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Review Paper

Screening of Antifungal Proteins from Plants of *Cucurbitaceae* family against *Fusarium oxysporum*: Potential as Biofungicides

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Abstract

Natural enemies like microbes and insects cause severe damage to the crops globally, resulting in a large economic loss. Plants of Cucurbitaceae family are known as the richest sources of carbohydrates, proteins, water and oil. Fusarium oxysporum causes the 'fusarium wilt' in the plants of this family and regarded as the major threat in growing cucurbits and thus, ensuing reduction in the total productivity of the crop. To overcome this problem, chemical fungicides are commonly spread over the crops which control the spreading of fungal diseases and subsequently increase the overall productivity. However, these synthetic chemicals are associated with various diseases and genetic disorders and are considered very lethal and risky for human beings, plants, animals and their environments. There are several proteins reported in the various plants with potential anti-fungal properties. We believe that these proteins can serve as useful resources for the formulation of future bio-fungicides which are natural, non-toxic and non-hazardous for us and our environment and surely merit over chemical fungicides. In this review, we have discussed about anti-fungal proteins reported in plants of Cucurbitaceae family which in near future, can be tested to get rid of 'fusarium wilt'.

Keywords: Bio-fungicides, *Fusarium oxysporum*, pathogen related proteins, ribosomal inactivating proteins, vicilin like proteins.

Introduction

One of the major problems in growing crops successfully is coming from natural enemies like fungi, bacteria, protists and insects. Plants are the wealthy source of nutrients for these types of organisms. There are approximately 250,000- 500,000 known plant species and only few of them show anti-microbial and anti-fungal activities[1,2]. It is well known that plants do not have their own immune system and when they are exposed to various pathogens, they defend themselves by varying defence mechanisms. In plants, the first line of defence includes the physical barriers such as cuticles and cell wall. Chemical barriers make the second line of defence which include the constitutive as well as induced compounds in response to the foreign particles. The well-known chemical barriers are phytoanticipins, saponins, phenols, quinines, defensins, peptides and proteins. The Phytoanticipins are low molecular weight compounds, produced after the microbial infections. The term 'phytoantacipin' was coined by Van Etten *et al.* [3]. They may present on the plant surface or in vacuole and are released from the vacuole after infection with the help of the hydrolytic enzymes. Saponins are secondary metabolites and are produced in many plant species. They are reported to play important roles in plant defence. They show strong anti-fungal activity against various fungal species. Phenols and quinines make another class of anti-microbial compounds produced by few plant species that affect the vectors of plant pathogen either by excreting into the external environment or by accumulating into the dead cells[4,5]. Defensins, another group of defensive compounds, hamper with the pathogen food and impede their growth and development.

There are a wide range of novel medicinally important proteins known which play important roles towards plant defense by inhibiting the fungal growth. Various plant parts including seeds, stems, leaves and roots of many plants contain antifungal proteins that potentially inhibit the growth of pathogenic fungi. Infact, plants are the richest source of chitinases, glucanases, ribosome inactivating proteins and pathogen related proteins that particularly needed for protecting seeds and seedlings from fungal infection[6-8]. It is well known that fungi are the organisms which are unable to produce their own food; thus, they are dependent on other organisms, specifically on plants and animals for fulfilling their nutritional needs. They can distress any part of the plant, kill plant in a short period of time and even sometimes destroy the whole crop. In *Cucurbitaceae* family, fungus *Fusarium oxysporum* causes the fusarium wilt which destroys the crops of family including watermelon, muskmelon, cucumber and many more. *Cucurbitaceae* is one of the largest families of plant kingdom and considered as the richest source of carbohydrates, proteins, water and oil. Fusarium wilt causes severe damages to the crop, resulting in huge losses in terms of yield, quality and revenues. This reduction in total productivity of family due to fungal invasion accounts for high economic loss worldwide and is a subject of major concern among

agriculture and food biotechnologists. To overcome this problem, fungicide or the synthetic chemicals are more commonly used which further highlight a major scientific concern as they are generally considered very toxic and hazardous for human beings, plants and animals along with their surroundings.

