

APPLICATION OF MICROORGANISMS WITH INSECTICIDAL PROPERTIES FOR PESTS' CONTROL IN AGRICULTURE

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Abstract: The interest in finding new and environmentally safe pest control technologies is constantly growing among farmers. The use of biological pesticides is the most attractive crop protection method, which is a safe alternative to the use of chemical pesticides. Considering the relevance of the pest prevalence biocontrol methods, the development of insecticides based on microorganisms and fungi is promising research direction. The purpose of the article is to analyse existing data on the use of the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana*, as well as the bacteria *Bacillus thuringiensis* and *Streptomyces avermitilis* as bioagents for the pest control in agriculture. To comprehensively gather information on biopesticides, we employed a systematic and rigorous approach. We utilized a wide range of electronic sources, including Google Scholar, PubMed, Scopus (Elsevier), Web of Science, Semantic Scholar, Academia, and other relevant websites, to conduct extensive literature searches. Our analysis of over 100 scientific papers and other relevant online resources enabled us to amass a comprehensive archive of pertinent literature. The target objects of the micromycetes *Metarhizium anisopliae*, *Beauveria bassiana*, as well as the bacteria *Bacillus thuringiensis*, *Streptomyces avermitilis* are a wide range of crop pests, which includes species of coleopteran and lepidopteran insects, as well as nematodes. The complex use of several species of these microorganisms at once allows to achieve the highest efficiency in the fight against both specific species of insect pests and to expand the list of pests that are their target objects. The integrated use of microorganism association of several species to control a wide range of agricultural pests is a promising technology for use in the agricultural sector. Further research is needed to select optimal combinations of microorganisms to achieve the highest efficiency in combating both specific types of insect pests and to expand the range of applications of such insecticides.

Keywords: microbial pesticides; *Metarhizium anisopliae*; *Beauveria bassiana*; *Bacillus thuringiensis*; *Streptomyces avermitilis*

Introduction

Agricultural productivity depends significantly on the effectiveness of plant protection, as insect pests cause significant economic losses worldwide. Annual crop yield loss caused by pests reaches up to 42%, making them one of the main problems of agricultural production [1, 2]. Ensuring food security in the face of population growth, climate change and agricultural intensification makes pest control one of the central tasks of modern agricultural science. Traditional chemical insecticides have played a key role in increasing yields during the 20th century, but their widespread and long-term use is accompanied by numerous problems: the accumulation of insecticide residues in products, toxicity to non-target organisms (including pollinators and natural predators of pests), resistance development and buildup in pest populations and negative impacts on biodiversity and ecosystems. Current reviews emphasize that crop loss reduction should occur not through new chemical compounds alone, but preferably through integrated, envi-

ronmentally sound approaches – among which biological methods of pest control are one of most promising [3–5].

In response to these challenges, interest in the development and implementation of environmentally safe and sustainable strategies for pest control is growing. Among such approaches, biological control using microorganisms with insecticidal properties has a special place, which is considered as one of the most promising alternatives to chemical insecticides [6]. Biological pesticides, or bioinsecticides, are created based on natural microorganisms – bacteria and fungi, capable to efficiently infect or inhibit insect pests development, without causing harm to other populations and the environment [7]. In this context, microorganisms with insecticidal properties (entomopathogenic fungi, toxin-producing bacteria, actinomycetes that synthesize nematocidal and insecticidal molecules) are perceived as a promising alternative or addition to chemical insecticides.

Four groups of microorganisms that have the most developed scientific and applied base deserve

special attention: *Metarhizium anisopliae* and *Beauveria bassiana*, *Bacillus thuringiensis* and *Streptomyces avermitilis*. Each of these groups demonstrates high efficiency against a wide range of pests in laboratory and field studies, but has its own limitations related to formulation, stability, range of action, risk of resistance acquisition by pests and other interactions with the agroecosystem. These microorganisms implement wide variety of action mechanisms - from the formation of specific toxins to direct parasitism on insect hosts, which provides targeted pest suppression with minimal environmental impact [5–8]. Cultivation of such microorganisms with combined use with other methods open new opportunities for the creation of effective, sustainable and economically viable plant protection technologies.

Despite the promising research results, the widespread use of insecticidal microorganisms requires solving several key problems: Required optimization of the composition and delivery technologies to increase stability and persistence in the field; Limited understanding of ecological interactions and consequences for non-target organisms and soil/phytosphere microbiomes; need in further development of strategies to minimize the risk of resistance acquisition; and harmonization of regulatory approaches and economic feasibility for farmers.

The aim of this work and the main objective of the publication are to analyze and summarize current data on the use of *Metarhizium anisopliae*, *Beauveria bassiana*, *Bacillus thuringiensis* and *Streptomyces avermitilis* as biological pest control agents in agriculture. Special attention is given to their biological characteristics, cultivation conditions, ability to synthesize bioactive metabolites and prospects for combined use under different environmental conditions.

1. The application of entomopathogenic fungi in pest control

1.1. *Metarhizium anisopliae* as an agent of bioinsecticide

Metarhizium anisopliae is a naturally occurring fungus that affects a wide range of insect pests. The sources for this fungus isolation include insects and their cadavers, various soil samples. Some representatives of *Metarhizium* spp. are facultative saprophytes or plant root symbionts in rhizosphere [9]. The fungus infects insects by conidiospores, which during the germination form appressorium and penetrate through insect cuticula and colonize the body. On the terminal step of infection, the host dies due to the nutrients depletion. For the further spreading to new hosts fungus forms hyphae with conidia on the surface of cadaver[10].

