Archives of Ecotoxicology, Vol. 6, No. 1, pp. 17-21, 2024

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Archives of Ecotoxicology

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Microbial Pesticides and Inoculants Production - A Promising Technology to Chemical Pesticides

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Article info
Received 12 February 2024
Revised 8 May 2024
Accepted 21 May 2024
Published online 29 June 2024

Mini Review

Keywords:
Microbial pesticide,
microorganisms, substrates,
Submerged culture,
solid state culture,
immobilized cell,
batch culture,
Fed-batch culture

Abstract

The conventional agrochemical pesticides tend to be broad-spectrum products that impact many different kinds of organisms. These products allow farmers to control numerous pests with one agrochemical, but can also negatively impact other non-harmful species in the environment. Conventional chemical pesticides can also cause environmental pollution because of the residual chemical. Therefore, Microbial pesticides use microorganisms such as bacteria, fungi, viruses, protozoans and yeasts. Microbes use the toxic metabolites produced to destroy and prevent the growth of pests.They also tend to decompose quickly and leave fewer residues on food and in the environment. Microbial pesticides tend to be highly targeted to specific pests. Because they are so targeted, they are generally considered to be more environmentally friendly than synthetic agrochemicals. For example, *Bacillus thuringiensis* (Bt), a bacteria that is frequently employed as a microbial pesticide comes in a variety of strains and subspecies, and each one is capable of killing a single insect or a small number of closely related insect species while remaining harmless to other creatures (including people and animals). Nevertheless, this review discusses the media for microbial pesticide production, use of organic and inorganic carriers for microbial pesticide production, inoculation of the substrate for microbial pesticide production, use of microorganisms as microbial pesticides, culture techniques for microbial pesticides, and inoculants production, as well as the benefits of microbial pesticides over chemical pesticides.

1. Introduction

The application of plant beneficial microorganisms has been widely accepted as an efficient alternative to chemical pesticides (**Vassileva *et al.*, 2021**). Microbial control of insects offers significant advantage in comparison with chemical pesticide (**Ujvary, 2010**). Insect pathogens are harmless and non-toxic for other forms of life, and hence leave no objectionable residues. Microbial pesticide demonstrates a relatively high degree of specificity and hence tends to protect beneficial insects, such as pollinators, parasites, and predators **(Ujvary, 2010)**. Moreover, a susceptible insect host develops resistance to microbial pathogens only quite slowly, if at all. Microbial pesticide are effectively applied as dusts or sprays, may be introduced or colonized, may be used with chemical insecticides, both compatibly and synergistically, and may be used in combination with parasites and predators **(Charles *et al.*, 1996).**

Infectious diseases have long shown promise as a means of controlling insect pests. By contaminating insects with a pathogenic fungus using a surface culture approach, microbial pesticide has been successful in infecting wheat cockchafer larvae. This method is made to keep all harmful substances, both soluble and insoluble **(Boomsma *et al.*, 2014)**. According to **Samada and Tambunan (2020)**, microbial pesticide refers to the entire culture, which includes the spores, soluble and insoluble metabolic by-products of the microorganisms, and the residue of the nutrient medium on which fungi and bacteria are grown and sporulated **(Samada and Tambunan, 2020)**

Pesticides produced using microorganisms have a higher spore and microbial count than those produced using some prior art techniques. All of the bacteria in the culture, their metabolic byproducts, and the remaining nutritional medium are included in this. Processing yields a product with a high potential for efficacy and no loss of soluble components **(Boomsma *et al.*, 2014)**. The existence of a component that is heat stable, soluble, toxic to insects, and more especially poisonous to insect larvae, distinguishes microorganisms' spores or biomass. The addition of this element is what makes the microbial products' extraordinarily high potency possible **(Berini *et al.*, 2018; Samada and Tambunan, 2020).**

In general, the process of making a microbial pesticide involves the following steps: creating an inoculant of bacteria that produce toxins, such as Bacillus thuringiensis; inoculating a nutrient medium with the bacteria; propagating the bacteria; reducing the moisture content of the bacteria; and grinding the dried bacteria and their metabolic products to a particle size suitable for use as an inseminant **(Yousten and Wallis,1987; Xiao and Wu, 2019).**

