
# Asian Journal of Biochemistry

ISSN 1815-9923

Academic
Journals Inc.

www.academicjournals.com

Asian Journal of Biochemistry 6 (1): 29-37, 2011
ISSN 1815-9923 / DOI: 10.3923/ajb.2011.29.37


# Role of Chitinase in Plant Defense

N. Sharma, K.P. Sharma, R.K. Gaur and V.K. Gupta
Department of Biotechnology, MITS University, Lakshmangarh, Rajasthan, India

*Corresponding Author: Dr. Vijai Kumar Gupta, Department of Biotechnology, MITS University, Lakshmangarh, Rajasthan, India Tel: 00919352215170*

## ABSTRACT

Plants represent the major component of biota and have the capability to synthesize their food through the process of photosynthesis. Physiological and environmental changes affect their health and make them vulnerable to variety of diseases thus directly or indirectly affect other components of ecosystem. A large number of environmental issues are linked with the eradication of plant diseases with chemical compounds. Most of these diseases are caused by fungal and insect pathogens. Chitin is the main structural component of these organisms and thus the enzyme responsible to hydrolyze chitin content are receiving attention in regard to their development as biopesticides or chemical defense proteins in transgenic plants and in microbial biocontrol agents. Therefore, understanding the overview of chitinase will provide a basis for improving the pathogenic activity of potential biocontrol strains, for developing novel biological control strategies and for exploring their roles in the plant defense. The present review describes the properties of chitinase with respect to plant health improvement.

**Key words:** Chitinases, plant defense, genetic engineering

## INTRODUCTION

A multitude of microorganisms are the pathogens of plant species worldwide (Hopkins and Purcell, 2002). Crop losses due to pathogens are often more severe in developing countries (e.g., in cereals, 22 percent of total loss) and is it is roughly estimated an equal to loss of 200-300 US$ billion money on the basis of data provided by Oerke *et al.* (1994). The most regular methods used to protect plants rely on the chemical compounds those are available in various forms such as insecticide, fungicide etc. The major problem associated with the use of such compounds is the loss natural microflora of the soil which could be involved in the protection of plants against infection caused by secondary pests. Besides this, the generation of insecticidal resistance, safety risks for human and wildlife, contamination of groundwater and riparian habitats and decrease in biodiversity are other key issues which were commonly seen with the advent these compounds and need to be thought prior to their use. The public concern over the harmful effects of these chemical compounds on the environment and human health has enhanced the search for safer, environment friendly control alternatives. Thus control of plant pathogens like fungus and insect by the application of biological agents holds great promise as an alternative to chemicals in this context.

Plants are equipped with a variety of defense mechanisms to protect themselves against the attack of pathogens. Some of these are constitutive while others are induced upon the attack by pathogens. The interaction between plants and pathogens induces a variety of defense mechanisms which includes cell wall strengthening (Bradley *et al.*, 1992), de novo production of antimicrobial

compounds (pathogenesis response proteins and secondary metabolites (Hammerschmidt, 1999; Misra and Gupta, 2009; Gupta *et al*., 2010a) and rapid localized cell death etc. (Alverez, 2000). In the category of pathogenesis related proteins chitinase and glucanse (Sela-Buurlage *et al*., 1993) have very important role since they attack directly on the fungal and insect structural component. Besides these two, other enzymes of plant secondary metabolite pathway including Chalcone synthase and isomerase (Hahlbrock *et al*., 1981) Phenylalanine ammonia lyase (Cramer *et al*., 1985) are also significant due to antimicrobial nature of secondary metabolites.

Chitinase attack on chitin molecules which are the main structural component in fungal cell wall and insect's skeleton. In nature chitin is found to be in the form of complex with other biomolecules such as carbohydrates and proteins (Sietsma and Wessels, 1979). In Sponges it forms a complex with silica (Ehrlich *et al*., 2007). In arthropods, chitin is an integral part of skeleton (Merzendorfer and Zimoch, 2003) and digestive tract lining (Lehane, 1997). It is known to be present in the eggshell (Mansfield *et al*., 1992) and microfilarial sheath (Fuhrman and Piessens, 1985) of nematodes also.

