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Antifungal and insecticidal potential of chitinases: A credible choice for the eco-friendly farming

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ABSTRACT

Numerous biopesticides based on live microbes, their enzymes and other bioactive compounds have been evaluated against different pests in laboratories and also in experimental fields. Many of them are available at commercial scale as an eco-friendly substitute of the hazardous chemicals or synthetic pesticides. Chitinases are one of them, as important biocatalysts with potential to dissolve chitin layer of cell wall of several phytopathogenic fungi and integument of insects. Implications on the use of chitinases as a biopesticide are significantly acceptable but lack of interest of the agriculturalists is the major hitch. This review is critically based on the encouraging and successful studies performed by researchers for the evaluation of biocontrol potential of chitinases alone or in the combination with other bioactive compounds.

1. Introduction

Since many decades, usage of strong chemical pesticides against agricultural pests have been practiced, but still fungi and insects constantly being considered as the biggest threat for damaging cash crops (fruits, cotton, coffee and tobacco etc.) and important staple crops (wheat, rice, maize and potatoes etc.) all over the world. Application of chemical pesticides is widely considered as the reliable and beneficial option for the inhibition/killing of pests. Haplessly, excessive and irrational consumption of pesticides brings toxicity and negative effects to soil and ground water, especially in developing countries (Singh et al., 2014). Many pesticides recognized as the persistent chemicals, bind strongly to the soil particles and remain immobile for a long time. Cleaning of the immobile synthetic pesticides from soil and groundwater is highly cost intensive and many times impossible to clean at large scale. Meanwhile, the effect of synthetic pesticides is lethal on non-targeted organisms that disturb the ecosystem. Adverse effects of the chemical pesticides on non-targeted arthropods have been widely reported. Unfortunately, natural insect enemies e.g., parasitoids and predators are most susceptible to synthetic insecticides and are severely affected (Gill and Garg, 2014). On the other hand, it has been reported the consistent exposure of human body to sub-lethal quantities of chemical pesticides for a long run (months to decades), causes some chronic illness like different kinds of cancers, negative impacts on nervous, reproductive, renal, cardiovascular, and respiratory systems (Table 1) (Pan-Germany, 2012; Gill and Garg, 2014). Entire dependence on the hazardous chemical pesticides for the future agricultural purposes mean further loss of the soil quality, more possibilities of water contamination and unsustainable burden on the fiscal system.

Apart from synthetic pesticides, several other proper options are also available that work eco-friendly, biodegradables, and cost effective too; these are microbes or their products used as biopesticides with an exclusive selectivity towards the targeted harmful pests (Berini et al., 2019). Chitinases are one of them, harmless for the plants and vertebrates, which do not possess chitin $(C_8H_{13}O_5N)n$ in their tissues. Chitinases belongs to the class of hydrolytic enzymes with a potential to inhibit or degrade the chitin containing microorganisms like fungi and insects. Chitin is present in different organisms providing the structural framework and shape but always associated with other bio-molecules. Chitin microfibrils combine with other sugars, proteins, glycoproteins and proteoglycans to form fungal septa and cell wall as well as arthropod cuticles. In animals, chitin is associated with proteins, while in fungal cell wall it is associated with glucans, mannans or other polysaccharides. In fungal wall, chitin bound covalently to the glucans, either directly or via peptide bridges. In insects and other invertebrates, the chitin is always associated with specific proteins, with both covalent and non-covalent bonding (Roberts, 1992; Prabu and Natarajan, 2012). Regarding the mode of action, chitinases can be differentiated widely as

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Table 1
The List of chronic diseases that are linked to the exposure of human body to synthetic Pesticides (Gill and Garg, 2014).

S. No.	Nature/Class of pesticides	Diseases caused by the synthetic pesticides	References
1	Triazine-herbicides (atrazine, simazine, propazine and degradants)	Reproductive disorders	Greenlee et al. (2003)
2	Organophosphate, organochlorine, carbamate, and pyrethroid	Hormonal imbalances including infertility and breast pain	Xavier et al. (2004)
3	Organophosphates and carbamate	Respiratory diseases (Asthma, Chronic obstructive pulmonary disease (COPD))	Chakraborty et al. (2009)
4	Agrichemicals like nitrates, atrazine and other pesticides	Birth defects	Winchester et al. (2009)
5	Arsenic and halogenated compounds	Cancer (childhood and adult brain cancer; renal cell cancer; lymphocytic leukaemia (CLL); prostate cancer)	(Heck et al., 2010; Cocco et al., 2013)
6	Organophosphate and organochlorine pesticides	Neuro degenerative diseases including Parkinson and Alzheimer disease	(Hayden et al., 2010; Tanner et al., 2011)
7	Organochlorine pesticides	Diabetes (Type 2 Diabetes)	Son et al. (2010)
8	Organophosphate pesticides	Cardio-vascular disease including artery disease	Andersen et al. (2012)