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https://doi.org/10.36547/ae.2024.6.1.17-21

2. Media for production of microbial pesticides

Production of microbial pesticides depends heavily on nutrient media that is useful for the growth of bacteria that produce toxins. These media suitably include the protein and carbohydrates needed for sufficient biomass development and toxin generation. Alternately, media primarily composed of necessary vitamin, mineral, and nitrogen-supplying components, such as those found in regular nutrient agar, may be used. A nutritional media that has been proven to be especially helpful in producing the microbial insecticides. Soybean flour 30 to 40 Add dextrose or sugar to fish meal 15 to 20 stale milk 15 to 20 In common with other Bacillus species, Bacillus thuriugiensis, as a result, an acceptable nutrient medium may contain a combination of bran and expanded perlite in place of carbohydrates other than starches **(Guven *et al.*, 2021)**. Such a substrate is used by bacteria that produce toxins to create amylase, which then converts the starch content of the bran into easily digestible carbohydrates like sugars. According to a preferred embodiment, the nutritional substrate and particulate inorganic carrier are coated with the nutrient medium **(Berini *et al*., 2018)**. Bran, wheat middlings, red dog flour, alkalfa meal, corn meal, peanut meal, oat hulls, rice hulls, oatmeal, corn stalks, corn cobs, kudzu vines, sorghum vines, beet pulp, soybean vines, sweet potato vines, sweet potatoes, Irish potatoes, cottonseed meal, and similar materials make suitable organic substrates **(Pandey *et al*. 2007)**. Preferably, vegetable materials used as a carrier for or as a component of the nutrient medium are minced to provide a high ratio of surface area to volume and so promote active bacterial growth. According to experimental data, organic carrier materials like bran provide bacteria (*Bacillus thuringiensis*) with significant amounts of nutrients **(Ujvary, 2010; Ruiu, 2018)**.

3. Use of organic and inorganic carriers for microbial pesticides production

A microbial pesticides culture can be more easily comminuted to a desired mesh size when using inorganic carriers as opposed to equivalent cultures grown on media made completely of organic materials **(Fukui and Tanaka, 1982)**. Expanded volcanic glasses, such as volcanic ash, calcined diatomaceous earth, and similar materials are preferred inorganic carriers **(Vassileva *et al*., 2021)**. These materials are preferably characterized by a high degree of friability, which is necessary to aid the comminution of microbial pesticides.

Combinations of inorganic and organic carrier materials yield the best outcomes. The blends in all appropriate ratios. Preferable mixed carrier media have an inorganic content of 20 % to about 20 % by volume and an organic content of 80 % to about 20 % by volume. Expanded perlite and an organic substance, such as bran, provide up between 80 % and 20 % of the volume of a carrier medium that is particularly suitable **(Vassileva *et al.*, 2021).**

Making the Inoculunt Culture A nutritional medium of the 7 containing sodium hydroxide in about 100 ml, heat-sterilized, and cooled to 28 º C. After adding a pure *Bacillus thuringiensis* culture from a slant tube to the sterilized media, the flask was shaken for eight hours to create a flask culture from which a seed-tank culture was created (**Berini *et al*., 2018).**

4. Inoculation of the substrate for microbial pesticides production

Inoculation of *Bacillus thuringiensis* BAR 3 for co-production of parasporal crystal toxins, antimicrobial substances and insecticidal compounds was investigated by **Chuka-Ogwude *et al*. (2017)**. Either dried spores or tank inoculum can be used to inoculate the substrate. When using dried spores, the moisture is adjusted after the dried spores are inserted, shortly before the mass is put in the sporulation bins. The dried spores should ideally be inoculated at a temperature of around 50 º C because this appears to heat shock the spores and start a more rapid germination. On a dry basis, 300 pounds of substrate require about one pound of 150 BSG materials. Cultivation and optimization experiments of *Bacillus thuringiensis* pre-culture ($1.5x10^7$cfu) were inoculated into 100 mL of medium in 250 mL Erlenmeyer flasks **(Chuka-Ogwude *et al*., 2017).** After inoculation, the temperature is dropped to approximately about 28 º to 34 º C. With either Wet or dry inoculation it is advantageous to carry out the early portions of the sporulation at a suitable and high temperature and later the temperature may be dropped to about 32 º C (Chuka**-Ogwude*etal*., 2017).** The relative humidity of the air used for the second stage should be significantly lower than that of the air used for the first stage. It should advantageously be considerably below 50 to 60 % and be in the range of 10 to 20 %. The second stage treatment's temperature should be greater than 35 º C, ideally between 50 and 55 º C. This encourages faster drying. To avoid or reduce the chance of material contamination with other organisms, the process should be carried out as quickly as possible in both the first and second stages. Use of clean air with little contamination from other organisms that might grow in the mass should be taken into consideration during the sporulation.