It is shown that *Metarhizium anisopliae* is an effective bioinsecticide and is used against various agricultural pests (Table 1) [11–24].

One of the advantages of bioinsecticides with *Metarhizium anisopliae* is strict host specificity. The fungus has been shown to be highly selective for its host, infecting only a limited number of insect species [25]. This specificity is important because effects on non-target organisms such as beneficial insects and mammals are minimized.

In general, multiple researchers reported mortality rates between 30 and 90% for various pests, including *Plutella xylostella*, *Tuta absoluta*, *Helicoverpa zea*, *Anthonomus grandis*, *Helicoverpa armigera*, *Ceratomya trifurcata*, *Aphis glycines* [11–24].

The percentage of crop pest mortality observed while applying *Metarhizium anisopliae* vary depending on several factors, such as a target pest, a dose of microfungus, biopesticide form and content, environmental conditions such as temperature, humidity and exposure to sunlight. It has been shown that high humidity 60–75 % and moderate temperatures (approximately 24–25°C) are favorable for fungal growth and infection of target pests [21, 22]. Application in oil or granular form of *M. anisopliae* have shown higher efficacy against pests such as *Bemisia tabaci* and *Spodoptera exigua* [26]. At the same time encapsulation of conidia *M. anisopliae* (sodium alginate capsules) protects spores against UV light but decreased insect mortality (only 10 % for *Diatraea saccharalis*) [27].

M. anisopliae has shown potential as a bioinsecticide to control various crop pests. However, further research is needed to optimize its performance under different environmental conditions and to assess its potential for large-scale use in agricultural systems.

1.2. The use of *Beauveria bassiana* as an agent of bioinsecticide preparations

Beauveria bassiana is a well-known entomopathogenic fungus that has been extensively studied for its potential use as a bioinsecticide against various agricultural pests. Currently, there are many commercial biopesticides that contain *B. bassiana*. The fungus is known to infect and kill a wide range of insect pests, including aphids, whiteflies, thrips, beetles, caterpillars and many others. Below (Table 2) is a list of some of the agricultural pests against which *B. bassiana* has been used as a bioinsecticide [18–32].

The crop pest mortality observed with *Beauveria bassiana* varies depending on the target pest and the stage of insect development. Significant mortality rates of various agricultural pests have been reported following treatment with *Beauveria bassiana*. For example, in [21] it was shown that treatment of *Helicoverpa armigera* larvae with *Beauveria bassiana*

causes 20-90% mortality. The mortality of *Plutella xylostella* was 78% in [30]. High efficiency of *Beauveria bassiana* application (larval mortality rate 95.5%) was also shown for *Frankliniella occidentalis* under greenhouse conditions [31]. A relatively low death rate (50%) was observed for eggs of *Tuta absoluta* treated with *Beauveria bassiana* conidia [24].

It is worth noting that the effectiveness of *Beauveria bassiana* is affected by several factors, including the concentration and composition of the fungus, the method of application, and environmental conditions such as temperature and humidity.

For example, the pests that spent most of the time on the underside of the leaf are better affected by

powders, at the same time, pests that penetrate the leaves are more sensitive to nebulized liquid [33].

Humidity is known to be a very important environmental factor when using fungal pesticides like *Beauveria bassiana*. Under the greenhouse condition the infection rate of *Frankliniella occidentalis* increased by 17–25% after the humidity was increased by 15% [34].

In summary, the percentage of crop pest deaths observed when using *Beauveria bassiana* vary depending on several factors. However, it is clear, that *Beauveria bassiana* has the potential to be an effective bio-insecticide against a wide range of agricultural pests.

Table 1: The harmful effect of *Metarhizium anisopliae* on different pests

Pest	Habitat	Harmful effect	Reference
<i>Aphis glycines</i> (soybean aphid)	Soybean crops	The fastest death time (5 day), mortality 67.5%	[11]
<i>Plutella xylostella</i> (diamondback Moth)	Cruciferous crops	<i>Metarhizium anisopliae</i> proved to be the most effective by killing more than 90% of the population	[12, 13]
<i>Nilaparvata lugens</i> (brown planthopper)	Rice crops	The cumulative mortalities of adult ranged from 17.2 to 82.1%, 10 days after inoculation	[14, 15]
<i>Cerotoma trifurcata</i> (bean leaf beetle)	Soybean crops	Both insecticide seed treatments provided a high level of <i>C. trifurcata</i> control, as measured by significant differences in foliar damage ratings, compared to the untreated plots, for all planting dates tested	[16]
<i>Helicoverpa zea</i> (corn earworm)	Corn crops	Using four microbial insecticide formulations (<i>B. thuringiensis</i> var. <i>kurtaki</i> , <i>B. thuringiensis</i> var. <i>aizawai</i> , <i>M. anisopliae</i> , and <i>B. bassiana</i>) against <i>S. frugiperda</i> and <i>H. zea</i> at two locations in the Yucatan Peninsula, the microbial formulations protected maize against <i>S. frugiperda</i> and <i>H. zea</i> as well as the chemical insecticide emamectin benzoate.	[17, 18]
<i>Anthonomus grandis</i> (boll weevil)	Cotton crops	For <i>Anthonomus grandis</i> , the most virulent isolate was <i>Metarhizium anisopliae</i> BRM 2335, followed by <i>Beauveria</i> BRM 14527 and BRM 67744 (92.5 %)	[19]
<i>Spodoptera frugiperda</i> (fall armyworm)	Corn pest	An increased amount of conidia (1.5×10^9 conidia/mL) was found to be toxic to larvae, pupae, and adults after 9 days of treatment, resulting in a 100% mortality rate in eggs, 98% in larvae, 76% in pupae, and 85% in adults.	[20]
<i>Helicoverpa armigera</i> (cotton bollworm)	Cotton crops	The highest mortality was observed in Rawalpindi with the lowest pupation rate by applying the combined concentrations of <i>B. thuringiensis</i> and chlorantraniliprole	[21, 22]
<i>Frankliniella occidentalis</i> (western flower thrips)	Vegetable and fruit crops	The results from the experimental field trial showed that a single spray of <i>M. anisopliae</i> CQMa421 reduced thrips by 50–70% compared to the control	[23]
<i>Tuta absoluta</i> (tomato leafminer)	Tomato crops	Twenty-one days post treatment, spore concentration 1.0×10^6 conidia/mL, resulted in the highest death rate: 50 and 37.6% with eggs when treated by <i>B. bassiana</i> and <i>M. anisopliae</i> , respectively.	[24]