endochitinases, which splits the chitin randomly at internal sites, or as exochitinases that remove monomers or dimers (chitobiosidases) of N-Acetyl-D-Glucosamine (GlcNAc) from the non-reducing end of the chitin (Adrangi and Faramarzi, 2013; Oyeleye and Normi, 2018; Zhou et al., 2019). The use of chitinases as a biocontrol agent is one of the attractive and environmentally safe strategies (Singh et al., 2008, 2011, 2014; Okongo et al., 2019). In current scenario, biological control should be viewed seriously as an important component of the integrated disease management (IDM), the permanent reduction of synthetic pesticides will come only if farmers will be inclined more towards the use of biopesticides. In present review, we have critically documented the recent studies performed inside and outside of the laboratory for the evaluation of the biopesticidal impact of chitinases.

2. Mechanism of the chitinases as a biocontrol agent against fungi and insects

Based on the cleavage of chitin by chitinases, we can classify them into two forms, exo and endo chitinase (Fig. 1). The exochitinases have characteristics of a deep substrate binding cleft and processivity, while shallow binding cleft of endochitinases makes the reaction non processive (Frederiksen et al., 2013). A complete enzymatic degradation of insoluble chitin to free N-acetylglucosamine (GlcNAc) is performed by the complex of chitinases (Subbanna et al., 2018). Antifungal potential of chitinases depends on the morphology of the fungal cell walls i.e. very complex (Fig. 2). In many cases, chitin embedded in the cell wall in such a manner that easily can expose to the biocontrol chitinases. But in all cases of the fungi, it's not feasible; chitin layer is not always exposed to the chitinases as a result such a fungus species remains as the chitinase resistant fungi. Yan et al. (2008) demonstrated the mechanism of the purified recombinant rice chitinase from *Pichia pastoris*. This enzyme could efficiently inhibit the growth of *Rhizopus stolonifer* and *Botrytis squamosa* but it had no major destructive impact on *Aspergillus niger* and *Pythium aphanidermatum*. In fact, rice chitinase showed different spectrum of antifungal activities due to the surface of microstructure and the proportion of chitin in the fungal cell wall of these fungi. Enzyme had the more possibilities to interact with the chitin present in fungal cell wall when the scale-shaped structures were arranged in a manner allowing exposure of chitin fiber bundles on the surface of fungal cell wall.

In case of adult insects and their larvae, peritrophic membrane and exoskeleton work as a physicochemical barrier (Fig. 3) to protect against environmental hazards and predators. Both are composite materials and made up of chitin, protein, lipids, catecholamine metabolites and traces of minerals. However, some entomopathogenic fungi (such as *Metarhizium anisopliae*, *Beauveria bassiana*, and *Aspergillus flavus)* and bacteria (both Gram positive and negative prokaryotes) have overcome these kinds of barriers by producing multiple extracellular degradative enzymes, including chitinases and proteases causing the fatal infection (Kramer and Muthukrishnan, 1997). Working mechanism of chitinase shown in, Fig. 4, (Chandrasekaran et al., 2014). Tu et al. (2010) isolated the chitinase producing *Serratia marcescens* GEI strain from the gut of workers of Chinese honey bee *Apis cerana* and evaluated in laboratory to control insect parasite, *Varroa destructor* of western honey bee *Apis mellifera*. The supernatant and the collected proteins by ammonium sulfate precipitation from the bacterial culture showed 100% mite mortality on the female mites in 5 days. The workers of both honey bee species were not sensitive to the spraying and feeding of chitinases. Zhong et al. (2015) isolated the *Pseudomonas* sp. strain TXG6-1 from soil

