 60.90±0.79$^{b}$ |
| | 0.2 | 29.16±1.77$^{i}$ | 43.87±0.89$^{c}$ |
| Control (*M. anisopliae*) | - | 83.28±0.78$^{efg}$ | 98.54±1.04$^{a}$ |
| S. Em± | | 2.04 | 2.18 |
| CD (*P*=0.05) | | 5.77 | 6.19 |

Note: * values represent means ± standard deviation of four replicates. Treatment means with letters in common are not significant by Duncan's New Multiple Range Test at a 5% level of significance

also registered high germination values after 24 h. It was observed that at 24 h after incubation, all adjuvants showed enhanced conidial germination in *M. anisopliae* as compared to control (without adjuvants) (83.28±0.78%), except for synthetic additive, which inhibited the germination of conidia with germination of 37.76±1.37%, 33.22±1.81% and 29.16±1.77%, respectively, at a concentration of 0.05%, 0.1% and 0.2% respectively. At 48 h, all the treatments showed germination values between 98.66 and 100% except for the synthetic adjuvant.

*Metarhizium anisopliae* showed the highest germination when growth media was supplemented with natural polysaccharides like gum acacia, guar gum, and CMC. Our results are similar to the findings of Flores-Villarreal *et al*. (2023) in which *Hirustella citriformis* formulated in media incorporated with gum acacia led to more than 90% germination 24 h after incubation. Among oil adjuvants, groundnut oil showed the highest germination. These results were similar to the findings of Alves *et al*. (1998), where *M. anisopliae* formulated in groundnut oil led to a mean germination percentage of 94.98±% at 24 h after incubation and 99.41±% germination at 48 h after incubation. Higher concentrations of synthetic adjuvant caused a significant reduction in the conidial germination. Alves *et al*. (2002), reported the adverse effects of chemical spreaders on the conidial viability of *M. anisopliae* without a good recovery. Boyette *et al*. (1996) also concluded that chemical adjuvants lowered the germination at concentrations higher than 0.1%.

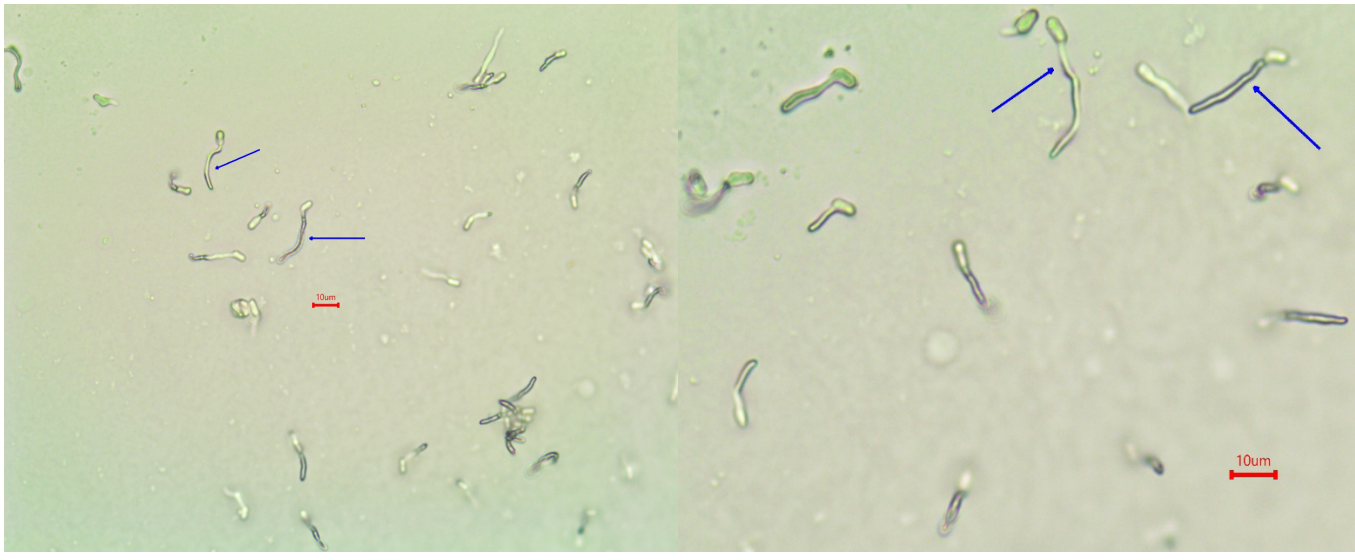


**Figure 1.** Germination of conidia harvested from the medium supplemented with gum acacia was observed at 4 h after incubation.