5. Microorganisms use as microbial pesticides

What types of microorganisms produce toxins? Bacteria and fungi are typically associated with toxin production. However, it should be noted that not all species of bacteria and fungi produce toxins. Bacteria such as *Bacillus cerus* produce bacterial toxin, *Staphylococcal* spp produce enterotoxin while Fungi, e.g. *Penicillium* expansum and other fungi produce Mycotoxin and Aflatoxin, Ochratoxin and Patulin. **(MFS, 2011)**.

Bacterial pesticides, *Bacillus thuringiensis* (Bt) is wellknown and have been made into products available for commercial purpose **(Ujvary, 2010; Ruiu, 2018)** table 1. *Bacillusthuringiensis* is a Grampositive bacteria that acts as an insecticide by producing exudates, such as poisonous parasporal crystals and endospores which when consumed by insects get dissolved in their midgut by the alkaline environment and release delta-endotoxin, a protein that has a lethal effect on insects **(Xiao and Wu, 2019).** *Bacillus thuringiensis* is used to reduce pest infestation in plants, such as cabbage and potato, and is capable of controlling lepidopterans in different plants **(Berini *et al.*, 2018; Samada and Tambunan, 2020).**

Beauveria bassiana is an example of an entomopathogenic fungus that has been widely used as biopesticide because it is highly efficacious against a lot of arthropod hosts **(Boomsma *et al*., 2014)** table 1. However, to understand their effectiveness and sustainability against pests, there is a need to fully evaluate their molecular mechanism of pathogenicity beyond the conventional approach. The mechanism of pathogenicity of B. bassiana begins with adhesion to the host pest, penetration of cuticle, and colonization of the pest heaemocoel **(Wojda *et al*., 2009).**

A fungi species, *Trichoderma* sp. has been reported to prevent the activity of numerous fungi inhabiting the soil that cause root rot, black gram, and green gram in chickpeas, and groundnu**t (Samada and Tambunan, 2020)** table 1. Likewise, Beauveria bassiana and M. brunneum have been reported in the control of thrips, beetles, weevil, aphids, whiteflies, and mites infestation in ornamental crops, fruits, and vegetables **(Dara, 2017; Arthurs and Dara, 2019)**

Generally, microbial pesticides exert no adverse effects on the environment, producers, and consumers of agricultural products because their ingredients are generally considered safe and are target specific **(Guven *et al.*, 2021)**. In addition, their usage lower greenhouse gas emissions compared to chemical pesticides **(Llamas *et a*l., 2021).** Process for producing microbial pesticides which comprises absorbing on a particulate carrier substrate a nutrient inoculum containing toxins producing microorganisms (Bacillus thuringiensis), the moisture content of substrate is about 55-62% by weight to form substrate-inoculated particles. Incubated at temperature of about 25 ^{o}C to about 35 ^{o}C with relative humidity of at least about 70%, for a period of time from about 25 hours to about 48 hours until substantial sporulation occurs.

Table 1 Summary of organisms use for microbial pesticides production

Bacteria	Fungi	Protozoa	Nematode
Bacillus thuringiensis var. kurstaki	*Beauveria bassiana*		*Steinernema feltiae*
Bacillus thuringiensis var. israelensis	*Trichoderma* sp	*Nosema locustae*	*Steinernema riobravis*
Bacillus thuringiensis var. tenebrinos	*Lagenidium giganteum*		*Steinernema carpocapsae*
Bacillus thuringiensis var. aizawai			*Heterorhabditis heliothidis*
Bacillus popilliae			*Steinernema scapterisci*
Bacillus lentimorbus			
Bacillus sphaericus			