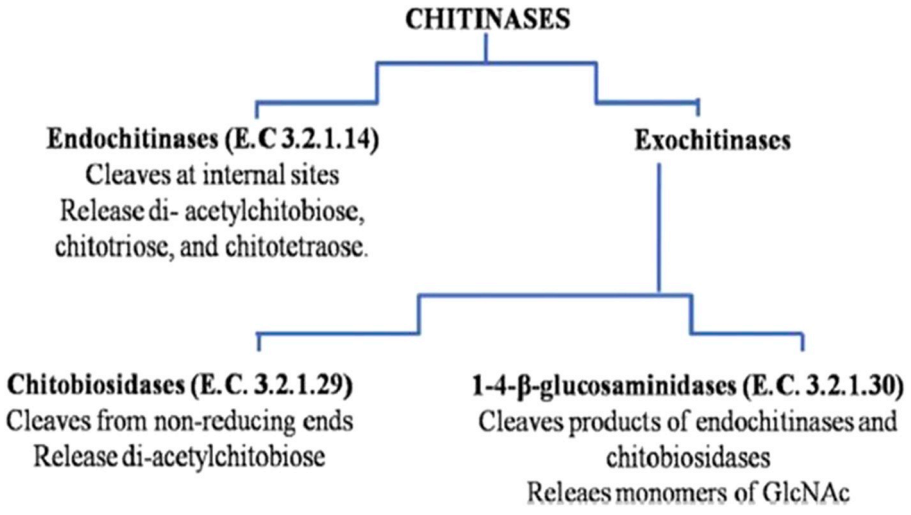

Fig. 1. Classification of chitinases based on the mode of their action (Subbanna et al., 2018).

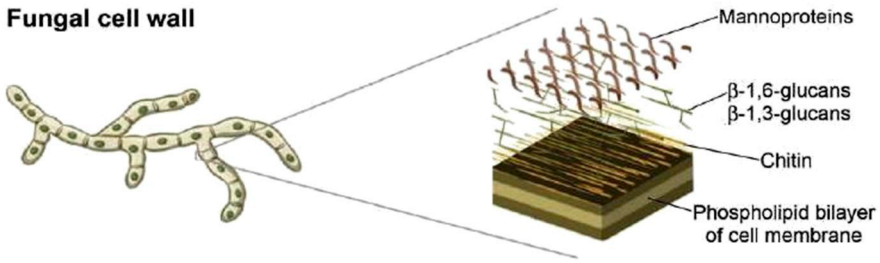

Fig. 2. General structure of the fungal cell wall (Berini et al., 2018).

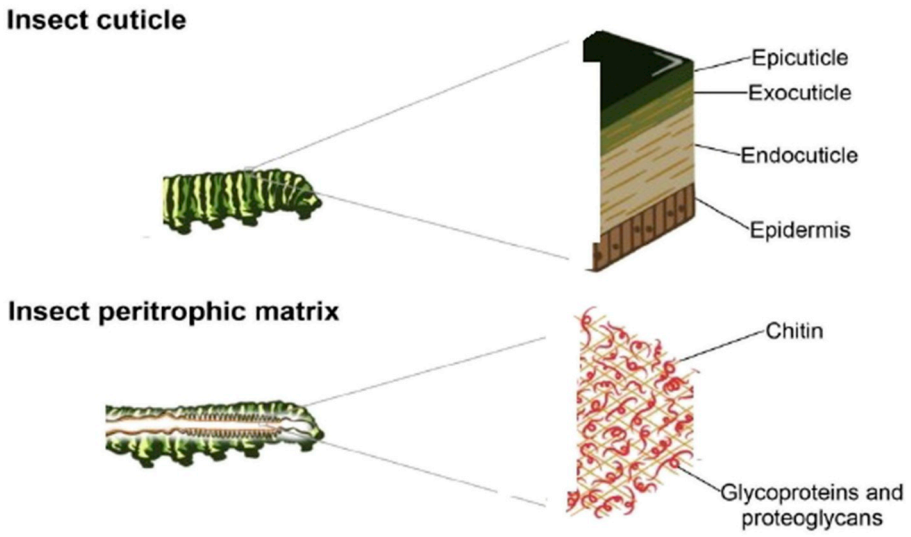

Fig. 3. Presence of chitin layers (cuticle and gut) in case of insects (Berini et al., 2018).