Continuous increase in global population along with urbanization, price rise, changing food habits and climatic disturbances has transformed food consumption, production and markets, causing global food crisis. This, in turn, is disturbing our society severely by pushing millions into poverty and malnutrition[9]. Continuous increase in global population has increased the demand of food and nutritional resources which has simultaneously built a pressure on agricultural production resulting in the excessive use of chemical pesticides and fungicides in last few decades. It has caused a serious threat to environment and has been considered as one of the root causes of several diseases, due to the presence of these chemicals in food[10]. To overcome this co-problem, the need of hour is to find safer methods and ways to replace chemicals with naturally available means and resources which may not harm us and our environment. It is now well known that many plants produce proteins which have shown anti-microbial activity and are part of their defense system. These proteins are highly sensitive and effective against various harmful pathogenic fungi and have great potential to be used as eco-friendly alternatives against toxic synthetic chemicals. These natural compounds are known to have minimal toxic effects towards living organisms and their environment [11].

In this review article, we have discussed various proteins and peptides which are present in different parts of *Cucucrbitaceae* family plants and show anti-fungal activity against *Fusarium oxysporum*, with a concluding remark that anti-fungal proteins present in plants can be used as a replacement of harmful chemical anti-fungal agents and will surely merit over them.

Anti-fungal proteins in plants of *Cucurbitaceae* Family

Cucurbitaceae is one of the largest and diverse plant families consisting of about 125 genera and 960 species, with worldwide distribution. The plants of this family are found in tropical and subtropical areas. The plants of family are well known for their traditional medicinal uses[12-15]. The major family members are melons, squashes and gourds. Different parts of the water melon are used as remedies in various types of ailments. Water melon contains almost 95% water and its high amount of water makes it a powerful diuretic diet. It is also traditionally used to treat cardiovascular disease and kidney problems. rind, root, Seeds and leaves extracts of water melon show painkilling and anti-inflammatory effects[16-22]. Valuable scientific studies have shown pharmacological importance of Muskmelon. In China, it is used as traditional medicine for the treatment of abdominal distension and constipation[23]. Bottle gourd and sponge gourd are the popular plants of this family. Since ancient times, bottle gourd is used as effective medicine in India, China, European countries and Brazil. It is a well-known diuretic, having anti-diabetic, anti-hyperlipidemic, analgesic, Hypertensive, cardio protective, anti-cancer, CNS depressant and anti-inflammatory activities[24-27]. Sponge gourd also possesses medicinal as well as nutritional values. It is used to treat asthma, sinusitis and fever[28]. Its seeds contain an abortifacient protein which has shown ribosomal inhibiting property as well as therapeutic potential against AIDS[29-31].

In general, members of this family have always been considered a subject of research due to the fact that they are rich source of proteins. For long, proteins with many biological activities like anti-fungal, anti-bacterial, anti-viral, anti-diabetic, anti-tumor and anti-AIDS has been known from this family[32-36]. In last few years, several proteins have been purified from different plant parts which are traditionally linked with the plants defence system. On the basis of their nature and biological activity, we have classified anti-fungal proteins known from *Cucurbitaceae* family as pathogen related (PR) proteins, ribosomal inactivating proteins (RIPs), vicilin like proteins and others.

Pathogen related proteins (PR)

Certain plants, in presence of various stress conditions such as infections (by insects or fungi), exposure to certain chemicals, wounding and atmospheric conditions (e.g. UV irradiation), defend themselves by expressing constitutive and induced proteins collectively called PR proteins[37,38]. To inhibit the spreading of the infection, plants show a hypersensitive response induced by PR proteins and characterized by localized and rapid cell death (necrosis) surrounding the site of pathogen invasion[39,40]. This pathogen inhibiting property makes PR proteins and associated phenolic compounds and other factors a great tool of commercial and economical importance in agriculture and medicine.

In plants, there are several types of inducible defence related proteins, classified into 17 families of PR proteins. PR proteins are widely distributed in plant kingdom and many of them have been isolated from different parts of the plants such as leaves, stem, root and seeds[41-43]. These low-molecular proteins (6-43kDa) are stable at very high temperature, remain soluble at low pH (<3) and are highly resistant to proteases[44]. PR proteins exhibit strong anti-fungal and anti-microbial activities which make them essential for the proper development and function of plant as a whole or in parts. In case of certain fungi, PR proteins diffuse towards the attacking pathogen and break their chitin supported cell wall. PR proteins are further classified into different families (PR-1 to PR-5) depending on their amino acid sequences and enzymatic or biological activity.

