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A REVIEW: CHITINOLYTIC BACTERIA AS A POTENT BIOCONTROL AGENT AGAINST AGRI INSECT-PESTS

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ABSTRACT

Importance: Chitin is one of the major component of exoskeleton of insects. Chitin degradation by microorganisms causes severe damage and death of insect-pests and their larvae. In present scenario, the most common method employed by farmers in management of insect pests is use of synthetic chemicals. The application of chemicals in the form of insecticides-pesticides and fertilizers in agriculture results in health hazardous and environment issues. Environmental safety and target specificity are the prime concerns nowadays, when we go for pest control. The use of biological toxins is one of the safest approaches to control the infestation rate in the field of agriculture. In this context, chitinolytic microbes are likely to play a key role as applied biocontrol agent due to their short life cycle and mass culturing in addition to their target specificity. These microbes secrete chitinase enzyme which act as a control mechanism against the insect-pests'. **Observation:** The use of chitinase helps in controlling the infestation rate of insect-pests. A significant reduction in larval and pupal population was observed when chitin degrading bacteria was used in combination with other bacterial genus which all together has a different mode of action. **Conclusion and Relevance:** The use of biological toxin helps in improving the environment health. At the same time the health hazardous risk caused due to subsequent use of chemicals can also be minimized. In addition, by employing organic approach independently, helps in sustenance of beneficial microorganisms and insects and will also improve the soil fertility by increasing the ability to hold water and nutrients.

KEYWORDS: Chitinolytic Bacteria, insect-pests, biocontrol agent, bioassay.

INTRODUCTION

Chitin is a commonly available natural renewable resource next to cellulose in nature.[1] It is a long chain of ß-1-4-linked N-acetyl-D-glucosamine homopolymer.[2] Chitin is the core structural component of many organisms few are, protozoans, fungal cell wall, shells of crustaceans, exoskeleton of insects. The innermost layer of insect fore and hindgut is made up of chitin.[3] The damage induced by chitinase and its over-expression in their gut lining causes significant increase in insect mortality and reduction in nutrient utilization along with the subsequent effect on insect growth.[4]

Chitin degrading microbes utilize chitin as a energy source and this will helpful in recycling these resources in soil natural ecosystem.[5] Bacteria present in soil are excellent source of chitinase and can be used for conversion of chitinaceous waste into several useful products for application in agronomy, biotechnology and drugs and medication.[6,7] Chitinolytic microbes have various applications as a potent biocontrol agent.[8] Bacteria from several genera like *Aeromonas*, *Bacillus*, *Pseudomonas*, *Serratia* and *Streptomyces* occurs frequently in soil and are suitable source of chitinase enzyme.[5]

In biological control, insect parasitoids, parasites and microbes are quite useful and most effective against several pests because of their higher multiplication rate, greater mobility as well as high degree of host specificity.

Long histories of safe use of biological agents are companionable with other approaches of pest control.[9] Facultative pathogens of some insect-pest species are usually used as biological control agents. Large portion of microbial species like viruses, fungi, bacteria, have been identified and exploited as potent bicontrol agents for the control of major portion of insect-pests, responsible for yield loss. One of the best example of microbial pathogen is *Bacillus thuringiensis* which employed more than 90% of the biopesticide marketplace and is virulent to several orders.[10] In 1985, the first evidence of insect resistance against *Bacillus thuringiensis*, the delta endotoxin was reported in farming fields.[11] To overwhelmed the problem of insect

resistance against *Bacillus thuringiensis* based biopesticides, it is essential to lessen the use of alike *Bacillus thuringiensis* strains or different strains having the similar mode of action.

Chitinolytic microbes will become a promising solution to serious issues of insecticide resistance, death of natural enemies, pest resurgence, health hazards, environmental pollution to list a few.

In this review, we aim to analyse the literature on potential of chitinolytic bacteria as a biocontrol agent, a one step forward towards the sustainable agriculture.

Chitinolytic Bacteria: an alternative to chemical insecticides

Mode of action: Pathogenic effects of chitinolytic bacteria generally ensue in insects, after its ingestion in the form of food. As a result host dies within few days as bacteria start multiplying in the gut and produce chitinase enzyme which punctures the peritrophic membrane of larval gut leading to distraction of its membrane integrity causing death of insect.[12] The main symptoms appears with cessation of feeding followed by paralysis. Though, in certain cases inhibition of feeding, causes death of insect within 36-48 hours.[13] The chitinase enzyme is also known to disturb the biology of insect-pests.[4]

CASE STUDY

Chitin based treatments were reported to show strong insecticidal activity against cotton leaf worm, *Spodoptera littoralis* (Boisduval) when blend with artificial diet at 5g kg^{-1}.[14] Among chitinolytic bacteria, *Serratia marcescens* (Grimont) has been reported a good chitinase enzyme producer.[12] The pathogenicity of *Serratia marcescens* and its strains towards the insects is mainly due to production of chitinase and other hydrolytic enzymes.[15]

