RESEARCH

Beauveria Bassiana as a Potent Biopesticide for Control of Locust: A Review

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ABSTRACT

Beauveria bassiana can be used as a biological pesticide to control a number of pests such as locust, grasshoppers, whiteflies, termites and other insects. It is spread on affected crops as an emulsified suspension. As a fungal species, Beauveria bassiana parasitizes a wide range of arthropod hosts. However different strains vary in their host ranges, some having narrow ranges, like the strain Bba 5653 which is very harmful to the larva of locust. White Muscardinae disease causing cosmopolitan fungus, Beauveria bassiana, naturally grows in soil and act as a parasite on numerous species of arthropods and thus it belong to entomopathogenic fungi. This fungus is cultured in laboratories under in-vitro conditions and spores are made from this fungus, which are sprayed in crop fields of rice, maize etc. When the microscopic fungal spores acquire contact with the body of an arthropod host, it penetrates the cuticle after germinating, grows inside it, and kills the insects within a few days. This is followed by emergence of a white mold from the cadaver which produces new spores. This paper discusses the role of Beauveria bassiana as a potent biopesticide against locusts.

KEYWORDS: Beauveria bassiana, White Muscardinae disease, Entomopathogenic fungi, Bba 5653, cadavar.

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INTRODUCTION

Locusts cause a havoc every year around the Middle-East and South-East Asian and some North-East African countries. Focusing on our country India, locusts destroy crops like paddy, maize, fodder grass and vegetables. The causatives include exceptional summer breeding and unusually favourable weather (Ramesh & Pandey, 2020). Medical and veterinary pests account for 41% of harmful resistant species whereas, agricultural pests account for a whopping 59%. However, the chemical killing of insects and pathogens cause severe side effects and accumulation of toxins in the human body. Hence, the need-of-the-hour alternative are Biopesticides. Biological methods exploit an insect's natural enemies and include use of insect predators, parasitoids, and pathogens. There are various ways to use an insect's natural enemies as biological control agents for that insect, one of the most common is extensively multiplying pathogens like bacteria or fungi and applying them to the vulnerable or infected area as a biopesticide. Biopesticide composition exhibits effective or more rapid knockdown pesticidal activity, and synergistic pesticidal activity than the conventional chemical pesticide, noting the point that most of the pests have now evolved and become resistant to many pesticidal chemicals.

Beauveria bassiana, despite being the focus of commercial-scale efforts to manage insect pests for the past 20-30 years, in the Russia, France, eastern Europe, China, United States and Canada, it still has a very limited use in India, specially in the lesser developed states (Jaronski & Goettel, 1997). When magnified, it can be useful more than thought of. *B. bassania* has an affinity for Anopheles mosquitoes. So, along with Locusts and pests, it also infects Anopheles mosquitoes which cause Malaria (Darbro *et al.*, 2012)

SPORULATION AND MASS PRODUCTION

Three types of spores produced by *Beauveria bassiana* in culture are - aerial conidia, blastospores, and submerged conidia. Aerial conidia are produced on insect cadavers and on solid substrates. Single-celled hyphal bodies called Blastospores with thin-walls, with varying amounts of mycelium are produced by most isolates in submerged culture. These smooth-walled propagules are larger than conidia, and contain different wall surface carbohydrates (Maheshwari 2008). Blastospores usually germinate much faster than either of the two other forms, 50% germination being reached within 5-6 hours. The fungus builds itself up as an endophyte either naturally, e.g., by stomatal penetration, or with the help of various inoculation methods such as seed coatings and immersions, soil drenches, root and rhizome immersions, radicle dressings, stem injection, and foliar and flower sprays (Sree & Verma 2015). Hence, it is widely recognized as a success in a variety of plants such as grasses; agricultural crops, viz., tomato, cotton, corn, and potato; the medicinal group of plants including coffee, cocoa, poppy, and opium; and trees such as western white pine and *Carpinus caroliniana* (Singh *et al.*, 2015).