PR-1 are the most abundant proteins which show anti-fungal properties at micromolar levels against various pathogenic fungi. The acidic or basic nature, resistance to proteases and

extracellular secretion are the characteristic features of PR-1 proteins[45]. PR-2 proteins weaken the fungal cell wall at the hyphal apex of filamentous molds by hydrolyzing structural (1,3) β-glucan present in it. PR -3 proteins weaken the fungal cell wall by destroying the chitin polymer present in cell wall that makes fungal cells osmotically sensitive and thus, inhibit the fungal growth. PR-4 proteins inhibit the fungal growth by binding with β-chitin present in attacking fungal cell wall. PR-5 proteins inhibit the hyphal growth and facilitate hyphal and spore lysis resulting into plasma membrane permeabilization and dissipation of the plasma membrane potential[46, 47]. These proteins are also known as Thaumatin like proteins due to homologous amino acid sequences.

Several PR proteins have been purified from plants of *Cucurbitaceae* family. A thermo stable PR-1 protein of 40 kDa was purified from pumpkin rind. It is relatively a high molecular weight anti-fungal protein in comparison to others. This protein is known to inhibit the growth of several pathogenic fungi including *Botrytis cinerea, Fusarium oxysporum, Fusarium solani and Rhizoctonia solani and Candida albicans*[48,49]. It has membrane permeability activity by which it disrupts the integrity of the cell plasma membrane and results in the leakage of the cytoplasmic component and thus, inhibits the fungal growth. A heat stable 14.8 kDa novel anti-fungal protein, PR-2, was identified from the Pumpkin rinds. This protein strongly inhibits the growth of *Botrytis cinerea, Colletotrichum coccodes, Fusarium solani, Fusarium oxysporum,*and *Trichoderma harzianum* at 10-20 μM[50]. This protein mainly inhibits the fungal growth by damaging the fungal cell membrane. Another potential antifungal PR protein was purified from the leaves of pumpkin and was designated as PR-5 protein. It is a 28 kDa protein which shows synergistic effects with the Nikkomycine, a chitin synthase inhibitor against *Candida albicum*[51].

Ribosomal inactivating proteins (RIP)

Plant RIPs arrest the synthesis of foreign proteins by inactivating fungal ribosome by N-glycosidase activity[52]. RIPs are further classified into three groups (Type1, Type2 and Type3) on the basis of their structure. Type -1 RIPs are single chain *N*-glycosidases having molecular masses of 11- 30 kDa. Type-2 RIPs have two chains, an N-glycosidase (chain A) and cell binding lectin (chain B). Type-3 RIPs consist of four chains arranged in the form of dimers. RIPs have been isolated from number of plants e.g. seeds of the *Caryophyllaceae saponaria ocymoides* (30.2kDa) and *Vaccaria pyramidata* (28.0kDa)[53].

Hispin, a 21 kDa RIP was purified from the hairy melon seeds[54]. It shows potent anti-fungal activity against various pathogenic fungal species, such as *Coprinus comatus, Fusarium oxysporum, Physalospora piricola, and Mycosphaerella arachidicola.* α-momorcharin protein is also an anti-fungal RIP, extracted and purified from *Momordica charantia*. It does not only inhibit the growth of fungi but also known to possess many important biological activities. It displays anti-fungal activity against *Fusarium solani* and *Fusarium oxysporum*[55]. Luffacylin, a 7.8kDa RIP was purified from sponge gourd seedswhich inhibits the growth of *Mycosphaerella arachidicola* and *Fusarium oxysporum*[56]. Luffangulin and Luffaculin are two another anti-fungal RIPs from *Luffa acutangula* seeds having molecular masses of 5.6kDa and 28 kDa respectively[57].

Vicilin like proteins: There are many plant proteins known which perform dual functions like storage and defence. Vicilin like proteins form such a family, for example, a noval 8kDa peptide purified from *Cucumis melo* has shown anti-fungal activity against *Fusarium oxysporum* and has high sequence homology with vicilins[58]. Anti-fungal proteins are the unique components in plants which play an important role in plant defence against pathogenic fungi and protect them during different stages of growth. Recently, a low molecular weight (11 kDa) vicilin like protein was purified from the *Citrullus lanatus* seeds, however functional characterization of this protein is not yet established[59].