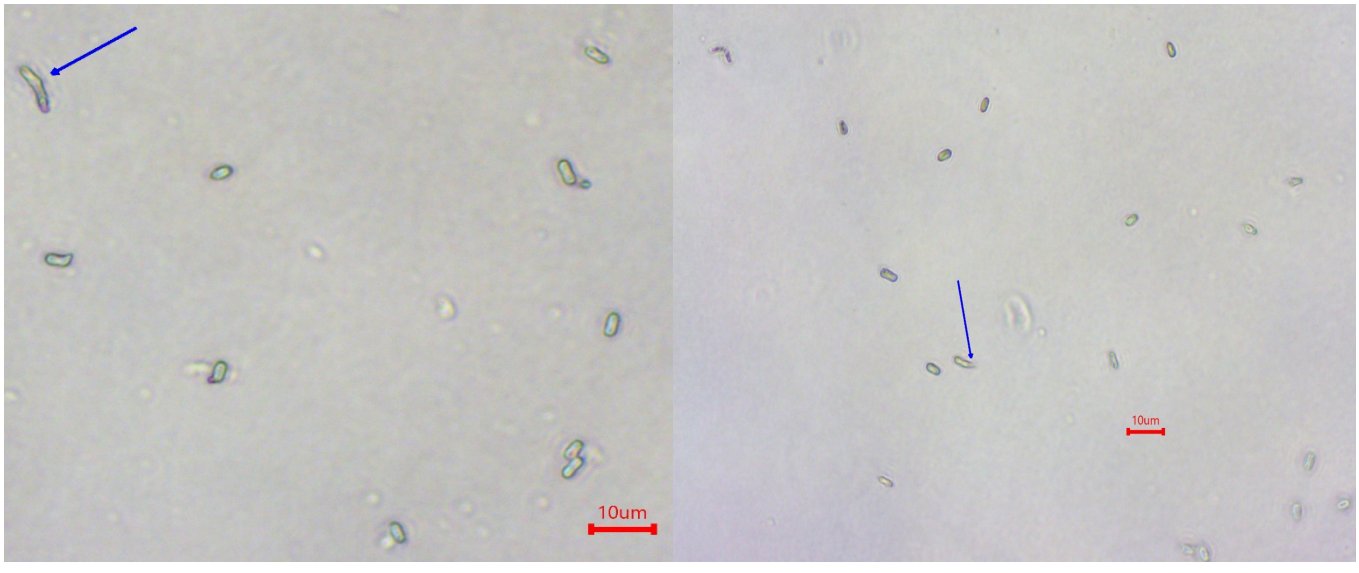


**Figure 2.** Germination of conidia harvested from the medium supplemented with synthetic adjuvant was observed at 4 h after incubation.
Note: Arrow indicates germinated conidia