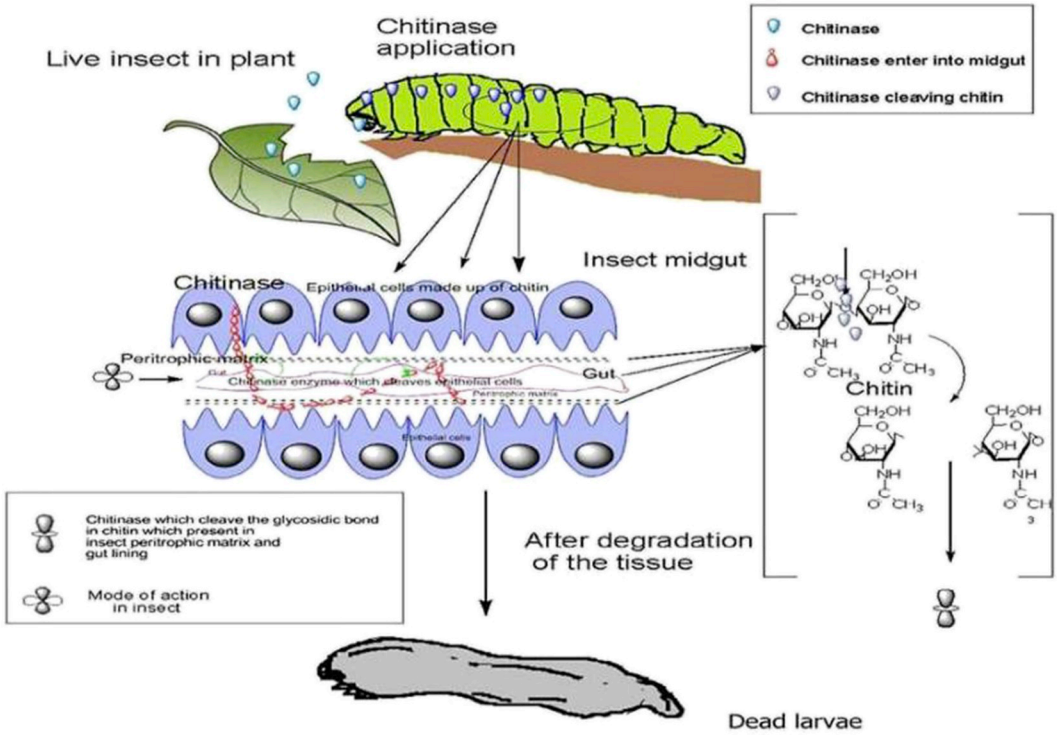

Fig. 4. Chitin hydrolysis by chitinases in the case of insect biocontrol (Chandrasekaran et al., 2014).

and purified the chitinase enzyme (PsChiC) of 52 kDa. The PsChiC alone did not express the potent insecticidal effect but in presence of *Spodoptera litura* multicapsid nucleo-polyhedro virus (SpltNPV) increased the insecticidal impact against the cotton leaf worm, *Spodoptera litura*. Suganthi et al. (2017) produced and purified a chitinase from *Pseudomonas fluorescens* and observed 100% mortality against *Helopeltis theivora* (tea mosquito bug) within 96h. Enzyme was sprayed on the fresh tea shoots in controlled environment then insects were allowed to feed on these shoots. Mortality was confirmed through the shrunken abdomen, because midgut of the insects covers with a layer of chitin.

3. Occurrence of the chitinases and biocontrol potential

Chitinases are available and frequently reported from prokaryotes, fungi and plants, purification of chitinases and structural insights have been well documented in the literature. But practical applications of the enzyme at experimental farming fields are very scarcely reported (Singh et al., 2014). In present discussion we have taken only the instances of those chitinases and their sources that were evaluated for antifungal or insecticidal impacts at laboratory scale or applied on the agricultural fields.