Table 2: The harmful effect of *Beauveria bassiana* on different pests

Pest	Habitat	Harmful effect	Reference
<i>Bemisia tabaci</i> (silverleaf whitefly)	Sweet potato, tomatoes, squash, broccoli, cauliflower, cabbage	The lengthy duration of 2–3 weeks to kill the insect pest population.	[28]
<i>Spodoptera exigua</i>	Horticulture crops, shallot plants	The concentration of the combination insecticide that caused the highest mortality of <i>S. exigua</i> at 7 days after observation was in treatment AB1 (BB 0.08% + EDM 0.627) which caused a mortality of 82.98%.	[29]
<i>Plutella xylostella</i>	Cruciferous crops	Highest mean corrected mortality (77.80%) was recorded	[30]
<i>Tuta absoluta</i>	Solanaceae plants	Twenty-one days post treatment, spore concentration 1.0×10^6 conidia/mL, resulted in the highest death rate: 50 and 37.6% with eggs when treated by <i>B. bassiana</i> and <i>M. anisopliae</i> , respectively.	[24]
<i>Frankliniella occidentalis</i> (western flower thrips)	Pests of greenhouse plants	These EPF were very effective on <i>T. urticae</i> eggs with a percentage reduction greater than 92.86%, whereas the percentage reduction in the <i>T. urticae</i> mobile forms varied between 95.11 and 98.52%	[31]
<i>Rhyzopertha dominica</i>	Stored wheat	The main effects were significant at $P < 0.001$ and after 8 d of exposure the adult mortality was 25.86% with the highest application rate of DE, which reached 32.64% after 24 d of exposure.	[32]
<i>Helicoverpa zea</i>	Cotton, soybean, tomato, sorghum and a wide range of wild species	Using four microbial insecticide formulations (<i>B. thuringiensis</i> var. <i>kurtaki</i> , <i>B. thuringiensis</i> var. <i>aizawai</i> , <i>M. anisopliae</i> , and <i>B. bassiana</i>) against <i>S. frugiperda</i> and <i>H. zea</i> at two locations in the Yucatan Peninsula, the microbial formulations protected maize against <i>S. frugiperda</i> and <i>H. zea</i> as well as the chemical insecticide emamectin benzoate.	[18]
<i>Helicoverpa armigera</i>	Soybean, pigeon pea, tomato, cotton, sorghum, maize, linseed, pea, chickpea and many vegetable crops	The present findings provide insight into <i>B. bassiana</i> at 10^9 conidia/mL was more virulent under laboratory and field conditions by reducing larval infestations, decreasing pod damage and subsequently increasing the production yield of chickpeas during both cropping seasons.	[21]

1.3. Use of *Bacillus thuringiensis* to produce bioinsecticide preparations

The bacterium *Bacillus thuringiensis* is the most successful bioagents used to control insect pests. Currently bioinsecticides Bacillus-contain compositions occupy about 2% of the total insecticide market. *Bacillus thuringiensis* has high activity against the larval stages of various types of insects and causes tissue destruction of the midgut of the insect and subsequent sepsis.

After sporulation, *Bacillus thuringiensis* produces crystalline inclusions, which are formed by toxins namely Cry and Cyt. These toxins have a very selective spectrum of activity, causing the death of a narrow

range of insect species. Cry and Cyt toxins belong to a class of bacterial toxins known as pore-forming toxins. They are secreted as water-soluble proteins and undergo conformational changes in insect's digestive tracts, which causes them to be inserted into the cell membrane of their hosts.

Products based on *B. thuringiensis* have limited application in agriculture, as Cry toxins are highly lethal only for young insect larvae; they are also sensitive to solar radiation. Nevertheless, an important breakthrough in reducing the use of chemical insecticides in agriculture occurred with the development of transgenic crops capable of expressing Cry toxins. Cry toxin synthesis genes have been introduced into transgenic agricultural plants (*B. thuringiensis* cotton,

corn, potato, and tobacco), which provides a more targeted and effective way to control insect pests in agriculture. This approach led to a significant reduction in chemical insecticides usage in areas where the cultivation of these transgenic crops was widespread. Transgenic plants continuously produce and accumulate the Cry protein in the cells, which protects the insecticidal toxin from degradation caused by UV light. The effect of the toxin is specifically aimed at insects that damage leaves and stem.

Worldwide, more than 40 million hectares of genetically modified crops with an embedded Cry toxin gene have been grown, leading to a significant reduction in the use of chemical insecticides and in some cases contributing to the suppression of certain insect pest populations, such as *P. gossypiella*.

