

# Compatibility of essential oils with Beauveria bassiana (Balls.) Vuil

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## Abstract

Insect pests are a primary agricultural concern, as they can cause productivity losses. The use of pesticides has been the main means of controlling pests. Despite its significant contribution to agriculture, its indiscriminate use harms the environment. The awareness about the risks of agrochemicals using natural enemies such as fungi and alternative controls, such as essential oils, can be an alternative form of control. The objective of this work was to evaluate the fungicidal activity of essential oils of *Cymbopogon citratus, Cinnamomum camphora* var. *linaloolifera*, and *Thymus vulgaris* on the entomopathogen *Beauveria bassiana*. Bioassays followed a completely randomized design in a 3x6 factorial scheme, in which one factor was the essential oils of C. *camphora*, C. *citratus*, and T. *vulgaris*, and the other factor was the four essential oil concentrations, plus two controls. The evaluations took place on the 3rd, 7th, and 14th day. Each treatment had five repetitions, and each repetition was a plate. When assessing compatibility with the entomopathogenic fungus, it was verified that the essential oils showed antifungal activity at all concentrations tested for that non-target organism. Thus, it is advised against the concomitant use of this entomopathogen and the tested essential oils. However, both control tools can be used at different times, with rotation and intercalation of the two methods, following the IPM practices.

Keywords: Entomopathogen; integrated pest management; sustainable practices; terpenes.

## Resumo

## Compatibilidade de óleos essenciais com Beauveria bassiana (Balls.) Vuil

Insetos-praga são uma grande preocupação para a agricultura, pois podem causar perdas de produtividade, sendo o uso de agrotóxicos o principal meio de controle deste tipo de praga. Apesar de sua significativa contribuição para a agricultura, seu uso indiscriminado é prejudicial ao meio ambiente. A conscientização acerca dos riscos associados aos agroquímicos, a implementação de agentes antagonistas naturais, como microrganismos e fungos, junto com a adoção de abordagens alternativas, exemplificadas pelos óleos essenciais, poderiam configurar uma alternativa sustentável viável para o controle agrícola de insetos. O objetivo deste



trabalho foi avaliar a atividade fungicida de óleos essenciais de Cymbopogon citratus, Cinnamomum camphora var. linaloolifera e Thymus vulgaris sobre o entomopatógeno Beauveria bassiana. Os bioensaios seguiram delineamento inteiramente casualizado, em esquema fatorial 3x6, sendo um fator os óleos essenciais de C. camphora, C. citratus e T. vulgaris e o outro fator as quatro concentrações de óleos essenciais mais dois controles. As avaliações ocorreram no 3°, 7° e 14° dia. Cada tratamento teve cinco repetições e cada repetição foi uma placa de Petri. Ao avaliar a compatibilidade com o fungo entomopatogênico, verificou-se que os óleos essenciais apresentaram atividade antifúngica em todas as concentrações testadas para aquele organismo não alvo. Assim, desaconselha-se o uso concomitante deste entomopatógeno e dos óleos essenciais testados. No entanto, ambas as ferramentas de controle podem ser utilizadas em momentos distintos, com rodízio e intercalação dos dois métodos, seguindo as práticas do IPM.

Palavras-chave: Entomopatógeno; manejo integrado de pragas; práticas sustentáveis; terpenos.

## Resumen

#### Compatibilidad de aceites esenciales con Beauveria bassiana (Balls.) Vuil

Las plagas de insectos son una gran preocupación para la agricultura, ya que pueden causar pérdidas de productividad. El uso de plaguicidas ha sido el principal medio de control de plagas. A pesar de su importante contribución a la agricultura, su uso indiscriminado es perjudicial para el medio ambiente. La concientización sobre los riesgos de los agroquímicos, el uso de enemigos naturales como los hongos y los controles alternativos, como los aceites esenciales, pueden ser un control alternativo. El objetivo de este trabajo fue evaluar la actividad fungicida de aceites esenciales de Cymbopogon citratus, Cinnamomum camphora var. linaloolifera y Thymus vulgaris sobre el entomopatógeno Beauveria bassiana. Los bioensayos siguieron un diseño completamente al azar, en un esquema factorial 3x6, en el que un factor fueron los aceites esenciales de C. camphora, C. citratus y T. vulgaris, y el otro factor fueron las cuatro concentraciones de aceites esenciales, más dos controles. Las evaluaciones se realizaron los días 3, 7 y 14. Cada tratamiento tuvo cinco repeticiones y cada repetición fue un plato. Al evaluar la compatibilidad con el hongo entomopatógeno, se verificó que los aceites esenciales mostraron actividad antifúngica en todas las concentraciones probadas para ese organismo no objetivo. Por lo tanto, se desaconseja el uso concomitante de este entomopatógeno y los aceites esenciales probados. Sin embargo, ambas herramientas de control se pueden utilizar en diferentes momentos, con rotación e intercalación de los dos métodos, siguiendo las prácticas de MIP.