**Table 2.** The effect of adjuvants on growth, conidiospore production and surface area of *M. anisopliae*

| Treatment | Concentration (%) | Radial mycelial growth (mm) | Conidiospore production (No. of conidia/ml) | Surface area (%) |
|---|---|---|---|---|
| Gum acacia | 0.05 | 70.75±0.08*$^{bcd}$ | 7.10**$^{fg}$ ($1.2x10^8$) | 91.42±0.99*$^{bc}$ |
| | 0.1 | 72.97±0.49$^{ab}$ | 7.51$^{abc}$ ($3.2x10^8$) | 95.13±0.51$^{ab}$ |
| | 0.2 | 74.63±1.65$^{a}$ | 7.62$^{a}$ ($4.2x10^8$) | 98.03±0.29$^{a}$ |
| Guar gum | 0.05 | 68.42±0.07$^{defg}$ | 6.98$^{gh}$ ($9.5x10^7$) | 90.73±0.41$^{c}$ |
| | 0.1 | 70.32±0.03$^{cde}$ | 7.45$^{abcd}$ ($2.8x10^8$) | 93.64±0.50$^{bc}$ |
| | 0.2 | 72.50±0.06$^{abc}$ | 7.52$^{ab}$ ($3.3x10^8$) | 95.01±0.19$^{ab}$ |
| CMC | 0.05 | 66.75±1.02$^{fg}$ | 6.94$^{gh}$ ($8.6x10^7$) | 77.81±1.81$^{e}$ |
| | 0.1 | 68.26±0.06$^{efg}$ | 7.31$^{cde}$ ($2.0x10^8$) | 82.87±1.38$^{d}$ |
| | 0.2 | 70.22±0.19$^{cde}$ | 7.39$^{bcde}$ ($2.4x10^8$) | 90.50±0.61$^{c}$ |
| Groundnut oil | 0.05 | 66.41±0.10$^{fg}$ | 6.93$^{gh}$ ($8.4x10^7$) | 90.39±0.27$^{c}$ |
| | 0.1 | 68.81±0.45$^{def}$ | 7.28$^{def}$ ($1.8x10^8$) | 92.43±0.10$^{bc}$ |
| | 0.2 | 70.38±0.15$^{cde}$ | 7.39$^{bcde}$ ($2.4x10^8$) | 98.08±0.50$^{a}$ |
| Cottonseed oil | 0.05 | 66.35±0.08$^{g}$ | 6.90$^{h}$ ($7.8x10^7$) | 90.28±0.25$^{c}$ |
| | 0.1 | 67.62±0.04$^{fg}$ | 7.23$^{ef}$ ($1.6x10^8$) | 92.43±0.10$^{bc}$ |
| | 0.2 | 68.37±0.06$^{defg}$ | 7.30$^{def}$ ($1.9x10^8$) | 98.08±0.50$^{a}$ |
| Synthetic adjuvant | 0.05 | 36.25±1.50$^{i}$ | 4.51$^{i}$ ($3.2x10^4$) | 35.60±0.13$^{g}$ |
| | 0.1 | 28.25±1.99$^{j}$ | 4.23$^{j}$ ($1.7x10^4$) | 31.78±0.30$^{h}$ |
| | 0.2 | 24.5±1.91$^{k}$ | 3.98$^{k}$ ($9.5x10^3$) | 28.02±0.61$^{i}$ |
| Control (*M. anisopliae*) | - | 40.91± 1.20$^{h}$ | 6.89$^{h}$ ($7.7x10^7$) | 52±1.23$^{f}$ |
| S. Em± | | 0.72 | 0.06 | 1.24 |
| CD (*P*=0.05) | | 2.04 | 0.17 | 3.51 |

**Note:** **Figures are log transformed values, whereas those in the parentheses are retransformed values. Treatment means with letters in common are not significant by Duncan's New Multiple Range Test at 5% level of significance

***** Values represent means ± standard deviation of four replicates

## CONCLUSION

This research explored the impact of various naturally derived adjuvants on both vegetative and spore characteristics of the entomopathogenic fungus, *M. anisopliae*. It was found that all the adjuvants tested had a positive effect, enhancing mycelial growth, conidiospore production, surface area, and conidial germination. The study has showed the detrimental effects of synthetic adjuvants and the present results forms a base for further investigations into the utilization and optimization of natural adjuvants, particularly gum acacia and guar gum, for the development of growth medium, bioformulations refinement, and spray applications. By doing so, it aims to enhance the efficiency of microbial biopesticides, thereby promoting sustainable pest management and facilitating the development of sustainable production systems.