One of the most common applications of *Bacillus thuringiensis* is the lepidopteran pests' control, including tobacco bollworm (*Heliothis virescens*), corn earworm (*Helicoverpa zea*), and European corn borer (*Ostrinia nubilalis*) [35, 36]. *B. thuringiensis* has also been shown to be effective against other important agricultural pests, including the Colorado potato beetle (*Leptinotarsa decemlineata*) [37] the diamondback moth (*Plutella xylostella*) [36], and the beet scale beetle (*Spodoptera exigua*) [38].

In addition to lepidopteran pests, *B. thuringiensis* has also been shown to be effective against other insect pests, including the western flower thrips (*Frankliniella occidentalis*) [39], the Mediterranean fruit fly (*Ceratitidis capitata*) [40] and green peach aphid (*Myzus persicae*) [41].

For example, a study by Crava et al. [38] found that the efficacy of *B. thuringiensis* against beet scale was improved when the bioinsecticide was delivered as a gel. Similarly, a study by Wu et al. [32] found that *B. thuringiensis* spray was more effective than dust in controlling the Colorado potato beetle.

When using *Bacillus thuringiensis*, a high mortality rate of various types of pests was observed. For example, a study by Abedi et al. [37] showed that a *B. thuringiensis*-based bioinsecticide (Dipel®) caused 100% mortality of tomato bollworm (*Helicoverpa armigera*) larvae under laboratory conditions. Similarly, *B. thuringiensis* formulations have been reported to cause high mortality rates in other pest species, including European corn borer moth (*Ostrinia nubilalis*) [42], diamondback moth (*Plutella xylostella*) [43] and cuckoo (*Cydia pomonella*) [44].

However, the effectiveness of *B. thuringiensis* is affected by several factors, including environmental conditions and developmental stage of the target pest. For example, a study by Liu et al. [41] found that mortality rates of beet armyworm (*Spodoptera exigua*) larvae treated with *B. thuringiensis* varied with temperature and moisture conditions. Another study by Wang et al. [45] reported that mortality rates of cotton

bollworm (*Spodoptera littoralis*) larvae were higher when *B. thuringiensis* was applied early in development.

Bacillus thuringiensis is an effective bioinsecticide for the control of a variety of agricultural pests, and its effectiveness may vary depending on the pest species, formulation, method of application, and environmental conditions. Further research is needed to optimize the use of *B. thuringiensis* in different farming systems and to address the challenges of developing resistance in target pest populations.

1.4. The use of *Streptomyces avermitilis* to produce biological preparations

One of the main advantages of bioinsecticides based on *Streptomyces avermitilis* is their broad spectrum of activity against various agricultural pests [46–53]. Several studies have demonstrated the effectiveness of these bioinsecticides against a variety of pests (Table. 3).

The potential of *S. avermitilis*-based bioinsecticides for nematode and tick control has been reported. For example, in laboratory studies, *S. avermitilis* was found to be highly effective against the gall nematode (*Meloidogyne incognita*), with 100% mortality observed in treated juvenile forms [54]. Similarly, *S. avermitilis* was found to be effective against two-spotted spider mite (*Tetranychus urticae*), with an adult mortality rate reaching 64% [55]. In world practice, the effectiveness of preparations based on avermectin against the following types of parasitic nematodes has been proven: *Meloidogyne incognita* on crops of corn, rapeseed, eggplant, tomatoes, zucchini; *Pratylenchus zae* on corn crops, *Heterodera schachtii* on sugar beet and zucchini crops, *Tylenchulus semipenetrans* on orange crops [56].

It is worth noting that the effectiveness of *Streptomyces avermitilis*-based bioinsecticides is affected by several factors, including the target pest, form, application method, and environmental conditions. For example, research shows that the efficacy of these bioinsecticides is enhanced by the use of synergistic agents such as chitosan or surfactants [57]. Similarly, the efficacy of these bioinsecticides is affected by temperature and humidity, with higher efficacy observed at higher temperatures and lower humidity. A study on *S. exigua* found that the efficacy of *S. avermitilis* was higher at higher temperatures and lower humidity levels. Another study found that the application of *S. avermitilis* as a soil drench was more effective in controlling fall armyworm than foliar application [58].

Several authors have reported high mortality rates of insect pests when treated with *S. avermitilis*-based bioinsecticides. For example, laboratory studies have found that the mortality rate of diamondback moth (*Plutella xylostella*) larvae after treatment *S. wit*

Table 3. The harmful effect of *Streptomyces avermitilis* on different pests.

Pest	Habitat	Harmful effect	Reference
Cyst nematode (<i>Globodera pallida</i>)	Potato	The researchers found that the doses of 18 and 36 µg/mL significantly reduced number of eggs, juveniles, cyst/g soil and reproduction rate in comparison to untreated control.	[48]
Tobacco caterpillar (<i>Spodoptera litura</i>)	Plants, which is present throughout the South and Eastern world	<i>S. avermitilis</i> alone (Mortality = 92 percent at 250 ppm; LC ₅₀ = 93.61 ppm; LC ₉₀ = 243.49 ppm; Antifeedant rate = 59.6 ± 15.13 percent).	[49]
Diamond moth (<i>Plutella xylostella</i>)	Cruciferous vegetables: cauliflower, cabbages, kale, garden cress, bok choy, broccoli, Brussels sprouts, mustard plant and similar green leaf vegetables.	It has a great affinity with the Cl ⁻ channels of the glutamate receptor, allowing the constant flow of Cl ⁻ ions into the nerve cells, causing a phenomenon of hyperpolarization causing death by flaccid paralysis	[50]
Fall armyworm (<i>Spodoptera frugiperda</i>)	Mais	At 48 HAT causing 95% mortality.	[51]
Green peach aphid (<i>Myzus persicae</i>), Cotton aphid (<i>Aphis gossypii</i>)	A wide range of cultures	Mortality 50%	[52]
Fruit fly (<i>Drosophila melanogaster</i>)	Fruits and vegetables, as well as carriers of various diseases	<i>Streptomyces</i> produce specialized metabolites that have potent larvicidal effects against the fly; larvae that ingest spores of these species die	[53]

avermitilis is 94% [59]. Similarly, the mortality rate of *Spodoptera exigua* larvae has been found to be 93% after treatment with *S. avermitilis*. Another study found that *S. avermitilis* is highly effective against *Spodoptera frugiperda*, with mortality rates of 80–100% observed in treated larvae [58].