Many species have countless resistant populations, of which, each resists many insecticides. Statistical analyses suggest that resistance evolves most readily, particularly for crop pests, in those with an intermediate number of generations every year that feed either by sucking on plant cell contents or by chewing (Karaagac 2012). Besides, the chemical pesticides used for pest control are non-selective and kill other insects beneficial to the crops. In short, chemical pesticides can adversely affect plant life or can upset insect-population balance by killing predators or parasitic insects that control the pest populations naturally. However this shall not be the case with biopesticides. Natural ways of killing will not create such problems.

PATHOGENECITY MECHANISM

Beauveria bassiana (strain Bba 5653) is a ubiquitous fungus that is infectious to a wide variety of insects from most orders. However, different genotypes exhibit separate individual virulence against different insect host species. Strain must be selected carefully, when choosing a potential microbial control agent (Jaronski *et al*., 1997). The insecticidal attribute of *B. bassiana* recommends its use in biopesticide industry with particular reference to the malaria-causing mosquito vector - Anopheles (Singh, *et al*., 2015). Hence, if used, malarial agents can be controlled simultaneously with Locusts and grasshopper species. These fungus are grown under in-vitro conditions and spores are made from this fungus, which are sprayed in crop fields of rice and maize etc. *Beauveria bassiana* secretes enzymes like amylase, protease, chitinase, and lipase when the microscopic spores of the fungus comes into contact with the body of locust, these enzymes penetrate the cuticle and grows inside killing the insects within a matter of days. This is followed by emergence of a white mold from the cadaver which in turn produces new spores (Fuguet *et al*., 2004).

Novel isolates of *B. bassiana* have been recommended for developing an efficient mycopesticide. Essentially, three basic types of formulations have been proposed for *B. bassiana*. First being the Conidia mixed with water, this bioformulation comprising of the fungal conidia (the biologically active form of the fungus) treated with oosporin (produced by submerged culture) provides an effective pest control measure particularly against Cicadellidae, Aphididae, Coleoptera, Delphacidae and Lepidoptera. Second being the Extracted Protein, either as granule, wettable powder, or dust combined with inert materials, such as inorganic or botanical, or in liquid form such as aerosol, foam, gel, suspension, or emulsifiable concentrates. The suggested protein content within the bioformulation ranges from 1 to 60% of the total weight of solids in liquid phase in the liquid formulation, while it ranges from 1 to 95% of the total weight of the pesticide in dry form (Hajek *et al*., 2017). The alternative third type is Endophytic Beauverial colonization, which not only enjoys a broad prey range, including wireworms, bugs, aphids, beetles, termites, and ants, but also inhabits a diverse range of hosts, such as the cash crops and the vegetable crops (Jaronki & Goettel, 1997).

From the conidium, emerges a short hypha which penetrates the cuticle by mechanical pressure combined with enzyme action. The fungus proliferates rapidly within the insect's haemocoel, either by hyphal growth or by multiplication of yeast-like blastospores. Within a few days of infection, the host insect dies; time of death depends upon dose as well as strain-specific pathogenicity. When relative humidity is high, soon after the insect dies, the fungus produces mycelium which quickly cover up the surface of the cadaver, giving rise to conidiogenous cells and conidia, which often appears like a white powder covering the insect (Maheshwari 2008). An illustration of a locust infected with *Beauveria bassiana* causingWhite Muscardinae disease is given below (Fig.1).

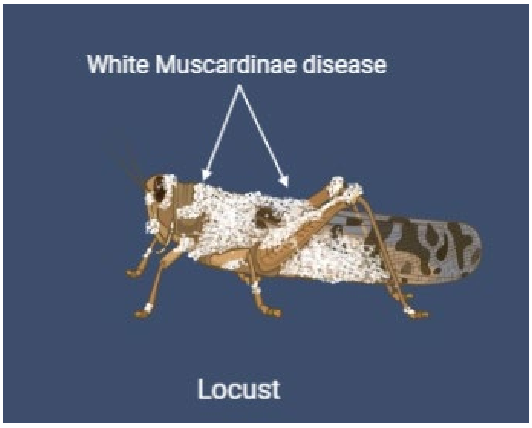

Figure 1. Locust infected with *B. bassiana* causing White Muscardinae disease (white spots).