Palabras clave: Entomopatógeno; manejo integrado de plagas; prácticas sustentables; terpenos.

## Introduction

Generally, the term pest refers to a population of insects whose individuals inflict some nuisance or injury to crops or animals. Injury is usually characterized as a harmful effect of insect activities (especially feeding) on host physiology, so harm is the loss of measurable usefulness of the host, both as quantity and/or quality of production (GULLAN; CRANSTON, 2017).

In Agriculture, the concept of 'pest' is directly related to the economic effects and reduction of crop productivity caused by the action of insects (NAKANO, 1981). When the level of damage reaches a certain threshold at which financial loss is important, then the population of a particular insect species is considered an economic pest. However, deciding when the damage level is important is subjective, varying according to the pest and damage (HILL, 1997).

Agricultural pests can compromise all production if they are not appropriately controlled. "Chemical" insecticides, e.g., those developed synthetically and made available commercially to act effectively as pesticides, are the most widely used form of control. Although, even if the farmer makes correct use of this control tool, there are issues inherent to the toxicity and persistence of synthetic chemical insecticides, causing health problems to farmers and consumers and environmental problems at micro and macro scales. Among the environmental issues there are the resistance to biological degradation of some pesticides and soil and groundwater contamination (BARZMAN et al., 2015).



The integration of several control tactics can be effective in reducing the infestation levels of pest insects. Integrated Pest Management (IPM) is a strategy that aims to minimize pest damage with minimal harm to human health and the environment. The control methods used in IPM are biological, chemical, cultural, mechanical, and physical, implemented following monitoring and more effective pest control (DREISTADT *et al.*, 2016). According to the Agricultural Research Service (ARS) of the United States Department of Agriculture (2018), IPM is a sustainable decision-making process based on science, which matches biological, cultural, physical, and chemical tools to reduce pest populations and bring tools and management strategies that minimize economic, health, and environmental risks.

Physical or mechanical control is generally indicated for small plantations, in which the adult insects are removed, and the eggs and pupae are crushed, e.g., in the management of *Ascia monusteorseis* (Lepidoptera: Pieridae) (Latreille, 1819) in small vegetable gardens by crushing eggs and removing caterpillars (GALLO et al., 2002). Regarding cultural control, the main control form is soil treatment. Since 1815, this measure has been used for pest control (CRUZ, 1995; DENT, BINKS, 2020). Some means commonly used include soil management, crop rotation, changing planting times, eradication of plants that serve as alternative hosts, fertilizing, and irrigation, among other forms of action that help in pest control (VARASCHINI, 2019).

Regarding chemical control, chemical substances are used to manage target organisms. Brazil is one of the largest food producers and exporters in the world, and Brazilian agriculture has intensively used transgenic seeds and various synthetic chemicals (agrochemicals and fertilizers) (GALLO *et al.*, 2002; MIRANDA, 2006; HOLTZ, 2015). However, the excessive and reckless use of insecticides and other pesticides has generated different economic and environmental problems. Due to their high toxicity, these products have caused problems for human health. In addition to the development of resistance by target insects, environmental imbalances due to the toxic action on non-target organisms, and the low degradation rate of these molecules in the environment, contaminating the soil and water tables (FINKLER, 2012; RIYAZ; SHAH; SIVASANKARAN, 2021).

Natural products, such as plant extracts and essential oils, also may be a tool to be used in IPM practices. Essential oils can be considered wide-spectrum pesticides because they have multiple modes of action and low-risk pesticides due to their quick volatilization and limited field permanence (KOUL; WALIA; DHALIWAL, 2008).

Biological control is a natural management method that regulates the number of plants and animals by natural enemies, which constitute agents of biotic mortality (PARRA *et al.*, 2002). It is a control method that uses natural enemies, such as predators, parasitoids, and entomopathogens, which act as population regulators of insects (SALLES, 1995; HOLTZ, 2015).

Microbial control is a method of biological control that uses entomopathogenic microorganisms, such as fungi, bacteria, and viruses, intending to cause illnesses to the insects, eventually causing the reduction of the pest population to levels that do not harm the culture in question (VARASCHINI, 2019). The use of entomopathogens has several advantages when compared to conventional insecticides, including cost-effectiveness, high yield, absence of harmful side effects for beneficial organisms, fewer chemical residues in the environment, and an increase in the biodiversity in man-managed ecosystems (GULLAN; CRANSTON, 2017).