In these organisms chitin metabolism is directly controlled by the activity of Chitin Synthases (CS) and Chitin Hydrolase (CH). Three types of chitin synthase were observed in *Saccharomyces cerevisiae*: CS I which is involved in repair functions at the end of cytokinesis; CS II, participating in the synthesis of primary septum and CS III, responsible for the formation of the ring or bud scar (Henar *et al*., 1999). Besides these enzymes, the recycling of cell wall components also depends upon the activities of a range of hydrolytic enzymes found intimately associated with the fungal cell wall. Most of the fungal cell wall hydrolases characterized to date belong to chitinase, glucanase and transglycosylase family (Adams, 2004). It is generally believed that chitinases are commonly found in organisms which are possessing chitin as a structural component.

The enzyme chitinase (EC 3.2.1.14) hydrolyzes the chitin polymer into to N-acetyl glucosamine by either endo or exo cleavages of the 1-3 and 1-4 bond (Van Aalten *et al*., 2000). The enzyme is classified into several categories on the basis of their isolation, structural and functional characteristics. It belongs to families 18 and 19 of glycosyl hydrolases (Henrissat and Davies, 1997) which are key enzymes for carbohydrate metabolism (Henrissat, 1991). Most of the prokaryotic and eukaryotic chitinases are grouped in Family 18 whereas chitinase of higher plants and some of the Gram positive bacteria like *Streptomyces* are included into Family 19 (Cohen-Kupeic and Chet, 1998). These two families contain both endo and exo chitinases. Endochitinases cleave randomly in the chitin chain while exochitinases cleave off chitobiose $(GlcNAc)_2$ or chitotriose $(GlcNAc)_3$ from the reducing or non reducing end of the chitin chain (Suzuki *et al*., 1999). In addition to endo- and exochitinases, chitin degrading organisms contain chitobioses (N-acetyl β-glucosaminidases), a third class of chitinolytic enzymes that convert GlcNAc dimers into their monomers (Tews *et al*., 1996a, b).

Chitinase was described for the first time in 1911 by Bernard in orchid bulbs in which it behaves like a thermo sensitive and diffusible antifungal factor. In animals the presence of chitinase was marked in snails by Flach *et al*. (1992). Since then these molecules are unanimously considered as a tool to strengthen plant immune response against a variety of pathogens by various workers owing to its property to lyse fungal cell wall and components of insect exoskeleton. Besides this, dramatic increase in chitinase levels by numerous abiotic agents (ethylene, salicylic acid, salt solutions, ozone, UV light) and by biotic factors (fungi, bacteria, viruses, viroids, fungal cell wall components and oligosaccharides) also proved their role in plant defense response (Punja and Zhang *et al*., 1993; Gupta *et al*., 2010b).

In addition to it, insect pathogenic fungi have considerable potential for the biological control of insect pests which apparently overcome physical barriers of the host by producing multiple extracellular enzymes including chitinolytic enzymes, which help to penetrate the cuticle and facilitate infection (Herrera-Estrella and Chet, 1999).

**Source of chitinase:** Chitinolytic microbes occur widely in nature and are preferred source of chitinase because their low production cost, easy availability of raw materials for their cultivation. The ability of a microbial community to degrade chitin is also important for the recycling of Nitrogen in the soil (Chandran *et al*., 2007). Bacteria like *Serratia marcescens, Xanthomonas maltophilia*, *Stenotrophomonas maltophilia* and *Paenibacillus illinoisensis* (Kobayashi *et al*., 1995; Zhang and Yuen, 2000; Jung *et al*., 2003, respectively) etc. have been proved as potent chitinolytic bacterial biocontrol agents while *Myrothecium verrucavia*, (Govindsamy *et al*., 1998) and *Trichoderma* sp. (Howell, 2003) were found as main source of chitinase among fungi. In insects and nematodes chitinases were found to be involved in molting process during their development (Adam *et al*., 1996). Chitinases were also reported in gastric juices of human being (Paoletti *et al*., 2007) where they were being thought to be involved catabolic activities. Further chitinase activity was also detected in human serum (Escott and Adams David, 1995) and found very similar to plant chitinases those are related in the process of inflammation and pathogen resistance (Chupp *et al*., 2007).