6. Culture techniques for microbial pesticides and Inoculants production

6.1 Submerged culture

Submerged culture process is one of the main biotechnological techniques to obtain microbial biomass or spores, which are further formulated into commercial pesticides. In general, fermentation is the art of mass-cultivation of microorganisms, in the majority of cases using specific media and controlled process parameters, such as temperature, pH, aeration, etc **(Willey *et al.*, 2008)**. During submerged culture process, microbial cells are grown homogenously in a bioreactor with liquid medium under agitation/aeration, using the medium nutrient components and releasing specific metabolites where the metabolites are the desired product. Biomass or spores is produced if any of them is the desired product.The overall biotechnological process depends on the type/form of the final formulated inoculant product. At the end of the fermentation process, microbial biomass and/or spores can further be used to formulate a solid commercial product based on solid carriers **(Vassileva *et al.*, 2020)**. Submerged fermentation processes should be optimized towards high cell/spore density and/or high metabolic activity for producing specific metabolites with plant beneficial properties **(Subramaniam *et al.*, 2018).**

6.2 Fed-Batch culture

Fed-batch, where feeding with substrate and supplements can extend the duration of culture for higher cell densities or switch metabolism to produce e.g. a recombinant protein. Fed- batch culture is one of the biotechnological culture techniques that can be used for bio-inoculants production. It could be the traditional single liquid batch fermentation operation model **(Vassileva *et al.*, 2021)**. Particularly the fed-batch mode of fermentation has been successfully experimented in bio-inoculants pesticides production, although this approach has been more frequently used in other biotechnological processes **(Vassileva *et al.*, 2021**). The fed-batch fermentation operation involves an intermittent feeding of substrate or DO-based and pH-based feeding to ensure a determined rate of consumption when the substrate concentration decreases to a minimum. In the field of plant beneficial microbial production, fed-batch fermentation is an efficient tool for reaching a sufficiently high biomass or high concentration of phyto-stimulating metabolites **(Subramaniam *et al.*, 2018)**. Fed-batch culture method provides approximately 20-fold higher viable cell counts of the microorganism or one fed-batch cycle is equal to 20 single batches **(Sarma *et al.*, 2013).** Manipulating the operational parameters and applying a fed-batch strategy, an increase of cell concentration of Azotobacter chroococcum was observed from 1.54 CFU/mL to 4.21 CFU/mL with a simultaneous energy reduction **(Quiroga *et al.*, 2015).**

6.3 Immobilized-Cell-Based inoculant development

Cell immobilization has been a realiable way of an inoculant development.The immobilization of cells can be defined as "the physical confinement or localization of cells to a certain defined region of space with preservation of some desired activity" **(Vassileva *et a*l., 2021; Karel *et al.*, 1985)**. An immobilized cell system is composed by three components: the cells, the matrix (carriers), where cells are immobilized, and the solution that occupies the rest of the matrix and may contain additives. The methods of immobilization are different, but mainly based on adsorption on solid carriers and (macro/micro)-encapsulation in gels. Immobilized systems are widely used in various biotechnological processes but are still limited for agricultural purposes **(Vassileva *et al.*, 2021).** Biotechnology offers a wide array of techniques leading to bioformulation realiable inoculants: starting from selection of promising microbial strains, characterizing their morphological, physiological and biochemical properties, testing their activity under fermentation and soil conditions and, finally, formulating them into commercial products **(Vassilev *et al.*, 2020)**. Scientific immobilization of toxins producing microorganisms for bioinoculants pesticides production mainly oriented towards isolation and screening of beneficial microorganisms that have the capacity to infect pest but remain nontoxic to humans and the environment. Immobilization of cells offers a number of advantages, such as a greater number of biomass/ cells in one volume unit, the possibility of easier continuous fermentation process, greater metabolic stability and activity, easier downstream operations, particularly in the field of formulation of bio-inoculants, the slow release of cells in soil-plant systems and the possibility to combine different types of bioeffectors in one product **(Fukui and Tanaka, 1982; Vassileva *et al.*, 2021).**