## REFERENCES

Abdukerim, R., Xiang, S., Shi, Y., Xie, X., Li, L., Chai, A., and Fan, T. 2023. Seed pelleting with gum arabic-encapsulated biocontrol bacteria for effective control of clubroot disease in Pak Choi. *Plants*, **12**(21): 3702. https://doi.org/10.3390/plants12213702 PMid:37960058 PMCid:PMC10647673

Akbar, S., Freed, S., Hameed, A., Gul, H. T., Akmal, M., Malik, M. N., and Khan, M. B. (2012). Compatibility of *Metarhizium anisopliae* with different insecticides and fungicides. *Afr J Microbiol Res*, **6**(17): 3956-3962. https://doi.org/10.5897/AJMR12.417

Alves, R. T., Bateman, R. P., Gunn, J., Prior, C., and Leather, S. R. 2002. Effects of different formulations on viability and medium-term storage of *Metarhizium anisopliae conidia*. Neotrop Entomol, 31: 91-99. https://doi.org/10.1590/S1519-566X2002000100013

Alves, R. T., Bateman, R. P., Prior, C., and Leather, S. R. 1998. Effects of simulated solar radiation on conidial germination of *Metarhizium anisopliae* in different formulations. Crop Prot, **17**(8): 675-679. https://doi.org/10.1016/S0261-2194(98)00074-X

Band, S. S., Kabre, G. B., and Hajare, A. R. 2023. Shelf-life assessment through CFU counts of liquid formulations of Metarhizium anisopliae at ambient temperature and cold storage. The Pharm Innov J, **12**(7): 1030-1035.

Bateman, R. P., Carey, M., Moore, D. E., and Prior, C. (1993). The enhanced infectivity of *Metarhizium flavoviride* in oil formulations to desert locusts at low humidities. Ann Appl Biol, **122**(1): 145-152. https://doi.org/10.1111/j.1744-7348.1993.tb04022.x

Betty, A. B., Thatheyus, A. J., and Ramya, D. 2013. Biodegradation of carboxymethyl cellulose using Aspergillus flavus. Sci Internat, **4**, 85-91. https://doi.org/10.5567/sciintl.2013.85.91

Bhuiyan, M. Z. R., Mendoza, L. E. D. R., Lakshman, D. K., Qi, A., and Khan, M. F. 2024. evaluation of adjuvants added to fungicides for controlling Cercospora leaf spot on sugar beet. Crop Prot, **175**, 106471. https://doi.org/10.1016/j.cropro.2023.106471

Boyette, C. D., Quimby, P. C., Caesar, A. J., Birdsall, J. L., Connick, W. J., Daigle, D. J., and Abbas, H. K. 1996. Adjuvants, formulations, and spraying systems for improvement of mycoherbicides. *Weed Technol*, **10**(3), 637-644. https://doi.org/10.1017/S0890037X00040562

Chin, J. M., Lim, Y. Y., and Ting, A. S. Y. 2022. Biopriming chilli seeds with *Trichoderma asperellum*: A study on biopolymer compatibility with seed and biocontrol agent for disease suppression. *Biol Control*, **165**, 104819. https://doi.org/10.1016/j.biocontrol.2021.104819

Dhar, S., Jindal, V., and Gupta, V. K. 2016. Optimization of growth conditions and medium composition for improved conidiation of newly isolated *Beauveria bassiana* strains. *Indian J Exp Biol*, **54**(10), 634-643.

Flores-Villarreal, R. A., Orozco-Flores, A. A., Cantú-Bernal, S. H., Gomez-Flores, R., Pérez-González, O., and Tamez-Guerra, P. 2023. Increased *Hirsutella citriformis* conidia shelf life in Acacia and Hirsutella gum formulations. Appl Sci, **13**(13), 7912. https://doi.org/10.3390/app13137912

Gul, H. T., Saeed, S., and Khan, F. A. 2014. Entomopathogenic fungi as effective insect pest management tactic: A review. Appl Sci Bus Econ, **1**(1): 10-18.

He, Z., Chapital, D. C., Cheng, H. N., Klasson, K. T., Olanya, O. M., and Uknalis, J. 2014. Application of tung oil to improve adhesion strength and water resistance of cottonseed meal and protein adhesives on maple veneer. Ind Crop Prod, **61**: 398-402. https://doi.org/10.1016/j.indcrop.2014.07.031

Joshi, M., Gaur, N., and Pandey, R. 2018. Compatibility of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* with selective pesticides. J Entomol Zool Stud, **6**(4): 867-872.

Jyothi, P., Rao, N. S., and Lakshmipathy, R. 2014. Compatibility of vegetable oils with entomopathogenic fungi against lesser grain borer, *Rhyzopertha dominica* (F.) in paddy. J Biol Control, **28**(1): 35-42.