Besides this, a number of proteins demonstrating chitinolytic activity were also identified in plants. These proteins were isolated from all organs of plants and tissues including apoplast and vacuoles. In plants these represent a large and diverse group of enzymes which differ not only in spatial and temporal localization, but also in molecular structure and substrate specificity (Brunner *et al*., 1998). The apoplastic chitinases play a role in the early stage of pathogenesis. They were thought to be helpful in the generation elicitor molecules which are involved in the transfer of information from hyphae to the intercellular space (Gerhardt *et al*., 1997). While vacuolar chitinases degrade the newly synthesized chains of chitin and thus repress fungal growth (Collinge *et al*., 1993).

Moreover the vacuolar chitinases were more active against crystalline chitin, whereas apoplastic forms were found to be more active towards soluble chitin (Collinge *et al*., 1993). In addition to it, the chitinase gene was also expressed during seed developmental stages (Wu *et al*., 2001) and fruit ripening process (Robinson *et al*., 1997) which signifies its relation with plant defense.

**Chitinase in plant defense:** The exploitation of chitnase with respect to plant defense can be done by a variety of ways. The enzyme can also be used in free or immobilized form to kill fungi and insects in affected areas. The micorganisms producing chitinase can also be used in soil as rhizobacterial population or alternatively the gene encoding chitinase can be inserted in the native microflora of soil. Sundheim *et al*. (1988) inserted chitinase of *Serratia marcescens* into *Pseudomonas fluorescens* which is normally present as normal flora of soil and obtained resistance in radish plants against *Fusarium oxysporum* infection. Chernin *et al*. (1997) were also able to decrease the onset of *Rhizoctonia solani* infection in cotton plants by using recombinant *E. coli* in rhizosphere expressing chitinase gene of *Enterobacter agglomerans*. Kirubakaran and Sakthive (2007) also demonstrated a broad-spectrum antifungal activity in *Escherichia coli* expressing chitinase gene of barley against *Botrytis cinerea* (Blight of Tobacco), *Pestalotia theae* (Leaf Spot of Tea), *Bipolaris oryzae* (Brown Spot of Rice), *Alternaria* sp. (Grain Discoloration of Rice), *Curvularia lunata* (Leaf Spot of Clover) and *Rhizoctonia solani* (Sheath Blight of Rice).

Besides these, the phenomena of synergism (the interaction of organisms or proteins or elements that when combined, produce a total effect that is greater than the sum of the individual element contributions) can be meaningful to fasten the killing of pathogenic organism. Lorito *et al.* (1993a) demonstrated that chitinolytic enzymes from *Trichoderma harzianum* Rifai and the closely related fungus *Gliocladium virens* J.H. Miller, J.E. Giddens and A.A. Foster act synergistically to inhibit the growth of a variety of plant pathogenic fungi. Lorito *et al.* (1993b) also demonstrated that bacterial biocontrol strains may also act synergistically with chitinolytic enzymes to inhibit plant pathogenic fungi. They determined strong synergism between *Enterobacter cloacae* and chitinolytic enzymes of *Trichoderma harzianum* and found that chitinolytic enzymes enhance the bacterial growth and their ability to bind to the fungal hyphae. Mauch *et al.* (1988) observed rapid killing of fungal cells by combining chitinases with β-glucanase. In addition to above synergistic effects of a β-1, 6-glucanase and chitinase from *Trichoderma harzianum* on the hydrolysis of fungal cell walls had been also reported (De La Cruz *et al.*, 1992; Misra and Gupta, 2009).

Alternatively, the production of transgenic plants over expressing chitinase gene had been also demonstrated to get resistance against pathogens. It was achieved by manipulating the activity of extracellular enzymes through construction of over producing mutants, enzyme negative mutants or even transgenic plants expressing the enzyme. Brogue *et al.* (1991) showed an increased ability to survive in tobacco plants in *Rhizoctonia solani* infected soil and delayed development of disease symptoms in tobacco seedlings by expressing chitinase. Dunsmuir *et al.* (1993) also confirmed the reduction in occurrence of *Rhizoctonia solani* infection in transgenic tobacco plants in which two bacterial chitinase gene were over expressed at high levels. Besides the immunity against fungal pathogen, overexpression of chitinase was also found be effective to raise resistance plants against bacterial pathogens, salinity stress and heavy metals stress (Dana *et al.*, 2006).