3.1. Prokaryotes as a source of potent chitinases

Since many years, several bacterial species including *Actinomycetes* have been investigated for the synthesis and characterization of chitinases for their various applications. Chitinases from prokaryotes vary widely in their size, ranging from 20 to ~90 kDa. They are found to be active over the wide range of temperature and pH, depending on the source of bacteria from which they have been isolated (Singh et al., 2014; Subbanna et al., 2018). *Streptomyces coelicolor* A3(2) contained the 13 chitinase expressing genes, 11 of them were coding for the enzymes belongs to family 18 chitinases and two enzymes belonged to family 19 of chitinases. Among them, Chi19F showed the inhibition of hyphal elongation of *Trichoderma reesei, Trichoderma viride, Mucor javaniccus* and *Fusarium solani*, on PDA media at pH ~5.5, 30 °C. The antifungal activities in family 18 of chitinases were not so effective, but Chi18bA slightly inhibited the growth of *Trichoderma reesei, Trichoderma viride, and Mucor javaniccus* (Kawase et al., 2006). Bacterial isolates of *Bacillus licheniformis, Stenotrophomonas maltophilia,* and *Bacillus thuringiensis* were isolated from the soil of rhizosphere of undisclosed plant. Among them, *B. licheniformis* revealed a potent antifungal activity and suppressed *Rhizoctonia solani, Macrophomina phasiolina, Fusarium culmorum*, *Pythium* sp., *Alternaria alternata* and *Sclerotium rolfsii* by inhibiting their hyphal growth on PDA media at 28 °C (Kamil et al., 2007). Chitinase producing *Streptomyces vinaceusdrappus* S5MW2 was isolated from Chilika lake, India. S5MW2 showed an antifungal activity against *Rhizoctonia solani*. The isolate S5MW2 also worked as a plant (tomato) growth promoting factor when evaluated in the greenhouse experiments in presence and absence of colloidal chitin (CC). Presence of CC showed the better results because chitinases are inductive enzymes not constitutive (Yandigeri et al., 2012). Chitinase was produced and purified from the *Bacillus pumilus* MCB-7, crude as well as purified biocatalyst showed the antifungal activity against agriculturally important fungi like *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Ceratorhiza hydrophila* and *Fusarium oxysporum*. The chitinase also showed a biopesticidal potential against the larvae of *Scirpophaga incertulas* (Walker), (Lep.: Pyralidae), a severe farming pest of paddy (Rishad et al., 2016). The *Serratia marcescens* strain ETR17 synthesized many hydrolytic enzymes like chitinase, protease and lipase along with the plant growth promoting metabolites IAA and siderophore. Bacterium was not saprophytic not the pathogenic as confirmed by the non hemolytic colonies observed on the blood agar. As this bacterium was isolated from the rhizosphere of tea plant, it was also an effective against the root rot disease caused by *Rhizoctonia solani* in tea plants. A talc powder formulation containing bacterial isolate showed a significant effect to reduce the root rot disease in tea and to stimulate the plant growth also *in vivo* studies because of providing stability and feasibility in the transformation of the microbes (Purkavastha et al., 2018). There were four bacilli (*Bacillus simplex* 30N-5, *B. simplex* 11, *B. simplex* 237, and *B. subtilis* 30VD-1) evaluated for the plant-growth promoting (PGP) and biocontrol potentials. It was observed *B. subtilis* 30VD-1 showed the most effective antagonism against *Fusarium* sp. on plate assays. Additionally, 100 mg/ml of the crude 1-butanol extract of 30VD-1's cell-free culture filtrate demonstrated 40% inhibition in radial growth of *Fusarium* sp. Pea seed bacterization with 30VD-1 led to considerable reduction in wilt severity in plants about 35% increase in dry plant biomass over uninoculated plants growing in *Fusarium*-infested soil. Phase/contrast microscopy demonstrated distortions and abnormal swellings in *F. oxysporum* hyphae on co-culturing with 30VD-1(Khan et al., 2018). Seven chitinases were produced from the bacteria and the fungi with biocontrol abilities and their potentials were evaluated against the graminaceous stem borers, *Eldana saccharina*, *Sesamia calamistis* and *Chilo partellus*. All chitinases were stable at wide range of pH and temperature, but recombinant fungal chitinases were found acid-stable than the bacterial enzymes. Chitinases from the thermophilic fungi *Thermomyces lanuginosus* SSBP (Chit1) and *Bacillus licheniformis* (Chit lic) caused 70% and 80% mortality, respectively, in the second instar larvae of *E. saccharina*. Six of the seven partially-purified microbial chitinases inhibited *Aspergillus niger, A. flavus, A. alliaceus, A. ochraceus*, *Fusarium verticillioides and Mucor* sp. (Okongo et al., 2019).