*Beauveria bassiana* (Bals.) Vuillemin is an entomopathogenic fungus found in soil and insects. Being able to colonize many species, occurring in epizootic and enzootic forms in Coleoptera, Hemiptera, and Lepidoptera, and the enzootic form in Diptera, Hymenoptera, and Orthoptera (ALVES, 1998). Infection by this fungus can be via integumentary, which will depend on nutrients from carbon sources, nitrogen, glucose, and chitin, which are essential for developing this fungus. In some insect groups, infection can also start through the digestive and respiratory systems through the spiracle (DALZOTO; UHRY, 2009). After going through the cuticle, germ tubes and hyphae are formed that run through the integument. There is a considerable mass of hyphae where the fungus multiplies at insect hemolymph. So, the insect dies. With the lack of nutrients, if the conditions are favorable, the fungus emerges, externalizing the hyphae and forming a white mass at the surface of the dead insect (LAZZARINI, 2005).

Essential oils can be mentioned as alternative control tools, products obtained through parts of plants, such as leaves, flowers, and stems, extracted by steam distillation. Although all plant organs can accumulate essential oils, the composition varies depending on where they are extracted (LUPE, 2007). Many essential oils have toxic, repellent, stimulating, and phage-inhibiting properties (VICENÇO, 2020). Besides the insecticidal



effect, essential oils may negatively affect the growth, development, and reproduction of several insect species whose cycle occurs in stored products (PAULIQUEVIS; CONTE; FÁVERO, 2013).

Generally, essential oils can be absorbed through the cuticle, inhaled, or ingested by insects (VICENÇO *et al.*, 2021). Fumigation tests, which consist of observing the toxicity of volatile essential oil compounds on living organisms and spraying essential oils, have already been described in the literature (REGNAULT-ROGER; HAMRAOUI, 1995). Some essential oils have fungicide and fungistatic potential (PANSERA *et al.*, 2022). Therefore, it is important to study them to serve as raw materials and models for synthesizing new products aimed at phytosanitary control in agriculture (CELOTO *et al.*, 2008). There are countless examples of the efficiency of essential oils in the management of phytopathogens. On the other hand, there is little information about the impact of these products on fungi of agricultural relevance, such as entomopathogenic agents, which help in the control of pests of different crops with agronomic interest (GONÇALVES *et al.*, 2017).

Among the several plants that are used for the extraction of essential oil, it can be pointed out those of the genera *Cymbopogon, Cinnamomum*, and *Thymus*, already widely studied and which, in general, have some biological activity (NERIO; OLIVERO-VERBEL; STASHENKO, 2010).

Given the above, the present work aimed to evaluate the compatibility of the essential oils of *Cymbopogon citratus* (DC.) Stapf, *Cinnamomum camphora* Nees e Eberm var. *linaloolifera* Fujita, and *Thymus vulgaris* L. with the entomopathogenic fungus *Beauveria bassiana* (Balls.) Vuil.

# **Materials and Methods**

The antifungal activity of essential oils from *C. citratus*, *C. camphora*, and *T. vulgaris* on *B. bassiana* was evaluated in the second half of 2022, following the procedures described by Pansera et al. (2022). The entomopathogen *Beauveria bassiana* (isolate UCS B05) was used, obtained from the collection of entomopathogens from the UCS Pest Control Laboratory. Entomopathogen multiplication was done through the subculture process in the PDA (potato-dextrose-agar) culture medium. The plates were maintained for 15 days in a BOD at a constant temperature of 25 °C and a photoperiod of 12 h (LOPEZ-PEREZ; RODRIGUEZ-GOMEZ, LO-ERA, 2015).

Bioassays were carried out following a completely randomized design in a 3x6 factorial scheme to assess the effect of the essential oils on the entomopathogen. Factor A was constituted of the essential oils of *C. camphora* (ho-sho), *C. citratus* (lemongrass), and *T. vulgaris* (thyme). Further details about the composition of the essential oils can be obtained from a previous study (VICENÇO et al., 2021). Factor B was composed of four concentrations of essential oils (0.5 %, 1.0 %, 1.5 %, and 2.0 % v/v) plus two control treatments, consisting of distilled water and Tween-80<sup>®</sup> (0.5 % v/v), which was used to emulsify the essential oils. The essential oil concentrations were added to 100 mL of melting PDA medium, previously autoclaved, with a micropipette. Afterward, the medium was poured into glass Petri dishes with a diameter of 8.0 cm.