It is not only the microbial chitinase but the plant chitinase had been also used to improvement plant health by various workers. In oil seed rape (*Brassica napus var. oleifera*) the importance of chitinase was also shown by various researchers. Grison *et al.* (1996) were able to increase tolerance in these plants against *Cylindrosporium concentricum, Phoma lingam, Sclerotinia sclerotiorum* infection by expressing chitinase gene. Transgenic wheat plants expressing chitinase of plants were also raised. Oldach *et al.* (2001) observed enhanced resistance to powdery mildew infection in wheat upon expression of chitinase of barley. In another experiment the transgenic wheat lines carrying a combination of a wheat β-1, 3- glucanase and chitinase exhibited delayed symptoms of *Fusarium* Head Blight (Anand *et al.*, 2003).

Chitinases were also used as a method to control insect and pest population which indirectly suggests its role in plant defense. Lawrence and Novak (2006) showed that the expression of poplar chitinase in tomato leads to inhibition of development in colorado potato beetle. Lipmann *et al.* (2009) investigated the secretome of a tobacco cell suspension by a combined proteomic and metabolomic approach and identified chitinase alongwith peroxidase and beta- 1,4,-xylosidase among the major defense protein. Wasano *et al.* (2009) observed that the presence of 56-kDa defense protein consisted of chitin like domain in mulberry latex was responsible to provide strong insect resistance to lepidopteran caterpillars, including the cabbage armyworm, *Mamestra brassicae* and the Eri silkworm, *Samia ricini*. Similarly Kitajima *et al.* (2010) also reported two chitinase like proteins LA-a and LA-b (latex abundant) from the latex of Mulberry (*Morus* sp.) and found them associated with insecticidal activities against larvae of *Drosophila melanogaster*.

Chitinases were also isolated from insects and they were also found to hold equal promise in the improvement of plant defense. Chitinases have been isolated from the tobacco hornworm and

several other insect species. Ding *et al.* (1998) developed transgenic tobacco plants expressing insect's chitinases and found them resistant to infection of tobacco budworm *Heliothis virescens*. Transgenic plants constitutively expressing hornworm chitinase had been generated and found to be resistant against infection (Kramer and Muthukrishnan, 1997). Besides this a recombinant baculovirus expressing chitinase of hard tick (*Haemaphysalias longicornis*) had been also used as a bio acaricide for tick control by Assenga *et al.* (2006).

Although the relationship between chitinase production and generation of reactive oxygen species is not clear, yet some experiments showed that these two both act in a co-ordinated way to protect plant against a challenge. Ghaouth *et al.* (2003) treated the peach fruit with UV-C light and noted a rapid induction of chitinase, beta-1,3-glucanase and phenylalanine ammonia lyase (PAL) activities and concluded that the response of peach fruit to elicitor treatment is similar to that seen in other plant-elicitors interactions and suggested the involvement of peach biochemical defense responses in UV-C-mediated disease resistance. Xu *et al.* (2008) observed significant stimulation in the activities of chitinase, beta-1,3-glucanase, catalase (CAT), peroxidase (POD) genes in seeds upon infection with *Pichia membranaefaciens*, *Cryptococcus laurentii*, *Candida guilliermondii* and *Rhodotorula glutinis* and suggested that antioxidant defense response may be involved in the mechanisms of microbial biocontrol agents against fungal pathogen. Gorii *et al.* (2009) found appearance of acidic chitinases and basic chitinase along with basic constitutive 1,3-beta-glucanases in Annona fruits by high levels of $CO_2$ and upon storage at chilling temperature (6°C). They finally suggested that these modifications in the proteome level were to enhance the synthesis of cryoprotectant proteins *in vivo*. Kumar *et al.* (2009) also observed enhanced level of chitinase activity along with increased level of Reactive Oxygen Species (ROS), increase in the expression level of several defense-related genes, activation of some pathogenesis response protein, increase in the level of enzymes of the terpenoid pathway in various tissues as well as in the medium surrounding the roots of transformants and hypothesized that elevated defensive state of the transformants act synergistically with the potent transgene encoded chitinase activity to confer a strong resistance to *Rhizoctonia solani* infection.