Others: Cucurmoschin (8kDa), a novel anti-fungal peptide abundant in arginine, glutamate and glycine residues was purified from black pumpkin seeds. It is reported to inhibit mycelial growth in the fungi *Botrytis cinerea*, *Fusarium oxysporum* and *Mycosphaerella oxysporum*[60]. List of these above mentioned anti-fungal proeins from *cucurbitaceae* family against *Fusarium oxysporum* is given in table-1.

Conclusion

As we all know, fungi are disease causing agents for plants which are major threat in growing them successfully. The fungal strain *Fusarium oxysporum* causes a number of disastrous diseases in plants of different families. Fusarium wilt is one of the oldest diseases of water melons, muskmelons and many other cucurbits that affect the production of fruits and seeds, causing huge economic loss worldwide. To overcome these problems, chemical pesticides are in great practice. No doubt, they are important to kill the disease causing pathogens, but definitely not sustainable for human health and friendly environment as long exposure to them is associated with various diseases, specifically carcinomas. Among number of anti-fungal plant proteins known and researched, proteins from plants of *Cucurbitaceae* family are the most promising because of their specific pathogen related activities. This is one of the largest plant families whose fruits and seeds are largely consumed by human beings in various forms because of their nutritional and medicinal values as well as good taste and availability. Many species of this family produce very effective anti-fungal proteins which inhibit the growth of *Fusarium oxysporum*. During their course of evolution, these proteins are developed by the plants to defend themselves against various pathogens. The potentials make them one of the very useful resources for the formulation of future bio-fungicides which are natural and non-hazardous for us and our environment.

Table-1
List of anti-fungal proteins isolated from plants of *Cucurbitaceae* family

Proteins	Source	Anti-fungal activity against	Molecular Weight (kDa)
PR-1	Pumpkin rinds	*Botrytis cinerea, Fusarium oxysporum, Fusarium solani, Rhizoctonia solani and Candida albicans*	40
PR-2	Pumpkin rinds	*Botrytis cinerea, Colletotrichum coccodes, Fusarium solani, Fusarium oxysporum,and Trichoderma harzianum*	14.8
PR-5	Pumpkin leaves	*Candida albicum*	28
Cucurmoschin	Black pumpkin seeds	*Botrytis cinerea*, *Fusarium oxysporum* and *Mycosphaerella oxysporum*	8
Hispin	Hairy melon seeds	*Coprinus comatus, Fusarium oxysporum, Physalo sporapiricola, and Mycosphaerella arachidicola*	21
α-Momorcharin protein	Bitter gourd seeds	*Fusarium solani* and *Fusarium oxysporum*	31.5
Luffacylin	Sponge gourd seed	*Mycosphaerella arachidicola* and *Fusarium oxysporum*	7.8
Vicillin like protein	Muskmelon	*Fusarium oxysporum*	8

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References

1. Borris R.P., Natural products research: perspectives from a major pharmaceutical company, *J. Ethnopharmacol.*, **51**, 29-38 **(1996)**

2. Tivy J., Biogeography: a study of plants in the ecosphere, Longman Scientific and Technical, J. Wiley, Burnt Mill, Harlow, New York **(1993)**

3. Van Etten H.D., Mansfield J.W., Bailey J.A. and Farmer E.E., Two classes of plant antibiotics: Phytoalexins versus phytoanticipins, *Plant Cell*, **6**, 1191-1192 **(1994)**

4. Rocio G.L., Gabriel M., Mariza G., *et al.,* Plant Antimicrobial Agents and Their Effects on Plant and Human Pathogens, *Int. J. Mol. Sci.*, **10(8)**, 3400-3419 **(2009)**

5. Osbourn A.E., Saponins in cereals, *Phytochemistry*, **62(1)**, 1-4 **(2003)**

6. Schlumbauam, Mauchf, Vogeliv and Bollert, Plant chitinases are potent inhibitors of fungal growth, *Nature*, **324**, 365-367 **(1986)**