MATERIALS AND METHOD

Materials and Method of preparing the above given biopesticide is as follows:

Materials:

- Pure culture of fungus (*Beauveria bassiana*)
- Agar media (Here, PAC Media - 300 gm potato, 20 gm dextrose, 20 gm agar agar and 1000 ml distilled water for 1L)
- Chemicals: Dextrose, distilled water, mineral oil (ground nut oil), tween-80 emulsifier mixture.
- Equipments: Incubator, Shaker Seed Fermentor, Grinder, Vibro Shifter, Tank Agitator, Cyclone Separator/Homogenizer.
- Others: Petriplates, conical flask, poly propylene bags, test tubes, Sponge, Sieve (75/300 microns).

Methods:

Step-1 Nucleus culture (Maintain culture)

Pure culture of fungus (*Beauveria bassiana*) is inoculated aseptically on plate having 20 ml PAC-00 agar Media; such two to three plates are generally inoculated. This inoculated plate is to maintained in the BOD incubator at 26 ± 1°C for six to seven days.

Step-2 Starter culture (In flask)

Culture grown on plate is inoculated aseptically in a flask (1000 ml capacity) having 500 ml PAC-00 broth media via cork borer (having 5 mm diameter cutting capacity), and permit it to grow at 26 ± 1°C for a minimum of three to four days. For increased growth, the flask is put for agitation of media on a shaker at 150 RPM. Such two flasks are maintained.

Step-3 Seed Fermentor (In small fermentor)

1 Litre culture grown in step-2 is aseptically inoculated in a small fermenter having 10 litre of PAC-00 media, and allow for further growth at 26 ± 1 °C for at least three to four days on motor for agitation at 100 RPM.

Step-4 Add to solid substrate (poly bags)

From fermenter, 20ml of culture is aseptically inoculated in previously sterilized poly- propylene bag (12 x 10 inch) having 180 gm broken rice grain. After inoculation of culture, seal the opening of the polybag with sponge and allow it to develop. Such 500 bag can be inoculated from 10L broth recovered from fermenter.

Step-5 Allow to develop

After inoculation, each bag is to be transferred to the growth room and allowed to develop at 26 ± 1°C for 9-10 days. During growth (4 days after growth) to break the clump of mycelium developed on grains, all bags are crushed smoothly by hand. After 10 days of growth, all the bags are transferred to cold room after removing the content in trays and are maintained at 5°C till the grain having fungal growth is submerged completely in Ground nut oil.

Step-6 Grinding

After sufficient cooling and drying (in cold room) for 10 to 15 days all the grain with the growth of microbes are put in a grinder or brought to submerge technique wherein all the grains were submerge directly in fixed quantity of Ground nut oil and then by using commercial Tank Agitator, the oil-grain mixture is agitated.

Step-7 Sieving

Ground material is sieved off 75 micron net with the help of Vibro Shifter and the resulting powder is then collected by using Cyclone Separator, designed especially for collection of spore of microbes. For submerge technique, sieve the oil-grain mixture from 300 micron sieve and the collected material which has fungal spores is brought to the Homogenizer.

Step-8 Mixing

10% fungal spore powder is then mixed in mineral oil (ground nut oil) and tween-80 emulsifier mixture (90% ground nut oil+10% tween-80) or in the case of submerged technique, homogenized mixture having fungal spores is again homogenized by adding 10%-15% tween-80 (Patel C. *et al.*, 2010).

Step-9 Curing

Mixed formulation is allowed to develop for two-three days in drums and then sent for storage or use.