## CONCLUSION

Chitinases are prime molecules of interest for plant pathologist and can be utilized by variety of ways to improve plant health. These enzymes are classified into various types on the basis of their structural and functional properties. One class of chitinase was not found equally active against chitin of another source. Thus there is a need to isolate and identify chitinase of broad spectrum activity. In addition to it, reactions conditions of the enzyme must be known prior to its use in open environment and in affected site. Besides these, there is an utmost requirement to enhance the basal level of chitinase production by using recent approaches of genetic engineering. Therefore a coordinated strategy by using above plans may be meaningful to assess the full potential of chitinolytic organisms in rendering plant defense.

## REFERENCES

Adam, R., B. Kaltmann, W. Rudin, T. Friedrich and T. Marti, 1996. Identification of chitinase as the immunodominant filarial antigen recognized by sera of vaccinated rodents. J. Biol. Chem., 271: 1441-1447.

Adams, D.J., 2004. Fungal cell wall chitinases and glucanases. Microbiology, 150: 2029-2035.

Alverez, M.E., 2000. Salicylic acid in the machinery of hypersensitive cell death and disease resistance. Plant Mol. Biol., 44: 429-442.

Anand, A., T. Zhou, H.N. Trick, B.S. Gill and W.W. Bockus *et al.*, 2003. Greenhouse and field testing of transgenic field plants stably expressing genes for Thaumatin like protein, chitinase and glucanase against *Fusarium graminearum*. J. Exp. Bot., 54: 1101-1111.

Assenga, S.P., M. You, C.H. Shy, J. Yamagishi and T. Sakaguchi *et al.*, 2006. The use of a recombinant baculovirus expressing a chitinase from the hard tick *Haemaphysalias longicornis* and its potential application as a bioacaricide for tick control. Parasitol. Res., 98: 111-118.

Bradley, D.J., P. Kjellbomand and C.J. Lamb, 1992. Elicitor- and wound-induced oxidative cross-linking of a proline-rich plant cell wall protein: A novel, rapid defense response. Cell, 70: 21-30.

Brogue, K., I. Chet, M. Holliday, R. Cressman and P. Biddle *et al.*, 1991. Transgenic plants with enhanced resistance to the fungal pathogen *Rhizoctonia solani*. Science, 254: 1194-1197.

Brunner, F., A. Stintzi, B. Fritig and M. Legrand, 1998. Substrate specificities of tobacco chitinases. Plant J., 14: 225-234.

Chandran, S., B. Parameswaran and A. Pandey, 2007. Microbial Chitinases: Effective Biocontrol Agents. In: Biological Control of Plant Diseases, Chincholkar, S.B. and K.G. Mukerji (Eds.) The Haworth Press, Inc., New York, pp: 381-382.

Chernin, L.S., L. De-la-Fuente, V. Sobolev, S. Haran, C.E. Vorgias, A.B. Oppenheim and I. Chet, 1997. Molecular cloning, structural analysis and expression in *Escherichia coli* of a chitinase gene from *Enterobacter agglomerans*. Applied Environ. Microbiol., 63: 834-839.

Chupp, G.L., C.G. Lee, N. Jarjour, Y.M. Shim and C.T. Holm *et al.*, 2007. A chitinase like protein in the lung and circulation of patients with severe asthma. New Engl. J. Med., 357: 2016-2027.

Cohen-Kupeic, R. and I. Chet, 1998. The molecular biology of chitin digestion. Curr. Opin. Biotechnol., 9: 270-277.

Collinge, D.B., K.M. Kragh, J.D. Mikkelsen, K.K. Nielsen, U. Rasmussen and K. Vad, 1993. Plant chitinases. Plant J., 3: 31-40.

Cramer, C.L., J.N. Bell, T.B. Ryder, J.A. Bailey and W. Schuch *et al.*, 1985. Co-ordinated synthesis of phytoalexin biosyntheyic enzymes in biologically-stressed cells of bean (*Phaseolus Vulgaris* L.). The Embo J., 4: 285-289.