6.4 Batch culture

Batch fermentation is a process where all the substrate and nutrients are added at zero time or soon after inoculation takes place, and the vessel is allowed under a controlled environment to proceed until maximum end product concentration is achieved. In Batch culture, there is no extra feeding from beginning to end of the process. **Yousten and Wallis (1987)** reported on Batch and continuous culture production of the mosquito larval toxin of *Bacillus sphaericus* 2362. In their report, batch culture was used to investigate the production of *Bacillus sphaericus* mosquito larvicide. In batch culture, control of the pH at 7.2–7.3 rather than allowing the normal rise to about 8.6 decreased the toxicity of the cells. Oxygen was required for toxin formation but increasing the level of dissolved oxygen in the medium by use of pure oxygen in the gas stream lowered toxin production. Sporulation and toxin production occurred in continuous culture and were greater at lower dilution rates. However, toxin yield in continuous culture was too low to be a likely alternative to batch culture. Batch fermentation involves the fermentation of the substrate into products by microbial cells. This process occurs within a closed system. Unlike continuous culture, it requires the supply of limited nutrients into the fermentor. Fermentation utilizes a living microbial cell suspension. It produces various valuable products and biomass through fermentation of the substrate.

6.5 Solid-State culture

Solid-state culture is a fermentation process based on substrates in solid forms and carried out in the absence of free water **(Pandey, 2003)**. This mode of cultivation of microorganism has attracted the attention of many scientists because it is in fact a natural process, with high economic potential and high yield of both the biomass and spores inoculant. It can be easily performed in laboratory and industrial conditions to produce various microbial products, including microbial pesticides, while recycling residual agro-industrial materials **(Pandey *et al.*, 2007)**. During the last 20 years, a wide number of studies appeared on the utilization of SSF in the production of plant beneficial microorganisms **(Vassilev *et al.*, 2018).** Although an economic comparison between submerged and sold state culture has not been analyzed and published, it seems that solid state culture is widely accepted as an advantageous fermentation tool in microbial pesticides and biocontrol production (Vassileva *et al.*, 2021). Microorganisms belonging to the same genus have shown better compatibility with each other, hence are extensively used to produce various metabolites. Microorganisms such as *Bacillus cereus* and *Bacillus thuringiensis*, *Aspergillus niger* MS23, and *Aspergillus terreus* MS105, *Clostridium thermocellum* ATCC 27405 and *Clostridium beijerinckii* ATCC 51743, *Aspergillus flavus* and *Aspergillus penicillioides* have been co-existed with other organisms in the solid state culture For efficient products formation like biopesticides and its inoculant development **(Prabhu *et al.*, 2022)**.

7. Advantages of microbial pesticides over chemical pesticides

Biopesticides are usually inherently less toxic than conventional pesticides. Biopesticides generally affect only the target pest and closely related organisms, in contrast to broad spectrum, conventional pesticides that may affect organisms as different as birds, insects and mammals. Biopesticides, also known as biological controls or biocontrols, are organisms used to manage pests or diseases and are most notably used in agriculture. Biopesticides use natural food chains to manage pests and diseases by taking advantage of predator-prey or parasite-host relationships. With this method, growers can sustainably manage crop pests without harming non-target organisms, the environment or humans. The following are the advantages of microbial pesticides:

Effect on non-target species

Biological control products typically target a narrow range of pests or diseases while non-target organisms, such as birds, bees, fish, humans and beneficial soil organisms, remain unaffected.

Pollution

Since biological controls are naturally occurring organisms, at the end of their life they completely biodegrade and leave no harmful residues on the crop or in the environment. This feature helps promote the safety and well-being of people who work on farms and the environment.

Cost

Biological control, as a part of IPM, works to achieve sustainable management of pests and diseases, keeping the pressure well below economically damaging levels. Pests and diseases do not develop resistance to biological controls. Since the rate of application will only change with pest or disease pressure, farmers can accurately predict input costs.

Pest resistance

Records have shown that pests tend to become resistant to conventional pesticides thus proving that it is not a long-term solution, something that never happens with the use of organic pesticides.

Market

As the ordinary consumer became aware of the dangers posed by synthetic chemicals, demand for farm products that have undergone organic treatments rose. This makes the use of these chemicals a potential risk as there's a glaring possibility of incurring huge losses due to the consumer shunning your product.