7. Roberts W.K. and Selitrennikocf F.P., Plant proteins that inactivate foreign ribosomes, *Biosci. Rep.*, **6**, 19-29 **(1986)**

8. Mauch F., Mauch M.B. and Boller T., Antifungal hydrolases in pea tissue II. Inhibition of fungal growth by combinations of chitinase and β- 1,3-glucanase, *Plant Physiology*, **88**, 936-942 **(1988)**

9. Bhattacharya S., Chattopadhyay D. and Mukhopadhyay A., Changing Dimensions of Food Security in a Globalized World: A Review of the Perspectives for Environment, Economy and Health, *Int. Res. J. Environment Sci.,* **2(3),** 67-73 **(2013)**

10. Hrelia P., Fimognari C., Maffei F., *et al.,* The genetic and non-genetic toxicity of the fungicide Vinclozolin, *Mutagenesis*, **11**, 445-453 **(1996)**

11. Loper J.E., Henkels M.D., Roberts R.G., *et al.,* Evaluation of streptomycin, oxytetracycline, and copper resistance of *Erwinia amylovora* isolated from pear orchards in Washington State, *Plant Disease*, **75**, 287-290 **(1991)**

12. Arawwawala M., Thabrew I., Arambewela L. and Handunnetti S., Anti-inflammatory activity of *Trichosanthes cucumerina* Linn. in rats, *J. Ethnopharmacol.*, **131**, 538-543 **(2010)**

13. Huyen V.T., Phan D.V., Thang P., *et al.,* Antidiabetic effect of *Gynostemma pentaphyllum* tea in randomly assigned type 2 diabetic patients, *Horm. Metab. Res.*, **42**, 353-357 **(2010)**

14. Asghar M.N., Khan I.U. and Bano N., In vitro antioxidant and radical-scavenging capacities of *Citrullus colocynthes* (L) and Artemisia absinthium extracts using promethazine hydrochloride radical cation and contemporary assays, *Food Sci. Technol. Int.*, **17**, 481-494 **(2011)**

15. Santos K.K., Matias E.F., Sobral S.C.E., *et al.,* Trypanocide, cytotoxic, and antifungal activities of *Momordica charantia, Pharm. Biol.*, **50**, 162-166 **(2012)**

16. Xu X. and Ma J.K., The clinical observation of watermelon seeds on 52 cases of benign prostatic hyperplasia, *Pract.*

Journal of Integration Chin. Mod. Med., **1(5)**, 462-463 **(1998)**

17. Bai H. and Bai G.X., The treatment of watermelon rind for 17 cases of surface ulcers, *Journal of Extern. Ther. Tradit. Chin. Med.*, **10(2)**, 51 **(2001)**

18. Yang Z.X., Watermelon healed 2 cases of chronic diarrhea, *Chin. Commun. Health*, **18(14)**, 36 **(2002)**

19. Gao M.X., Ma H.L. and Guo K.Q., The bactericidal test of watermelon juice pulsed magnetic field, *Food Fermentat. Ind.*, **30(3)**, 14-17 **(2004)**

20. Peng W.Y., The treatment of croton and watermelon seeds through Shikishimaing the Yintang point for the thrush of children, *Chin. Journal of Pract. Chin. Mod. Med.*, **17(19)**, 2927 **(2004)**

21. Zhou Y.J., The clinical observations of watermelon frost spray on cervical erosion, *Pract. J. Integration Chin. Mod. Med.*, **7(6)**, 34 **(2007)**

22. Deng J.G., Wang S., Guo L.C. and Fan L.L., Anti-inflammatory and analgesic effects of extract from roots and leaves of *Citrullus lanatus*, *Chinese Herbal Medicine*, **2(3)**, 231-235 **(2010)**

23. Gao Y., Cai R.L., Xie C., *et al.,* Pharmacological basis for the medicinal use of musk melon base (*Pedicellus Melo*.) for abdominal distention and constipation, *J. Ethnopharmacol.*, **142(1)**, 129-135 **(2012)**

24. Ghule B.V., Ghante M.H., Uparganlawar A.B. and Yeole O.G., Analgesic and anti-inflammatory activities of *Lagenaria siceraria* (Mol.) stand, fruit juice extract in rats and mice, *Pharmacogn. Mag.*, **2**, 232-238 **(2006)**