Dana, M.D.L.M., J.A. Pentor-Toro and B. Cubero, 2006. Transgenic tobacco plants overexpressing chitinases of fungal origin show enhanced resistance to biotic and abiotic stress agents. Plant Physiol., 142: 722-730.

De La Cruz, J., A. Hidalgo-Gallego, J.M. Lora, T. Benitez, J.A. Pintor-Taro and A. Llobell, 1992. Isolation and characterization of three chitinase in *Trichoderma harzianum*. Eur. J. Biochem., 206: 859-867.

Ding, X., B. Gopalakrishnan, L.B. Johnson, F.F. White and X. Wang *et al.*, 1998. Insect resistance of transgenic tobacco expressing an insect chitinase gene. Transgenic Res., 7: 77-84.

Dunsmuir, P., W. Howie, E. Newbigin, L. Joe and E. Penzes *et al.*, 1993. Resistance to Rhizoctonia solani in Transgenic Tobacco. In: Advance in Molecular Genetics of Plant-Microbe Interactions, Nester E.W. and D.P.S Verma (Eds.). Kluwer Academic Publishers, Netherlands, pp: 567-570.

Ehrlich, H., K. Manfred, H. Thomas, S. Paul and K. Christiane *et al.*, 2007. First evidence of the presence of chitin in skeletons of marine sponges. Part II. Glass sponges (Hexactinellida: Porifera). J. Exp. Zool. B Mol. Dev. Evol., 308: 473-483.

Escott, G.M. and J. Adams David, 1995. Chitinase activity in human serum and leukocytes. Infect Immun., 63: 4770-4773.

Flach, J., P.E. Pilet and P. Jolles, 1992. Whats new in chitinase research?. Experientia, 48: 701-716.

Fuhrman, J.A. and W.F. Piessens, 1985. Chitin synthesis and sheath morphogenesis in Brugia malayi microfilariae. Mol. Biochem. Parasitol., 17: 93-104.

Gerhardt, L.B., G. Sachetto-Martins, M.G. Contarini, M. Sandroni and R.D.P. Ferreira *et al.*, 1997. Arabidopsis thaliana class IV Chitinase is early induced during the interaction with Xanthomonas campestris. FEBS Lett., 419: 69-75.

Ghaouth, E.I., C.L. Wilson and A.M. Callahan, 2003. Induction of chitinase, beta-1,3-glucanase and phenylalanine ammonia lyase in Peach fruit by UV-C treatment. Phytopathology, 93: 349-355.

Gorii, O., M.T. Sanchez-Ballesta, C. Merodio and M.I. Escribano, 2009. Regulation of defense and cryoprotective proteins by high levels of $CO_2$ in Annona fruit stored at chilling temperature. J. Plant Physiol., 166: 246-258.

Govindsamy, V., K.R. Gunaratna and R. Balasubramanian, 1998. Properties of extracellular chitinase from *Myrothecium verrucaria*, an antagonist to the groundnut rust *Puccinia arachidis*. Can. J. Plant Pathol., 20: 62-68.

Grison, R., B. Grezes-Besset, M. Schneider, N. Lucante, L. Olsen, J.J. Leguay and A. Toppan, 1996. Field tolerance to fungal pathogens of *Brassica napus* constitutively expressing a chimeric chitinase gene. Nat. Biotechnol., 4: 643-646.

Gupta, V.K., A.K. Misra, R.K. Gaur, P.K. Jain, D. Gaur and S. Sharma, 2010a. Current Status of *Fusarium* Wilt Disease of Guava (*Psidium guajava* L.) in India. Biotechnology, 9: 176-195.

Gupta, V.K., A. Gupta, A.K. Misra, R.K. Gaur and B.K. Pandey, 2010b. RAPD-PCR of *Trichoderma* isolates and *in vitro* antagonism against *Fusarium* wilt pathogens of *Psidium guajava* L. J. Plant Protection Res., Volume, 50.

Hahlbrock, K., C.J. Lamb, C. Purwin, J. Ebel, E. Fautz and E. Schafer, 1981. Rapid Response of suspension-cultured parsley cells to the elicitor from *Phytophthora megasperma* var. sojae: Induction of the enzymes of general phenylpropanoid metabolism. Plant Physiol., 67: 768-773.