8. Limitations of microbial pesticides

Limitations of microbial include; high selectivity or host specificity, May require an additional control measures.The correct time of application. Delayed effect or mortality. Storage problem. Difficulty of culturing in large quantities. Short residual effectiveness.

9. Conclusion

The conventional chemical pesticides have helped to reduce a lot of crops pest diseases. However, the negative effect of chemical pesticides limit their use thereby promoting the use of microbial pesticides. Microbial pesticides are produced using cheap raw materials and microorganisms. Though microbial pesticides have some limitations but their advantages prevail over chemical pesticides. Microbial pesticides are usually inherently less toxic than conventional pesticides. Microbial pesticides generally affect only the target pest and closely related organism with no residual effect on the environment.

Acknowledgements

I remain thankful to Enugu State University of Science and Technology, though, there was no funding from the institution.

Declaration of interest

The author report no conflicts of interest.

References

1. Arthurs, S., and Dara, S. K. (2019). Microbial biopesticides for invertebrate pests and their markets in the United States. *Journal of Invertebrate Pathology,* 165: 13–21. https://doi.org/10.1016/j.jip.2018.01.008
2. Berini, F., Katz, C., Gruzdev, N., Casartelli, M., Tettamanti, G., and Marinelli, F. (2018). Microbial and viral chitinases: attractive biopesticides for integrated pest management. *Biotechnology Advance, 36*: 818–838. https://doi.org/10.1016/j.biotechadv.2018.01.002
3. Boomsma, J. J., Jensen, A. B., Meyling, N. V., and Eilenberg, J. (2014). Evolutionary interaction networks of insect pathogenic fungi. *Annual Rev. Entomology*. 59, 467–485. https://doi.org/10.1146/annurev-ento-011613-162054
4. Charles, J. F., C. Nielsen-LeRoux, and A. Dele´cluse. 1996. Bacillus sphaericus toxins:molecular biology and mode of action. *Annu. Rev. Entomo*l. 41:451–472
Chuka-Ogwude, D., Nwuche, C. O. and Ogbonna, J. C. (2017). Co-production of parasporal crystal toxins and antimicrobial substances by *Bacillus thuringiensis* BAR 3. *Nigeria Journal of Biotechnology, 32*: 41 – 52
5. Dara, S. (2017). "Microbial control of arthropod pests in small fruits and vegetables in temperate climate" in Microbial Control of Insect and Mite Pests. ed. L. A. Lacey (Amsterdam: Elsevier), 209–221.
6. Fukui S., Tanaka A. Immobilized microbial cells. *Ann. Rev. Microbiol.* 1982; 36:145–172. https://doi.org/10.1146/annurev.mi.36.100182.001045
7. Guven, O., Aydin, T., Karaca, I., and Butt, T. (2021). Biopesticides offer an environmentally friendly solution for control of pine processionary moth (Thaumetopoea wilkinsoni tams) larvae and pupae in urban areas. *Biocontrol Science and Technology*, 31: 35–52. https://doi.org/10.1080/09583157.2020.1826905
8. Llamas, B., Suarez-Rodriguez, M. C., Gonzalez-Lopez, C. V., Mora, P., and Acien, F. G. (2021). Techno-economic analysis of microalgae related processes for CO2 bio-fixation. *Algal Research*, 57:102339. https://doi.org/10.1016/j.algal.2021.102339
9. Karel S.F., Libicki S.B., Robertson C.R. The immobilization of whole cells: Engineering principles. *Chemical Engineering Science*, 40:1321–1354. https://doi.org/10.1016/0009-2509(85)80074-9
10. Pandey A. (2003). Solid-state fermentation. Biochemical Engineering Journal, 13:81–84. https://doi.org/10.1016/S1369-703X(02)00121-3
11. Pandey A., Soccol C. R., Larroche C. (2007). Current Developments in Solid State Fermentation. Asiatech Publishers Inc.; New Delhi, India: 2007. p. 517.
12. Prabhu, G., Bhat, D., Bhat, R.M. (2022). A Critical Look at Bioproducts Co-cultured Under Solid State Fermentation and Their Challenges and Industrial Applications. *Waste Biomass Valor 13,* 3095–3111. https://doi.org/10.1007/s12649-022-01721-0