Bioinsecticides based on *Streptomyces avermitilis* have shown great potential as an effective and environmentally friendly alternative to chemical insecticides. Their broad spectrum of activity against a wide range of agricultural pests makes them a valuable tool for pest control in agriculture.

2. Cultivation methods and metabolites isolation of *Metarhizium anisopliae*, *Beauveria bassiana*, *Bacillus thuringiensis*, *Streptomyces avermitilis* on laboratory and industrial scales

2.1. *Metarhizium anisopliae* cultivation

Biotechnological cultivation of *M. anisopliae* requires growth conditions optimization to achieve high yields and spore quality. Various factors, such as temperature, pH value, aeration and substrate composition, might affect fungal growth and sporulation [60]. Several authors have examined the effects of these factors on the growth and sporulation of *M. anisopliae*. For example, *M. anisopliae* has been shown to grow optimally at 25–30°C and pH 5.5–7.5. Additionally, aer-

ation and agitation are crucial to supply oxygen and prevent the formation of fungal granules [61]. In addition, the type and composition of the substrate used for cultivation play an important role in determining the yield and quality of spores. Several substrates such as rice, wheat and corn were used for cultivation of *M. anisopliae*, and their composition was optimized to increase the yield and quality of spores. The results of the study showed that the most effective substrate composition was a 2:1:1 ratio of rice, wheat and corn. Additional ingredients such as salt, glucose and calcium caseinate were used to improve the cultivation efficiency of *M. anisopliae* [62].

Selection of the appropriate strain is critical for successful cultivation of *M. anisopliae*. Different strains of *M. anisopliae* exhibit variations in spore yield, virulence, and resistance to environmental stress. Thus, selection of a strain with desirable characteristics is crucial to produce high-quality biocontrol. Several authors have reported the isolation and characterization of new strains of *M. anisopliae* with improved spore yield and insecticidal activity [63]. Additionally, genetic and metabolic engineering has been used to modify *M. anisopliae* strains to improve their insecticidal properties and spore yield [64]. For example, the study reported that overexpression of the chitinase gene in *M. anisopliae* enhanced the fungus's insecticidal activity against cotton bollworm. Another study showed that disruption of the *ssn6* gene in *M. anisopliae* improved spore yield and virulence against western

flower thrips [65]. Thus, strain selection and genetic modifications are promising strategies for improving *M. anisopliae* for commercial production.

Various cultivation methods have been developed and optimized for the biotechnological production of *M. anisopliae*. The most common cultivation methods include deep culture (SmF), solid phase culture (SSF), and co-culture methods. Submerged culture (SmF) involves the growth of *M. anisopliae* in a liquid medium under aerobic conditions. SmF has the advantage of high spore yields and easy scale-up. However, it requires the use of complex media and sophisticated equipment, which increase the cost of production. Solid-phase culture (SSF): SSF is a cultivation method that involves the growth of *M. anisopliae* on a solid substrate. SSF has several advantages such as low cost, simple equipment and high spore yield, the possibility of using agricultural waste as a substrate [66]. Studies have been conducted to establish optimal conditions for solid-phase cultivation of *M. anisopliae*. In the study of Rangel et al. [61] it was shown that the best results are obtained when rye straw treated with 0.5% (v/w) NaOH for 1 hour as a substrate was used. Under these conditions, the maximum productivity of *M. anisopliae* was achieved - 7.6×10^8 conidia per gram of substrate.

Co-cultivation methods: Co-cultivation methods involve culturing *M. anisopliae* with other microorganisms, such as bacteria and yeast, to increase the yield and quality of spores. Co-cultivation methods also reduce production time and increase the biomass of *M. anisopliae*. For example, co-cultivation of *M. anisopliae* with *Bacillus thuringiensis* increase spore yield and its virulence against desert locusts [67–68].

Further processing of *M. anisopliae* involves recovering and purifying spores from the culture medium. Several methods, such as filtration, centrifugation and drying, have been used to recover *M. anisopliae* spores. Additionally, spore purification is important to remove contaminants such as mycelium and waste that affect the effectiveness of the biocontrol agent [69]. The most common purification methods include density gradient centrifugation, size exclusion chromatography, and electrophoresis.

Therefore, biotechnological cultivation of *M. anisopliae* is crucial for the commercial production of this important biocontrol agent. Optimization of growth conditions, strain selection and subsequent processing are essential to achieve high spore yield and quality. The use of various cultivation methods, such as SmF, SSF, and co-cultivation methods, has increased spore yield and reduced production costs. In addition, genetic and metabolic engineering have been used to improve the insecticidal properties and spore yield of *M. anisopliae*. The development of automated monitoring systems and bioreactors has revolutionized the scaling-up process, enabling large-scale production of

M. anisopliae. Thus, biotechnological cultivation of *M. anisopliae* is a promising approach for the sustainable production of this important biocontrol agent.