After medium solidification, a 5 mm disc of *B. bassiana* was transferred to the center of each Petri dish. The plates were sealed and incubated in a BOD at 25 °C and a photoperiod of 12 h for 14 days. The evaluations were carried out on the 3rd, 7th, and 14th day by measuring colony diameter using a digital caliper. Each treatment had five repetitions, and each repetition was a Petri dish, totaling 25 Petri dishes per treatment.

The discs of *B. bassiana* were removed from the Petri dishes that contained the treatments on the 14th day of evaluation to determine whether the biological activity of the essential oils was antifungal or fungistatic. The discs were transferred to new Petri dishes containing only the PDA medium, and the Petri dishes were incubated for seven days in a BOD at 25 °C and a photoperiod of 12 h. The assessment was carried out on the 7th day after substrate replacement.

Data underwent ANOVA, followed by Tukey's post hoc test of multiple comparison of means at a 5 % probability of error, using the software SPSS 2.0.

# **Results and Discussion**

The results regarding the compatibility of the essential oils on B. bassiana are presented in Table I.

Table I - Average mycelial growth (mm) of the entomopathogenic fungus Beauveria bassiana exposed to increasing concentrations of the essential oils C. citratus, C. camphora, and T. vulgaris, with evaluations at the 3rd, 7th, and 14th days after incubation.

Treatment –	Mycelial growth of B. bassiana (mm)		
	3rd day	7th day	l4th day
Water	10.04 a	19.05 a	39.16 a
Tween-80® (0.5 % v/v)	5.93 b	16.15 b	25.68 b
Cymbopogon citratus			
0.5 % v/v	0 c	0 c	0 c
1.0 % v/v	0 c	0 c	0 c
1.5 % v/v	0 c	0 c	0 c
2.0 % v/v	0 c	0 c	0 c
Thymus vulgaris			
0.5 % v/v	0 c	0 c	0 c
1.0 % v/v	0 c	0 c	0 c
1.5 % v/v	0 c	0 c	0 c
2.0 % v/v	0 c	0 c	0 c
Cinnamomum camphora var. linalolifera			
0.5 % v/v	0 c	0 c	0 c
1.0 % v/v	0 c	0 c	0 c
1.5 % v/v	0 c	0 c	0 c
2.0 % v/v	0 c	0 c	0 с

Means in column followed by the same letter do not differ statistically by Tukey's test at a 5 % error probability.

It could be observed that there was fungal growth in the negative controls (water and Tween-80<sup>®</sup> 0.5 % v/v), which differed statistically from each other. Note that there was no mycelial growth of the entomopathogen *B. bassiana* when it was exposed to the treatments with essential oils, regardless of the concentration and the assessment day, presenting a fungistatic behavior.

On the 7th day after substrate exchange, aiming to verify the behavior of the entomopathogen *B. bassiana*, it was noted that there was no mycelial growth, proving an antifungal activity of the essential oils tested at all concentrations observed (Figure 1). Thus, regardless of the concentration, the essential oils tested had an antifungal, rather than a fungistatic action, on the entomopathogen *B. bassiana*.

Studies demonstrated the compatibility of alcoholic and aqueous extracts of jaboticaba (*Myrciaria cauliflo-ra* (Mart.)), guava (*Psidium guajava* (L.)), and three dilutions of the commercial disinfectant peroxytane 1512L on the viability of *B. bassiana*, conidia. However, only the extracts had little impact on inoculum potential (MARTINS; ALVES; MAMPRIM, 2016). The compatibility of essential oils from the bark of *Dugetia lanceolata* and the entomopathogenic fungus *B. bassiana* was described by Pompermayer (2020), who observed mode-rate toxicity in the fungus.



Figure I - Aspect of Beauveria bassiana inoculums, transferred after 14 days of exposure to increasing concentrations of C. camphora, C. citratus, and T. vulgaris essential oils, assessed seven days after incubation in fresh BDA medium.



In addition, studies carried out to test the compatibility of the essential oils of *Eucalyptus globulus*, and *E. citriodora* on *B. bassiana* indicated that concentrations higher than 0.4 % v/v and 0.2 % v/v, respectively, could inhibit the mycelial growth of the entomopathogen (IMMEDIATO et al., 2016). Ummidi and Vladamani (2014) found that mustard (*Sinapis alba*), coconut (*Cocos nucifera*), and eucalyptus (*Eucalyptus grandis*) oils at a concentration of 2.0 % v/v reduced the growth of *B. bassiana* colonies in 20.4 %, 17.4 %, and 20.4 % relative to the control (distilled water), showing that some oils have an antifungal effect on this entomopathogen.