3.2. Fungi as a source of the potent chitinases

Chitinase synthesizing fungi (*Conidiobolus coronatus* NFCCI 1235, *C. couchii* NFCCI 719, *C. coronatus* NFCCI 718, *Basidiobolus haptosporus* NFCCI, 1922 and *Basidiobolus haptosporus* NFCCI, 1923) were isolated from the two different genera of fungus, *Conidiobolus* and *Basidiobolus*. *B. haptosporus* NFCCI 1922 hydrolyzed the mycelia of tested fungal isolates as a major carbon source to produce chitinase. Maximum hydrolysis of fungal mycelia was noticed with the *Aspergillus niger* after 72h (Mishra et al., 2011). Chitinase produced by the *Trichoderma asperellum* UTP-16 inhibited the mycelial growth of *Fusarium oxysporum, F. ciceri, F. solani and F. udum* confirmed by the well diffusion assay technique (Kumar et al., 2012). The isolate B.bAT17 of *Beauveria bassiana* highly pathogenic against engorged *Rhipicephalus* (Boophilus) (R. B) microplus females, resulting in lethal time (LT50 and LT90) of 7.14 and 9.33 days at a concentration of 10^9 conidia/ml. *R. (B.) microplus* females treated with *B. bassiana* B.bAT17 significantly had a lower the amount of ovipositioning and most ticks died before they could begin to oviposit. Proteases and chitinases were analyzed in order to establish a screening method for identification of high virulent strains (Sun et al., 2013). The *Trichoderma asperellum* GDFS1009 exhibited the high growth rate, more sporulation efficiency and strong inhibitory properties against the pathogens that cause cucumber fusarium wilt and corn stalk rot. GDFS1009 synthesized the chitinase, glucanase, and protease, which can hydrolyzed the fungal cell walls. Xylanases were also produced and found better candidates to induce the plant resistance and to enhance plant immunity against pathogens. RNA sequencing (RNA-seq) and gas chromatography-mass spectrometry (GC-MS) showed that GDFS1009 secreted primary metabolites that are precursors of many antimicrobial compounds; this fungus also produced a variety of antimicrobial secondary metabolites, including polyketides, alkanes and six peptides (Wu et al., 2017). Recently, Alves et al. (2018) characterized a ~44 kDa chitinase from *Aspergillus niveus* works well at 65 °C and pH 5.0. This enzyme showed an antifungal potential against *Aspergillus niger*, *A. fumigates*, *A. flavus*. *A. phoenicis* and *Paecilomyces variotii*. Also, it was stable in many organic and inorganic compounds.

3.3. Plants as a source of potent chitinases

Plants do not have the immune system as they are vulnerable to the

fungal and insect pathogens, but many plants contained the antifungal chitinase genes. A chitinase gene from wheat plant was subcloned and expressed in *Escherichia coli*. Purified (33 kDa) recombinant wheat chitinase exerted a broad-spectrum antifungal potential against *Colletotrichum falcatum* (red rot of sugarcane), *Pestalotia theae* (leaf spot of tea), *Rhizoctonia solani* (sheath blight of rice), *Sarocladium oryzae* (sheath rot of rice) *Alternaria* sp. (grain discoloration of rice) and *Fusarium* sp. (scab of rye) (Singh et al., 2007). Generally chitinases belongs to the grains functioned as endochitinases and also contained lysozyme activity. In contrast, the bacterial chitinases acted as the exochitinases (Roberts and Selitrennikoff, 1988). Many chitinases have been purified from different plants and their molecular weights were found between 25 and 40 kDa, best working pH (4.0–6.0) and temperature (50–60 °C), several of them also showed strong antifungal activity against phytopathogenic and non-pathogenic fungi (Malik and Preety, 2019). The recombinant chitinase of 35 kDa from barley significantly inhibited the severe phytopathogenic fungi *Alternaria solani*, *Fusarium* spp, *Rhizoctonia solani* and *Verticillium dahliae* (Toufiq et al., 2019). Barley-derived chitinase gene was proved as an excellent candidate that can be used for generation of fungal resistant sugar cane plants. The crude protein extracts from the transgenic sugarcane plants SCT–15 and SCT–20 inhibited mycelial growth of *Colletotrichum falcatum* by 56%, and 52%, respectively. Further, transgenic lines SCT–15 and SCT–20 revealed the strong resistance against inoculated *C. falcatum* during *in vitro* bioassay, as they remained healthy and green in comparison to the control sugarcane plants, which turned yellow and finally died on third week after infection (Tariq et al., 2018).

4. Conclusion

Presence of chitin in insects and fungi make them logical target of chitinases. Several reports on potent chitinases are available in the literature but only few of them pertaining to their detailed studies applied as biopesticides in the agricultural fields. In the current scenario, there is a need of more field trials of novel chitinolytic bacteria and their enzymes as a biopesticide against the pests.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.bcab.2019.101289.

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