Hammerschmidt, R., 1999. Phytoalexins: What have we learnt after 60 years?. Annu. Rev. Phytopathol., 37: 285-306.

Henar, V.M., A. Duran and C. Roncero, 1999. Chitin synthases in yeast and fungi. EXS, 87: 55-69.

Henrissat, B. and G. Davies, 1997. Structural and sequence-based classification of glycosyl hydrolases. Curr. Opin. Structure Biol., 7: 637-644.

Henrissat, B., 1991. A classification of glycosyl hydrolases based on amino acid Sequence similarities. Biochem. J., 280: 309-316.

Herrera-Estrella, A. and I. Chet, 1999. Chitinases in biological control. EXS, 87: 171-184.

Hopkins, D.L. and A.H. Purcell, 2002. Xylella fastidiosa: Cause of pierces disease of grapevine and other emergent diseases. Plant Dis., 86: 1056-1066.

Howell, C.R., 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: The history and evolution of current concepts. Plant Dis., 87: 4-10.

Jung, W.J., K.N. An, Y.L. Jin, R.D. Park and K.T. Lim *et al.*, 2003. Biological control of damping-off caused by *Rhizoctonia solani* using chitinase-producing *Paenibacillus illinoisensis* KJA-424. Soil Biol. Biochem., 35: 1261-1264.

Kirubakaran, S.I. and N. Sakthive, 2007. Cloning and overexpression of antifungal barley chitinase gene in *Escherichia coli*. Protein Exp. Purif, 52: 159-166.

Kitajima, S., K. Kamei, S. Taketani, M. Yamaguchi and F. Kawai *et al.*, 2010. Two chitinase-like proteins abundantly accumulated in latex of mulberry show insecticidal activity. BMC Biochem., 11: 6-6.

Kobayashi, D.Y., M. Guglielmoni and B.B. Clarke, 1995. Isolation of the chitinolytic bacteria *Xanthomonas maltophilia* and *Serratia marcescens* as biocontrol agents for summer patch disease of turfgrass. Soil Biol. Biochem., 27: 1479-1487.

Kramer, K.J. and S. Muthukrishnan, 1997. Insect chitinases: Molecular biology and potential use as biopesticides. Insect Biochem. Mol. Bio., 27: 887-900.

Kumar, V., V. Parkhi, C.M. Kenerley and K.S. Rathore, 2009. Defense-related gene expression and enzyme activities in transgenic cotton plants expressing an endochitinase gene from *Trichoderma virens* in response to interaction with *Rhizoctonia solani*. Planta, 230: 277-291.

Lawrence, S.D. and N.G. Novak, 2006. Expression of poplar chitinase in tomato leads to inhibition of development in colorado potato beetle. Biotechnol. Lett., 28: 593-599.

Lehane, M.J., 1997. Peritrophic matrix structure and function. Annu. Rev. Entomol., 42: 525-550.

Lipmann, R., S. Kaspar, T. Rutten, M. Melzer and J. Kumlehn *et al*., 2009. Protein and metabolite analysis reveals permanent induction of stress defense and cell regeneration processes in a tobacco cell suspension culture. Int. J. Mol. Sci., 10: 3012-3032.

Lorito, M., G.E. Harman, C.K. Hayes, R.M. Broadway, A. Tronsmo, S.L. Woo and A. Di Pietro, 1993a. Chitinolytic enzymes produced by *Trichoderma harzianum*: Antifungal activity of purified endochitinase and chitobiosidase. Phytopathology, 83: 302-307.

Lorito, M., A. Di Pietro, C.K. Hayes, S.L. Woo and G.E. Harman, 1993b. Antifungal, synergistic interaction between chitinolytic enzymes from *Trichoderma harzanium* and *Enterobacter cloacae*. Mol. Plant Pathol., 83: 721-728.

Mansfield, L.S., H.R. Gamble and R.H. Fetterer, 1992. Characterization of the eggshell of Haemonchus contortus-I. Structural components. Comp. Biochem. Physiol. B, 103: 681-686.

Mauch, F., B. Mauch-Mani and T. Boller, 1988. Antifungal hydrolases in pea tissue II: Inhibition of fungal growth by combinations of chitinase and β-1,3 glucanase. Plant Physiol., 88: 936-942.