2.2. *Beauveria bassiana* cultivation

Substrate selection is crucial for the successful cultivation of *Beauveria bassiana*. Several substrates have been used to culture this fungus, including wheat bran [28], rice bran [70], corn cobs [71], soybean meal [9], and various agro-industrial wastes such as sugarcane bagasse [10], coffee husks [11], and empty oil palm bunches [12]. The composition of the substrate affects the growth and sporulation of the fungus, as well as its insecticidal activity.

Temperature, humidity, pH and aeration are important factors that affect the growth and development of *Beauveria bassiana*. The optimum temperature for cultivation is between 25°C and 30°C [13], and the relative humidity should be maintained at around 70% [14]. The pH of the medium should be between 6.0 and 7.5 [15], and adequate aeration is necessary to ensure the supply of oxygen to the fungus.

There are several types of cultivation methods that is used to produce *Beauveria bassiana*, including solid-phase cultivation (SSF) [16], submerged cultivation (SmF) [17], and a combination of SSF and SmF [18]. SSF is a process where the fungus is grown on a solid substrate, while SmF involves culturing the fungus in a liquid medium. The combination of SSF and SmF has been shown to improve *Beauveria bassiana* production because it allows for the advantages of both solid and liquid substrates. *Beauveria bassiana* is a promising tool for controlling the abundance of various insect pests. Cultivation on different substrates and under different conditions has been widely studied, and several factors have been identified that affect its growth and virulence. Solid-phase cultivation using agro-industrial waste has been found to be a cost-effective method for cultivating *B. bassiana*, while submerged cultivation allows for more precise selection of cultivation parameters to maximize biomass yield but is more expensive.

2.3. *Bacillus thuringiensis* cultivation

Biotechnological cultivation of *B. thuringiensis* is a cost-effective way to produce large quantities of the bacteria for use for biopesticides.

The optimal conditions for the highest *B. thuringiensis* biomass yield depend on several factors, such as the *B. thuringiensis* strain used, the type of medium, temperature, and pH. Different *B. thuringiensis* strains have been shown to have different requirements for optimal growth conditions. For example, some *B. thuringiensis* strains have been shown to grow best at pH 7.0, while others prefer a slightly more acidic pH of

6.5. In addition, the type of medium used to grow the bacteria have a significant impact on biomass yield. For example, some authors have shown that *B. thuringiensis* grown in a medium containing corn steep liquor and yeast extract produce higher biomass yields than when grown in other media.

Temperature is another important factor that affect biomass yield. *B. thuringiensis* is a mesophilic bacterium with optimal growth temperatures between 25 and 30°C. Higher temperatures lead to reduced biomass yields, while lower temperatures lead to slower growth rates. In addition, the availability of oxygen also plays a role in biomass yield. *B. thuringiensis* is an aerobic bacterium and requires adequate oxygen supply for optimal growth. There are several different types of cultivation methods that is used to grow *B. thuringiensis*, including submerged fermentation (SmF), solid-phase fermentation (SSF), and biofilm cultivation. SmF is the most used method for large-scale *B. thuringiensis* biomass production. It involves growing the bacteria in a liquid medium in a fermenter with continuous stirring to ensure sufficient oxygen supply. SSF involves growing the bacteria on a solid substrate such as wheat bran or rice husk. Biofilm cultivation involves growing bacteria on a surface, such as a membrane or packed bed reactor.

Each of these cultivation methods has advantages and disadvantages. SmF is usually the most efficient method for producing large amounts of biomass, but it is expensive due to the high cost of the liquid medium and the need for specialized equipment. SSF is a more cost-effective method for smaller production, but it is more difficult to control the conditions for optimal growth. Biofilm cultivation is a relatively new method that has shown promise for producing high yields of *B. thuringiensis* biomass, but it is still in the early stages of development.

The next step after *B. thuringiensis* biomass producing is purification of the crystalline toxins for use as a biopesticide. Purification typically involves series of procedures, such as filtration, centrifugation, and chromatography, to separate the toxins from the rest of the biomass. The purified toxins can then be formed into a biopesticide product.

There are several different types of crystalline toxins produced by *B. thuringiensis*, each with special mechanism of action against insect pests. The most common types of toxins are the Cry group, which are specific to insect species. For example, Cry1A toxins are toxic to Lepidoptera pests, while Cry3 toxins are toxic to Lepidoptera pests. It is necessary to select the appropriate *B. thuringiensis* strain and grow it under optimal conditions to produce the desired toxin. In addition to producing crystalline toxins, *B. thuringiensis* also produce other metabolites with potential biotechnological applications. For example, *B. thuringiensis* has been shown to produce exopolysaccha-

rides, which have potential in the food, pharmaceutical, and cosmetic industries.

2.4. *Streptomyces avermitilis* cultivation

Streptomyces avermitilis is a Gram-positive soil-dwelling bacteria well known for its ability to produce avermectins, a group of macrocyclic lactone compounds with potent anthelmintic and insecticidal properties [72]. Considerable efforts have been made to optimize the culture conditions of *S. avermitilis* to obtain maximum yields of the target compounds due to the high demand of these compounds in the pharmaceutical and agricultural industries.

The cultivation conditions of *S. avermitilis* vary depending on the desired product and the scale of production. A study by Liu et al. [65] found that the optimal temperature for avermectin production by *S. avermitilis* is 28°C with a pH range of 6.0–7.0. Another study by Guo et al. [66] showed that supplementing the culture medium with trace elements such as copper, zinc, and iron can significantly increase the yield of avermectins [73, 74]. In addition, the use of fed-batch fermentation methods has been shown to improve the yield of avermectins [55].