Among bacterial stains *S. marcescens* is one of the well-known producer of hydrolytic enzymes with multiple chitinase.[16] Interestingly, about 64 per cent of farmers followed at least one of the IPM tools in different agro-ecosystems of India.[17] Perforation of peritrophic membrane was also observed in 5th instar *Spodoptera* larvae when allowed to fed upon a diet blend with recombinant endochitinase encoded by *S. marcescens*.[18] The chitinase from fungus *Tricoderma harzianum* and *Metarhizium anisopliae* showed negatively affected growth and metamorphosis when tested against *Heliothis* and *P. xylostella* larvae.[19] Insect toxicity bioassays with larvae of DBM, showed that chitinase enzyme from *Brevibacillus laterosporus* (Shida) reduced the lethal mortality time upon infection, which contribute to insecticidal activity.[20] The reports were available where *Bacillus* sp. I.5 and *Bacillus cereus* I.21 was mentioned as a potent biocontrol agent against whitefly when used directly on plants in the form of spray.[20] *Serratia entomophila* AB_2 showed 89.5 percent mortality in *P. xylostella* larvae when treated with the formulation containing 0.95×10^7 cells/ml.[19] The bacterial strain *Serratia* sp. EML-SE_1 was reported to cause mortalities in third and fourth instar larvae of DBM.[21] The chitinase used as bio based insecticides are purified from few species and strains of *Bacillus*, *Pseudomonas* and *Streptomyces*.[22] Chitinase from *Bacillus* spp. was found effective against *S. litura* larvae when used singly and in combination with chitinase of *Bacillus thuringiensis*.[4]

Regardless of these many positive findings and ubiquitous nature of insect-pests, there are still a limited number of studies on the effects of chitin as a Integrated Pest Management tool. Reports were also available where chitinase producing bacteria were used with synergistic toxic effect of *Bacillus thuringiensis* against *Plutella xylostella* and *P. brassicae*.[23]

Some of the chitinase producing species previously reported as bio control agent were *Bacillus cereus*,[24] *Bacillus licheniformis*,[25] *Bacillus megaterium*,[26] *Bacillus circulans*,[27] *Bacillus subtilis*,[8] *Bacillus thuringiensis* subsp. *aizawai*,[28] *Bacillus stereothermophilus*.[29] and *Bacillus thuringiensis* sub sp. *krustaki*.[30]

DBM larvae were tested against the *Serratia marcescens* strain MSCP10 and *Staphylococcaceae bacterium* strain MSCW8 showed reduced lethal mortality time upon infection which contribute to insecticidal activity.[31] Several bacteria like *Bacillus*,[32] *Pseudomonas*,[33] *Steptomyces* spp.[34] and *Serratia* spp.[12] were reported to produce chitinase and used as insecticides.

The combination of two *Bacillus* species (*Bacillus subtilis* and *Bacillus thuringiensis* sp *kurstaki*) improves the mortality rate in *Plutella xylostella* and *Spodoptera litura* larvae.[4] Reports were also available where chitinase enhanced the larvicidal activity of *Bacillus thuringiensis* and baculoviruses, when studied in combination.[35] Mahmood et al in their study reported the toxicity of *Xenorhabdus nematophila* strain ATCC19061, a chitinase producer, against the larvae of *Helicoverpa armigera*, a major agriculture pest. The strain also showed the adverse effect on physical growth and development of surviving larvae.[36] Korany et al reported the entomopathogenic efficacy of *Aeromonas hydrophila*, a chitin degrading bacterium against the first instar larvae of *Galleria mellenella* L. They found the significant reduction in larval and pupal mortality rate when treated with different concentrations 185, 205, 235, 265, 295 U/mg of crude chitinase protein.[37] The 100 % damage control in *Bactocera* sp. fruitfly pupae and larvae was observed when tested *invitro* with *Actinomycetes Merubetiri* 1 and 2 isolates and *Actinomycetes* Tomatoes_Pare, chitinase producing filamentous bacteria.[38]

The use of biological toxins seems like the most suitable proposition in the recent concept of Integrated Pest

Management due to its security and safety to human well beings and non-targeted organisms. Microbes and their toxins are pathogenic against several insect-pest species and are easily known to grown on artificial media without the loss of virulence. Chemical pesticides are dangerous and are ruining the health of farmers and are costly too. Subsequent use of lethal chemicals leads to the development of resistance in insect-pests. Keeping these facts in view, scientists are continuously working in area to find out more economical and safer ways to control the deadly and resistant insect pests of agriculture.

CONCLUSION

The overall conclusion drawn from the different studies showed that chitinolytic bacteria are potent biological control agent, known to affect several Lepidopteran pests, which includes a major portion of insect-pests. Their target specificity and environment friendly nature provides a great opportunity for application of these microbes as an insect-pests control agent. Chitinolytic bacteria play a significant role in controlling the infestation rate in agricultural crops. They have the potential to degrade insect chitin and utilize as source of energy. The application of bio control agent in agriculture field also helps in improving the soil texture and fertility. Their efficient use can reduce the cost of expensive available synthetic chemicals. And at the same time reduce the risk of health hazardous issues and provide environment friendly processes as well.

Future Prospective

In future, in-depth study of genes responsible for mortality and fecundity in insect population can be done by advanced techniques. Proteomic studies can also be carried out to check the effect of chitinase enzyme on insect-pests.

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