Merzendorfer, H. and L. Zimoch, 2003. Chitin metabolism in insects: Structure, function and regulation of chitin synthases and chitinases. J. Exp. Biol., 206: 4393-4412.

Misra, A.K. and V.K. Gupta, 2009. *Trichoderma*: Biology, biodiversity and biotechnology. J. Eco-Friendly Agric., 4: 99-117.

Oerke, E.C., H.W. Dehnf, F. Schonbeck and A. Webwe, 1994. Crop Production and Crop Protection Estimated losses in Major Food and Cash Crop. Elseiver, Amsterdam, pp: 808.

Oldach, K.H., D. Becker and H. Lorz, 2001. Heterologous expression of the genes mediating enhanced fungal resistance in transgenic wheat. Mol. Plant Microbe Interact, 14: 832-838.

Paoletti, M.G., N. Lorenzo, D. Roberta and M. Salvatore, 2007. Human gastric juice contains chitinases that can degrade chitin. Ann. Nutr. Metab., 51: 244-251.

Punja, Z.K. and Y.Y. Zhang, 1993. Plant chitinases and their roles in resistance to fungal disease. J. Nematol., 25: 526-540.

Robinson, S.P., A.K. Jacobs and I.B. Dry, 1997. A class IV chitinase is highly expressed in grape berries during ripening. Plant Physiol., 114: 771-778.

Sela-Buurlage, M.B., A.S. Ponstein, S.A. Bres-Vloemans, L.S. Melchers, P.J.M. van den Elzen and B.J.C. Comelissen, 1993. Only specific tobacco (*Nicotiana tabacum*) chitinases and-1, 3-glucanase exhibit antifungal activity. Plant Physiol., 101: 857-863.

Sietsma, J.H. and J.G.H. Wessels, 1979. Evidence for covalent linkages between chitin and β-glucan in a fungal wall. J. Gen. Microbiol., 114: 99-108.

Sundheim, L., A.R. Poplawsky and A.H. Hlingboe, 1988. Molecular cloning of two Chitinase gene from Serratia marcescens and their expression in Pseudomonas species. Physiol. Mol. Plant Pathol., 33: 483-491.

Suzuki, K., M. Taiyoji, N. Sugawara, N. Nikaidou and B. Henrissat *et al.*, 1999. The third chitinase gene (chiC) of Serratia marcescens 2170 and the relationship of its product to other bacterial Chitinases. Biochem. J., 343: 587-596.

Tews, I., R. Vincentelli and C.E. Vorgias, 1996a. N, Acetylglucosaminidase (chitobiase) from Serratia marcescens: Gene sequence and protein production and purification in *Escherichia coli*. Gene, 170: 63-67.

Tews, I., A. Perrakis, A. Oppenheim, Z. Dauter and K.S. Wilson *et al.*, 1996b. Bacterial chitobiase structure provides insight into catalytic mechanism and the basis of Tay-Sachs disease. Nat. Struct. Biol., 3: 638-648.

Van Aalten, D.M.F., B. Synstad, M.B. Brurberg., E. Hough and B.W. Riise *et al.*, 2000. Structure of a two-domain chitotriose from Serratia marcescens at 1.9-Å resolution. Proc. Natl. Acad. Sci. USA., 97: 5842-5847.

Wasano, N., K. Konno, M. Nakamura, C. Hirayama and M. Hattori *et al.*, 2009. A unique latex protein, MLX56, defends mulberry trees from insects. Phytochemistry, 70: 880-888.

Wu, C.T., G. Leubner-Metzger, F.J.R. Meins and K.J. Bradford, 2001. Class I β-1,3- glucanase and chitinase are expressed in the micropylar endosperm of tomato seeds prior to radicle emergence. Plant Physiol., 126: 1299-1313.

Xu, X., G. Qin and S. Tian, 2008. Effect of microbial biocontrol agents on alleviating oxidative damage of peach fruit subjected to fungal pathogen. Int. J. Food Microbiol., 126: 153-158.

Zhang, Z. and G.Y. Yuen, 2000. The role of chitinase production by *Stenotrophomonas maltophilia* strain C3 in biological control of *Bipolaris sorokiana*. Phytopathology, 90: 384-389.