The choice of substrate for growing *S. avermitilis* is crucial to achieve high yields of the target product. Several authors have investigated the use of different carbon sources, such as glucose, sucrose, and maltose, for the cultivation of *S. avermitilis* [75, 76]. In addition, nitrogen sources, such as soybean meal and corn steep liquor, have also been investigated for their suitability as substrates for the cultivation of *S. avermitilis*.

The composition of the culture medium is crucial for the growth and production of *S. avermitilis*. Tran et al. [69] found that the use of a complex medium containing yeast extract, peptone, and malt extract was most effective for the growth of *S. avermitilis*. However, the use of specific media, such as minimal media, might also be effective for the production of specific compounds [76, 77].

Avermectins purification from *S. avermitilis* culture media is a crucial step in the production process. Several purification methods as solvent extraction, adsorption chromatography and preparative high-performance liquid chromatography (HPLC) [78] have been described. In addition, membrane separation methods such as ultrafiltration and nanofiltration have also been investigated for the purification of avermectins [79].

Optimization of culture conditions, substrate selection, and culture medium composition are crucial for achieving high yields of avermectins from *S. avermitilis*. Furthermore, efficient purification methods are required to obtain pure compounds for pharmaceutical and agricultural applications. Continued research on these aspects will lead to further improvements in

the biotechnological cultivation of *S. avermitilis* and the production of valuable compounds.

2.5. *Streptomyces avermitilis* cultivation

The effectiveness of the integrated use of several types of microorganisms to control a wide range of agricultural pests

The growing demand for environmentally friendly, safe and sustainable methods of pest control has led to the development of bioinsecticides based on several microorganisms, including *Metarhizium anisopliae*, *Beauveria bassiana*, *Bacillus thuringiensis* and *Streptomyces avermitilis*. Use of a single bioinsecticide may not be sufficient to control several pest species simultaneously. So, the integrated application of these microorganisms solves the problem of controlling a wide range of agricultural pests.

The effectiveness of the combined use of various microorganisms for the control of agricultural pests has been investigated. For example, Asif et al. [72] reported that the combination of *B. thuringiensis* and *S. avermitilis* was effective against various insect pests, including *Helicoverpa armigera*, *Spodoptera litura* and *Mythimna separata*. Abdelgaleil et al. [73] reported the combination of *M. anisopliae* and *B. bassiana* was effective against root-knot nematodes (*Meloidogyne spp.*) in tomato plants. Ansari et al. [74] reported the combination of *Metarhizium anisopliae* and *Bacillus thuringiensis* was effective in the control of spruce budworm (*Choristoneura fumiferana*), a major forest pest. Treatment with the combined product resulted in significantly higher larval mortality than the application of these microorganisms alone. Larval mortality in the control variant, where no treatment was performed, was 5-10%, when treated with *Metarhizium anisopliae* mortality was 50%, and when treated with *Bacillus thuringiensis* - 10-20%. However, when treated with the combined preparation, larval mortality increased to 80-100% [80].

Ansari et al. [74] found that combination of *Beauveria bassiana* and *Bacillus thuringiensis* is effective against the cotton bollworm (*Helicoverpa armigera*) pest. Authors reported [75] that they worked out the combination of *Metarhizium anisopliae* and *Streptomyces avermitilis* to be effective against the coffee berry moth (*Hypothenemus hampei*), a major pest of coffee crops.

Another important factor is the dosage and route of applying. The optimal dosage and route of application vary depending on the pest species and the microorganism used. For example, the use of *M. anisopliae* and *B. bassiana* as soil drench at a concentration of 1×10^7 spores/mL has been reported to be effective against root-knot nematodes [81]. Similarly, using *B. thuringiensis* as a foliar spray with concentration of

0.1% (w/v) has been reported to be effective against the following insect pests of maize: green scoop butterfly (*Mythimna separata*), grain scab (*Procecidochares utilis*), and leafworm (*Orosius albicinctus*) [84].

The optimal conditions for the application of complex preparations based on these microorganisms may vary depending on the specific crop and the pest targeted. However, it is generally recommended to apply these bioinsecticides during periods of high pest activity, when conditions are favourable for fungal growth and insect feeding [82, 83].

The effectiveness of complex preparations depends on, for example, use period. For example, applying of *B. thuringiensis* at early stages of pest infestation is more effective than at later stages [84]. Similarly, adding *S. avermitilis* before the pest population reaches a threshold level has been reported to be more effective [85].

The integrated use of microorganisms such as *Metarhizium anisopliae*, *Beauveria bassiana*, *Bacillus thuringiensis* and *Streptomyces avermitilis* is an effective and environmentally friendly solution for controlling a wide range of agricultural pests (Fig. 1). The optimal conditions for their use are the timing of application, dosage, route of administration and form of the insecticide.

The integrated use of microorganisms such as *Metarhizium anisopliae*, *Beauveria bassiana*, *Bacillus thuringiensis* and *Streptomyces avermitilis* is an effective and environmentally friendly solution for controlling a wide range of agricultural pests (Fig. 1). The optimal conditions for their use are the timing of application, dosage, route of administration and form of the insecticide.