ENVIRONMENTAL COSTRAINTS

Although it's generally believed that moisture content may be the requirement for infection, this is not necessarily with several hosts, including locusts. The host insect dies within two to three days; time to death depends upon dose and strain-specific pathogenicity (Jaronski *et al.*, 1997). However, strain selection, formulation, and host targeting play an important role in the development of a successful microbial control agent along with the environmental constraints. The problem lies in the development of bioassay methods that provide information that might be used to predict efficacy under field conditions. Inoculation techniques and environmental conditions adopted should mimic as much as possible those conditions expected at the host level within the field situation. it may be possible to control some of the constraints to consistently high field efficacy through formulation and/or strain selection (Jaronski *et al.*, 1997). Thermoregulation has been implicated in the ability of locusts and flies to overcome infection by entomophthoralean fungi. Locusts thermoregulate by basking in the sun, increasing their temperature up to 18°C higher than ambient and elevation of body temperature may account for lower rates of mycosis in free-living versus caged locusts. Much more effort is needed to identify and evaluate biotic and abiotic factors that influence the development of fungus infections in grasshoppers and locusts, both in naturally occurring infestations, and as a result of artificial multiplication of inoculum. This may be accomplished only through more studies on epizootiology of the disease in grasshoppers and other insects (Kemp 1986).

RESULTS

The propensity of *Beauveria bassiana* to antagonize, parasitize and kill insects, endorses it as an potent biocontrol agent. Biopesticides usually affect not only the targeted pests, but as well as the closely related organism in contradiction to conventional pesticides which may affect organisms as different as birds, insects and mammals. Biopesticide are effective in very small quantities and often decompose quickly. When used as Integrated Pest Management (IPM) programs, biopesticides can greatly reduce the utilization of conventional pesticides, while keeping the crop yield high. *B. bassiana* can be used as a potent biopesticide for controlling wide and large numbers of arthropods. By using this, our dependability on chemical pesticides shall greatly reduce. Once these constraints are known, it is possible to control some of them through novel targeting strategies, strain selection, and/or formulation (Jaronski *et al.*, 1997). Identification of microclimatic constraints would besides allow development of predictive models which would identify plethora of opportunity thereby optimizing efficacious use of these biopesticides.