25. Ghule B.V., Ghante M.H., Saoji A.N. and Yeole O.G., Hypolipidemic and anti-hyperlipidemic effects of *Lagenaria siceraria* fruit extracts, *Indian J. Exp. Biol.*, **44**, 905-909 **(2006)**

26. Ghule B.V., Ghante M.H., Saoji A.N. and Yeole O.G., Diuretic activity of *Lagenaria siceraria* fruit extract in rats, *Int. J. Pharm. Science*, **69**, 817-819 **(2007)**

27. Saha P., Sen S.K., Bala A., *et al.,* Evaluation of anticancer activity of *Lagenaria siceraria* Aerail parts, *Int. J. Cancer Res.*, **7(3)**, 244-253 **(2011)**

28. Nagao T., Lanaka R., Iwase Y., *et al.,* Studies on the constituents of *Luffa acutangula* Roxb, *Clin. Pharm. Bull*, **39**, 599-606 **(1991)**

29. Bailey L.H., The Garden of Gourds. The Gourd Society of America, Inc. Mt. Gilead, Ohio, United States **(1989)**

30. McGrath M.S., Hwang S.E., Caldwell S.E., *et al.,* GLQ 223: An inhibitor of human immunodeficiency virus replication in acutely and chemically infected cells of lymphocyte and mononuclear phagocyte lineage, *Natl. Acad. Sci. USA*, **86**, 2844-2848 **(1989)**

31. Ng T.B., Feng Z., Li W.W. and Yeung H.W., Improved isolation and further characteristation of beta trichosanthin: A ribosome inactivating and abortifacient protein from tubers of *Trichosanthes cucumeroicles* (Cucurbitaceae), *Int. J. Biochem.*, **23**, 561-567 **(1991)**

32. Ng T.B., Chan W.Y. and Yeung H.W., Proteins with abortifacient, ribosome inactivating, immunomodulatory, antitumor and anti-AIDS activities from Cucurbitaceae plants, *Gen. Pharmacol.*, **23(4)**, 579-590 **(1992)**

33. Lee H.S., Huang P.L., Bourinbaiar A.S., *et al.,* Inhibition of the integrase of human immunodeficiency virus (HIV) type 1 by anti-HIV plant proteins MAP30 and GAP31, *Proc. Natl. Acad. Sci. USA*, **92(19)**, 8818-8822 **(1995)**

34. Au T.K., Collins R.A., Lam T.L., *et al.,* The plant ribosome inactivating proteins luffin and saporin are potent inhibitors of HIV-1 integrase, *FEBS Letters*, **471(2/3)**, 169-172 **(2000)**

35. Nielsen K. and Boston R.S., Ribosome-inactivating proteins: a plant perspective, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, **52**, 785-816 **(2001)**

36. Puri M., Kaur I., Kanwar R.K., *et al.,* Ribosome inactivating proteins (RIPs)from *Momordica charantia* for antiviral therapy, *Curr. Mol. Med.*, **9(9)**, 1080-1094 **(2009)**

37. Broekaert W.F., Terras F.R., Cammue B.P. and Osborn R.W., Plant defensins: novel antimicrobial peptides as components of host defense system, *Plant Physiol.*, **108**, 1353-1358 **(1995)**

38. Selitrennikoff C., Antifungal proteins, *Appl. Environ. Microbiol.*, **67**, 2883-2894 **(2001)**

39. Oku H., Plant pathogens and disease control, CRS Press, Boca Raton, Florida **(1994)**

40. Walton J.D., Biochemical plant pathology, In: Plant biochemistry, Academic Press, California, 487-502 **(1997)**

41. Edreva A., Pathogenesis-related proteins: research progress in the last 15 years, *Gen. Appl. Plant Phys.*, **31**, 105-124 **(2005)**

42. Van Loon L.C., Rep M. and Pieterse C.M., Significance of inducible defense-related proteins in infected plants, *Ann. Rev. Phytopathol.*, **44**, 135-162 **(2006)**

43. Stanislava G., Biological and Technological Functions of Barley Seed Pathogenesis- Related Proteins (PRs), *J. Inst. Brew.*, **115(4)**, 334-360 **(2009)**