Kumar, A. M., Reddy, K. N., and Sreevathsa, R. 2008. Influence of pesticides, plant oils and antagonistic entomopathogenic fungus, *Metarhizium anisopliae* (Metsch.) Sorokin. *Pest Technol*, **2**(1): 28-31.

Lovett, B., Bilgo, E., Diabate, A., and St. Leger, R. 2019. A review of progress toward field application of transgenic mosquitocidal entomopathogenic fungi. *Pest Manag Sci.* **75**(9): 2316–2324. https://doi.org/10.1002/ps.5385 PMid:30801913

Mwamburi, L. A., Laing, M. D., and Miller, R. M. 2015. Effect of surfactants and temperature on germination and vegetative growth of *Beauveria bassiana*. *Braz. J Microbiol*, **46**(1): 67-74. https://doi.org/10.1590/S1517-838246120131077 PMid:26221090 PMCid:PMC4512050

Patil, S. D., and Jadhav, R. S. 2016. Effect of various adjuvants on growth and development of the entomopathogenic fungi *Nomuraea rileyi* (Farlow) Samson. *Int J Plant Prot,* **9**(2): 593-602. https://doi.org/10.15740/HAS/IJPP/9.2/593-602

Patil, S. D., Kadam, J. R., Chandele, A. G., and Kulkarni, S. R. 2012. Effect of UVC rays on biomass production by *Metarhizium anisopliae* (Metschinikoff) Sorokin when mixed with various adjuvants. *Internat J Forestry and Crop Improv*, **3**(2): 105-108.

Petlamul, W., Sripornngam, T., Buakwan, N., Buakaew, S., and Mahamad, K. 2017. The capability of *Beauveria bassiana* for cellulase enzyme production. *Proc 7th Internat Conf Biosci, Biochem Bioinformat* (pp. 62-66). https://doi.org/10.1145/3051166.3051167

Portilla, P. J. G., and Torres, A. N. B. 2010. Genetic diversity of a collection of entomopathogenic fungi using AFLP molecular markers (Publication No. Quito/USFQ/2010) [Doctoral dissertation].

Prakash G. V. S. B., Sankar, U, V. R., and Padmaja, V. 2015. Development and testing of mycopesticide formulations of *Metarhizium anisopliae* (Metschnikoff) for shelf life and field application against *Spodoptera litura* (Feb) larvae. *Int J Bioassays*, **4**(9), 4284-4289.

Rahman, M., and Dutta, H. S. 2023. Microbial seed coating-an emerging strategy towards organic vegetable production: A review. *Agric Rev,* **44**(4): 553-557. https://doi.org/10.18805/ag.R-2272

Raypuria, N., Das S. B., Bhowmick, A. K., and Vibha. 2019. Compatibility of *Metarhizium anisopliae* (Metchnikoff) Sorokin, with various adjuvants. *J Entomol Zool Stud*, 7(2), 544-547.

Ritika, J. N., and Sangha, K. S. 2019. Effect of adjuvants on *Lecanicillium lecanii* against nymphs of *Lipaphis erysimi* (Kalt). *Indian J Entomol*, **81**(3): 597-602. https://doi.org/10.5958/0974-8172.2019.00125.1

Sinha, K. K., Choudhary, A. K., and Kumari, P. 2016. Entomopathogenic fungi. Ecofriendly pest management for food security (pp. 475-505). Academic Press. https://doi.org/10.1016/B978-0-12-803265-7.00015-4 PMid:26369776

Tahmouzi, S., Meftahizadeh, H., Eyshi, S., Mahmoudzadeh, A., Alizadeh, B., Mollakhalili-Meybodi, N., and Hatami, M. 2023. Application of guar (*Cyamopsis tetragonoloba L.*) gum in food technologies: A review of properties and mechanisms of action. *Food Sci Nutr*, **11**(9): 4869-4897. https://doi.org/10.1002/fsn3.3383 PMid:37701200 PMCid:PMC10494631

Usha, J., Babu, M. N., and Padmaja, V. 2014. Detection of compatibility of entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill. with pesticides, fungicides and botanicals. *Int J Plant Animal Env Sci*, **4**(2): 613-624.