The essential oil of *T. vulgaris* (thymol as the major compound, with a content of 50 wt.%) reduced the growth of several phytopathogenic fungi *in vitro*, among them *Rhizoctonia solani*, *Pythium ultimum var. ultimum*, *Fusarium solani*, and *Colletotrichum lindemuthianum*. Some studies observed that hyphae degradation is the toxic mechanism of essential oils on fungi (ZAMBONELLI et al., 1996; NAZZARO et al., 2017; LI et al., 2020).

*Cymbopogon citratus* essential oil had an in vitro inhibitory effect on *Colletotrichum gloeosporioides* from the concentration of 500 ppm, a result that resembled the synthetic fungicide tebuconazole, used as a positive control. It was also found that citral (the major compound in the essential oil in question) was responsible for the inhibitory action (GUIMARÃES et al., 2007).

Tomazoni et al. (2017) observed that ho-sho essential oil (Cinnamomum camphora var. linaloolifera), from



the concentration of 1.5 mL·L<sup>-1</sup>, inhibited completely (100 %) the mycelial growth of several isolates of the phytopathogen Alternaria solani.

The antifungal effect of essential oils reduces hyphal growth and induces cytoplasmatic lysis of the fungi. The inhibition of growth caused by essential oils is generally influenced by changes in the composition of the cell wall, disrupting the plasmatic membrane and disorganization of the mitochondrial and cell structure (BIL-LERBECK *et al.*, 2001; MAIA; DONATO; FRAGA, 2015; PANSERA; SILVESTRE; SARTORI, 2022).

The essential oils comprise several compounds with different insecticidal and repellent mechanisms. Some terpenes have a repellent effect, causing aversion in the individuals, while others can interfere with the nervous and digestive systems of the insects, killing them (REGNAULT-ROGER; VINCENT; THOR, 2012). Many studies demonstrate that these compounds possess biological activity and can cause adverse effects on many pests (DHIFI et *al.*, 2016).

Due to this biological activity, essential oils and their components can act as fungistatic and/or antifungal agents, activity this called antifungal, depending on the concentrations used. The same oil can be effective against a wide range of species of microorganisms, but the minimum inhibitory concentrations (MICs) can vary according to the inherent susceptibility of each fungal species (ANTUNES; CAVACOB, 2010).

Plants use monoterpenes to defend against different types of stress, pathogens, allelochemical action, and repellent activity against insects, among other functions (CHAND; JOKHAN; GOPALAN, 2017). These compounds in essential oils can be classified into two major chemical groups, differing according to the metabolic route in which they were synthesized. Among them, there are the terpenes (monoterpenes, sesquiterpenes, and diterpenes) and, at a lower degree, phenylpropanoids (REGNAULT-ROGER; VINCENT; THOR, 2012).

Studies show that, among the insecticides commonly used to control agricultural pests, except for some (profenofos, indoxacarb, imidacloprid, triazophos, and methyldemeton), all the rest can be used safely together with the entomopathogen *B. bassiana*. When comparing the toxicity of synthetic insecticides against *B. bassiana* at 14 days and 30 days after inoculation, the toxicity of the insecticides was reduced 30 days after inoculation. At 14 days, imidacloprid and triazophos were moderately toxic; after 30 days, the toxicity reduced to slightly toxic. The synthetic insecticide profenophos, highly toxic in 14 days, reduced its toxicity to moderate after 30 days. Although the different tested insecticides inhibited the growth of *B. bassiana* in the *in vitro* treated medium, the combined or concomitant use of entomopathogen and insecticides cannot be discarded entirely (AMUTHA, 2010).

This study showed that essential oils of T. *vulgaris*, *C. camphora* var. *linaloolifera*, and *C. citratus*, at the investigated concentrations, affect the development and survival of *B. bassiana*, not being recommended or possible to use them concomitantly. However, that does not mean essential oils cannot be used with *B. bassiana* during the entire cycle. Both control tools can be used at different times, with rotation and intercalation of the two methods. Thus, essential oils will not interfere with the entomopathogen's action against the pests in question.

# Conclusion

From the results presented, it can be observed that the essential oils of *C. camphora* (ho-sho), *C. citratus* (lemongrass), and *T. vulgaris* (thyme) are incompatible with the entomopathogenic fungus *B. bassiana*, occurring an antifungal effect at the tested concentrations and causing the death of the fungus. However, further research with other extracts of essential oils, trials under field conditions, and quality control studies must be carried out to determine the compatibility of the essential oils with *B. bassiana* and evaluate the adoption of rotation practices between these control methods. In addition, further research is needed to elucidate the potential of these products from plant origin as an alternative tool to chemical products and their production on broader scales.



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