13. Quiroga G., Diaz A., Gomez M. (2015). Improving biomass production for two biofertilizers strains of Azotobacter chroococcum at laboratory level. *J. Biotechnol. Biomater*. 5:6. https://doi.org/10.4172/2155-952X.C1.044
14. Ruiu, L. (2018). Microbial biopesticides in agroecosystems. *Agronomy* 8:235. https://doi.org/10.3390/agronomy8110235
15. Samada, L. H., and Tambunan, U. S. F. (2020). Biopesticides as promising alternatives to chemical pesticides: a review of their current and future status. *Online J. Biol. Sci*. 20, 66–76. https://doi.org/10.3844/ojbsci.2020.66.76
16. Samada, L. H., and Tambunan, U. S. F. (2020). Biopesticides as promising alternatives to chemical pesticides: a review of their current and future status. *Online J. Biol. Sci.* 20, 66–76. https://doi.org/10.3844/ojbsci.2020.66.76
17. Sarma M.V.R.K., Gautam A., Kumar L., Saharan K., Kapoor A., Shrivastava N., Sahai V., Bisaria V.S. (2013). Bioprocess strategies for mass multiplication of and metabolite synthesis by plant growth promoting pseudomonads for agronomical applications. *Proceeding Biochemistry*, 48:1418–1424. https://doi.org/10.1016/j.procbio.2013.04.017
18. Subramaniam R., Thirumal V., Chistoserdov A., Bajpai R., Bader J., Popovic M. (2018). High-density cultivation in the production of microbial products. *Chem. Biochemical Engineering*, 32: 451–464. https://doi.org/10.15255/CABEQ.2018.1394
19. Ujvary, I. (2010). "Pest control agents from natural products" in Hayes' Handbook of Pesticide Toxicology. ed. B. T. Ikem (Massachusetts: Elsevier), pp119–229.
20. Vassilev N., Mendes G. (2018). Solid-state fermentation and plant-beneficial microorganisms. In: Pandey A., Larroche C., Soccol C.R., editors. Current Developments in Biotechnology and Bioengineering: Current Advances in Solid-State Fermentation. Elsevier; Amsterdam, The Netherlands: 2018. pp. 402–416.
21. Vassilev N., Vassileva M., Martos V., Garcia Del Moral L.F., Kowalska J., Tylkowski B., Malusá E. (2020). Formulation of microbial inoculants by encapsulation in natural polysaccharides: Focus on beneficial properties of carrier additives and derivates. *Front. Plant Sci*. 11:270. https://doi.org/10.3389/fpls.2020.00270
22. Vassileva M., Flor-Peregrin E., Malusá E., Vassilev N. (. 2020). Towards better understanding of the interactions and efficient application of plant beneficial prebiotics, probiotics, postbiotics and synbiotics. *Frontier in Plant Science*,11:1068. https://doi.org/10.3389/fpls.2020.01068
23. Vassileva, M., Malusà, E., Sas-Paszt, L., Trzcinski, P., Galvez, A., Flor-Peregrin, E., Shilev, S., Canfora, L., Mocali, S. and Vassilev, N. (2021). Fermentation Strategies to Improve Soil Bio-Inoculant Production and Quality. *Microorganisms*, 9(6): 1254. https://doi.org/10.3390/microorganisms9061254
24. Willey J.M., Sherwood L.M., Wolverton C.J. Prescott, Harley, and Klein (2008) Microbiology 7th ed. McGraw-Hill; New York, NY, USA: Applied and Industrial Microbiology; pp. 1049–1088.
25. Wojda, I., Kowalski, P., and Jakubowicz, T. (2009). Humoral immune response of galleria mellonella larvae after infection by Beauveria bassiana under optimal and heat-shock conditions. *Journal of Insect Physiology*, 55: 525–531. https://doi.org/10.1016/j.jinsphys.2009.01.014
26. Xiao, Y., and Wu, K. (2019). Recent progress on the interaction between insects and bacillus thuringiensis crops. *Philos. Trans. R. Soc. B* 374:20180316. https://doi.org/10.1098/rstb.2018.0316
27. Yousten, A. A. and Wallis, D. A. (1987). Batch and continuous culture production of the mosquito larval toxin of *Bacillus sphaericus* 2362. *Journal of Industrial Microbiology, 2:* 277- 283