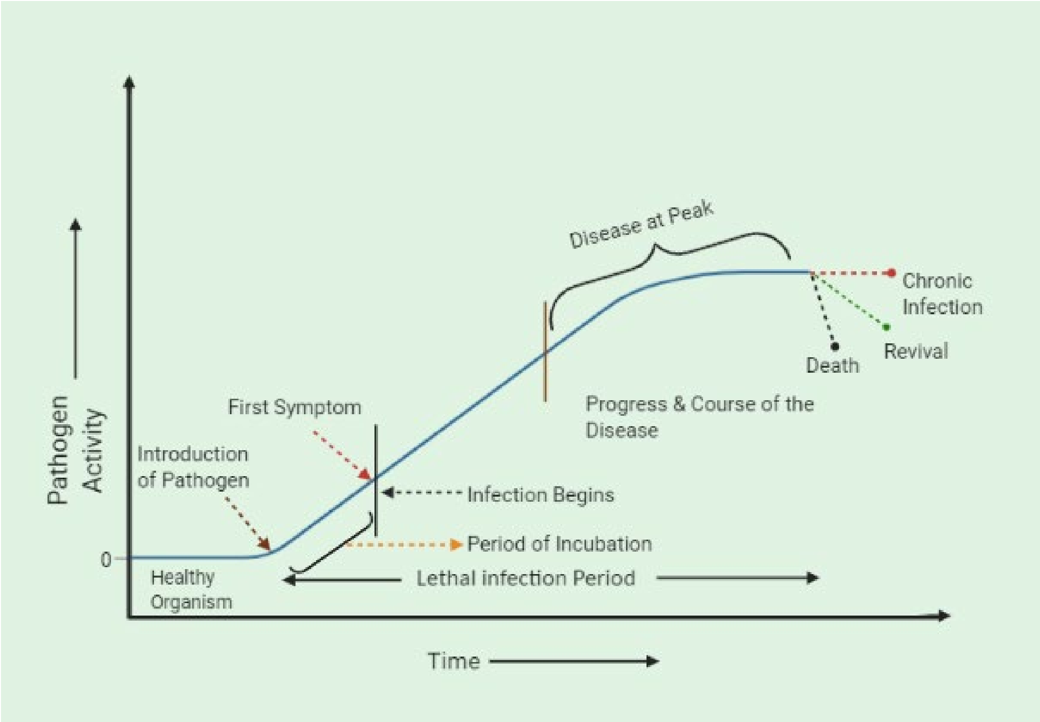

Figure 2. Progress and Course of the White Muscardinae disease in an insect.
When the insect is exposed to a lethal concentration of *B, bassiana* infection starts with its first symptom and intensifies as it progresses towards the peak of the disease within a number of days.

DISCUSSION

The use of chemicals for crop protection has been used for many years but the continuous use of the chemicals led to serious implications not only in human beings but also to the environment. More than 500 arthropod species now show resistance to one or more types of chemicals (MotaSanchez *et al.*, 2002). This has forced the researchers to seek towards to biological control agents. With wide range of applications on arthropod, *B. bassiana* can completely eradicate the use of chemical pesticides. Ultimately, success of *B. bassiana* as a microbial control agent will rely on our ability control some constraints and/or to predict its efficacy under various environmental conditions (Jaronski *et al.*, 1997). The continuous expansion of microbial control with regard to the entomopathogenic fungi, such as *B. bassiana*, is indispensable for promoting sustainable solutions to safeguard food and nutrition security. Genetic selection or genetic recombination for virulent and multistress abiotic tolerant strains coupled with reliable and cost-effective mass production and formulation strategies will contribute to generating resilient, safe and effective *B. bassiana* mycoinsecticides for use in different biocontrol approaches. Nevertheless, *Beauveria* and the other microbial biocontrol agents are not silver bullets and should be implemented within the context of integrated management programs (IPM) for pest control. Intelligent and effective delivery systems will also support the usefulness of *B. bassiana*. As stated by Ehlers 2011, "Since biopesticides are still mirrored in the legislation system designed for chemical pesticides, albeit with less stringent requirements, more favourable regulatory rules are needed to deal with microbial biopesticides in order to reduce time and costs for registration and, hence, promote wider commercial availability especially in the European Union". Due to the growing biopesticide industry and the recent involvement of large agro-chemical companies, a solid foundation is being layed down for progress in biological control strategies using *B. bassiana* and other microbial biocontrol agents (Mascarin & Jaronski, 2016). New findings on the versatile ecological roles displayed by *B. bassiana* with multiple effects add function and value to end users and will facilitate its eventual commercialization as a mycopesticide or as a plant growth promoter (biostimulant). Finally, we believe there is a need for improving education and extension of growers and extensionists about the availability and utility of *B. bassiana* in order to promote its extensive acceptance into overall pest management strategies.

CONCLUSION

Field trials conducted by Stefan *et al.*, 1997 show that *B. bassiana* is an appropriate and favourable microbial control bioagent of locusts and similar pests. However, more efforts are still needed to evaluate the potentials and harms of this fungus fully to commercialise it a biopesticide. A better understanding of veterinary epidermiology and the environmental constraints is needed. The fact which sets fungi apart from bacteria and viruses is it directly parasitise arthropods via penetration of the host integument, while the latter must be ingested to gain host entry (Bailey & Boyetchko, 2010) Currently, a number of pathogen defence-associated gene were revealed in the studies of insect–pathogen interactions. Also, novel pathogens and host response genes are intensively reported thanks to the applications of next-generation sequencing (Douglas *et al.*, 2018). Developing IPM strategies and continuing field studies that include microbial control as an important component will contribute to sustainable management practices.

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Conflicts of interest

The authors declare no conflict of interest.

Authors' contributions

VR conceptualized the draft & MS prepared the draft. AS critically reviewed the draft.

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