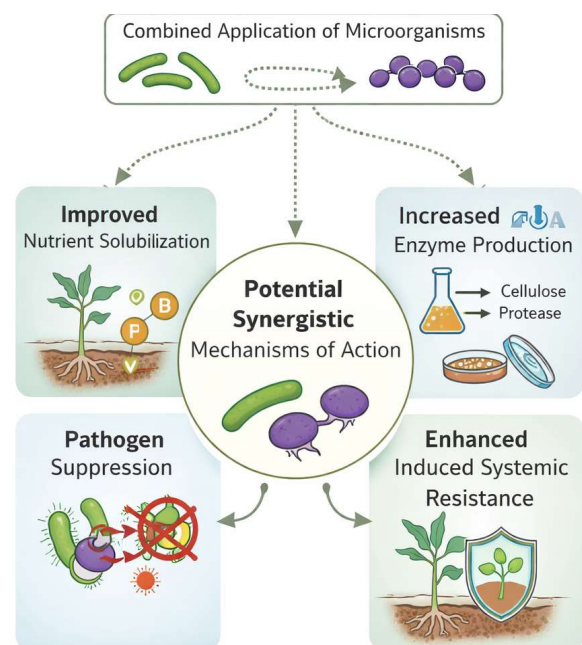


Figure 1. Conceptual schematic diagram illustrating potential synergistic mechanisms of action

Conclusions

As a result of scientific data analysis, it was established that the use of microorganisms *Metarhizium anisopliae*, *Beauveria bassiana*, *Bacillus thuringiensis* and *Streptomyces avermitilis* is a promising direction for the creation of environmentally safe plant protection products. Studies confirm their high efficiency against a wide range of agricultural pests, which makes these bioagents a competitive alternative to chemical insecticides.

It is shown that the efficiency of entomopathogenic fungi *M. anisopliae* and *B. bassiana* largely depends on environmental conditions, in particular humidity, temperature and application method. Under optimal parameters, they reach 30–95% lethality rate for various phytophages.

The high specificity of *M. anisopliae* for individual insect species and the wide range of pests susceptible to *B. bassiana* confirm the feasibility of using these fungi in biocontrol systems. *Bacillus thuringiensis* remains one of the most successful bacterial agents for pest control, primarily representatives of the Lepidoptera order. Its Cry and Cyt toxins provide high selectivity and efficacy, while transgenic plants capable of synthesizing Cry proteins have significantly reduced the use of chemical insecticides.

Streptomyces avermitilis exhibits a wide range of insecticidal and nematocidal activity and is character-

ized by high levels of pest mortality (80–100%) when using avermectin-based preparations. Its effectiveness can be increased by using synergists and adapting application technologies.

The analyzed data on cultivation methods indicate that each of the considered microorganisms requires specific conditions for maximum accumulation of biomass and target metabolites. Optimization of cultivation parameters (temperature, pH, aeration, substrate composition), the use of solid-phase or submerged cultivation, as well as the selection of high-yielding strains are key factors in increasing the effectiveness of biological preparations.

Overall, the results obtained confirm that *M. anisopliae*, *B. bassiana*, *B. thuringiensis* and *S. avermitilis* have significant potential as a components of bioinsecticides. Further research should be aimed at improving cultivation technologies, creating combined preparations and assessing their effectiveness in different agroecological conditions, which will contribute to the development of integrated plant protection systems and subsequently reducing anthropogenic load on the environment.

Interests disclosure

The authors declare no conflict of interests.

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ВИКОРИСТАННЯ МІКРООРГАНІЗМІВ З ІНСЕКТИЦИДНИМИ ВЛАСТИВОСТЯМИ ДЛЯ БОРОТЬБИ ЗІ ШКІДНИКАМИ СІЛЬСЬКОГОСПОДАРСЬКИХ КУЛЬТУР

Інтерес до пошуку нових та екологічно безпечних технологій боротьби зі шкідниками постійно зростає серед фермерів. Використання біологічних пестицидів є найпривабливішим методом захисту сільськогосподарських культур, який є безпечною альтернативою використанню хімічних пестицидів. Враховуючи актуальність методів біоконтролю поширення шкідників, розробка інсектицидів на основі мікроорганізмів та грибів є перспективним напрямком досліджень. Метою статті є аналіз існуючих даних щодо використання ентомопатогенних грибів *Metarhizium anisopliae* та *Beauveria bassiana*, а також бактерій *Bacillus thuringiensis* та *Streptomyces avermitilis* як біоагентів для боротьби зі шкідниками в сільському господарстві. Для всебічного збору інформації про біопестициди ми застосували систематичний та ретельний підхід. Ми використовували широкий спектр електронних джерел, включаючи Google Scholar, PubMed, Scopus (Elsevier), Web of Science, Semantic Scholar, Academia та інші відповідні веб-сайти, для проведення обширного пошуку літератури. Наш аналіз понад 100 наукових статей та інших відповідних онлайн-ресурсів дозволив нам зібрати вичерпний архів відповідної літератури. Цільовими об'єктами мікроміцетів *Metarhizium anisopliae*, *Beauveria bassiana*, а також бактерій *Bacillus thuringiensis*, *Streptomyces avermitilis* є широкий спектр шкідників сільськогосподарських культур, який включає види жесткокрилих та лускокрилих комах, а також нематод. Комплексне використання одразу кількох видів цих мікроорганізмів дозволяє досягти найвищої ефективності в боротьбі як з конкретними видами комах-шкідників, так і розширити перелік шкідників, які є їх цільовими об'єктами. Комплексне використання асоціації мікроорганізмів кількох видів для боротьби з широким спектром сільськогосподарських шкідників є перспективною технологією для використання в аграрному секторі. Необхідні подальші дослідження для вибору оптимальних комбінацій мікроорганізмів для досягнення найвищої ефективності в боротьбі як з конкретними видами комах-шкідників, так і для розширення спектру застосування таких інсектицидів.

Ключові слова: мікробні пестициди; *Metarhizium anisopliae*; *Beauveria bassiana*; *Bacillus thuringiensis*; *Streptomyces avermitilis*.