44. Van Loon L.C., Occurrence and Properties of plant pathogenesis-related protein, In: Datta S.K. and Muthukrishnan S. (eds), Pathogenesis-related proteins in plants, CRC Press, Boca Raton, 1-19 **(1999)**

45. Brederode F.T., Linthorst H.J. and Bol J.F., Differential induction of acquired resistance and PR gene expression in tobacco by virus infection, ethephon treatment, UV light and wounding, *Plant Mol. Biol.*, **17(6)**, 1117-1125 **(1991)**

46. Roberts W.K. and Selitrennikoff C., Zeamatin, antifungal protein from maize with membrane - permiabilizing activity, *J. Gen. Microbiol.*, **46**, 1771-1778 **(1990)**

47. Hejgaard J., Jacobsen S. and Svendsen I., Two thaumatin-like proteins from barley grain, *FEBS Letters*, **291**, 127-131 **(1991)**

48. Abad L.R., Urzo D., Liu M.P., *et al.,* Antifungal activity of tobacco osmotin has specificity and involves plasma membrane permeabilization, *Plant Sci.*, **118**, 11-23 **(1996)**

49. Park S.C., Lee J.R., Kim J.Y., *et al.,* PR-1, a novel antifungal protein from pumpkin rinds, *Biotechnol. Lett.*, **32(1)**, 125-130 **(2010)**

50. Park S.C., Kim J.Y., Lee J.K., *et al.,* Antifungal Mechanism of a Novel Antifungal Protein from Pumpkin Rinds against Various Fungal Pathogens, *J. Agric. Food Chem.*, **57(19)**, 9299-9304 **(2009)**

51. Cheong N.E., Choi Y.O., Kim W.Y., *et al.,* Purification and characterization of an antifungal PR-5 protein from pumpkin leaves, *Mol. Cells*, **7(2)**, 214-219 **(1997)**

52. Endo Y. and Tsurugi K., The RNA N-glycosidase activity or ricin A-chain: The characteristics of the enzymatic activity of ricin A-chain with ribosomes and with r RNA, *J. Bio. Chem.*, **263**, 8735-8739 **(1988)**

53. Bolognesi A., Olivieri F., Battelli M.G., *et al.,* Ribosome-inactivating proteins (RNA N-glycosidases) from the seeds of *Saponaria ocymoides* and *Vaccaria pyramidata, Eur. J. Biochem.*, **228(3)**, 935-940 **(1995)**

54. Ng T.B. and Parkash A., Hispin, a novel ribosome inactivating protein with antifungal activity from hairy melon seeds, *Protein Expr. Purif.*, **26(2)**, 211-217 **(2002)**

55. Wang S., Zhang Y., Liu H., *et al.,* Molecular cloning and functional analysis of a recombinant ribosome-inactivating protein (alpha-momorcharin) from *Momordica charantia*. *Appl. Microbiol. Biotechnol.*, **96(4)**, 939-950 **(2012)**

56. Parkash A., Ng T.B. and Tso W.W., Isolation and characterization of luffacylin, a ribosome inactivating peptide with anti-fungal activity from sponge gourd (*Luffa cylindrica*) seeds, *Peptides*, **23(6)**, 1019-1024 **(2002)**

57. Wang H. and Ng T.B., Luffangulin, a novel ribosome inactivating peptide from ridge gourd (*Luffa acutangula*) seeds, *Life Sci.*, **70(8)**, 899-906 **(2002)**

58. Ribeiro S.F.F., Agizzio A.P., Machado O.L.T., *et al.,* A new peptide of melon seeds which shows sequence homology with vicilin: Partial characterization and antifungal activity, *Scientia Horticulturae*, **111**, 399-405 **(2007)**

59. Yadav S., Tomar A.K., Jithesh O., *et al.,* Purification and partial characterization of low molecular weight vicilin-like glycoprotein from the seeds of *Citrullus lanatus, Protein J.*, **30(8)**, 575-580 **(2011)**

60. Wang H.X. and Ng T.B., Isolation of cucurmoschin, a novel antifungal peptide abundant inarginine, glutamate and glycine residues from black pumpkin seeds, *Peptides*, **24(7)**, 969-972 **(2003)**