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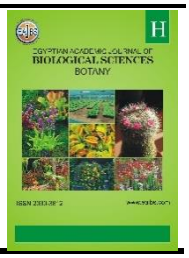

Isolation, Purification and Characterization of Antifungal Chitinase from *Phaseolus vulgaris* var. *paulista*

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ABSTRACT

Chitinase, a pathogenesis-related protein, from bean seeds was purified to homogeneity by ammonium sulphate precipitation and chitin affinity binding technique from common bean seeds. Chitinase showed a molecular mass of nearly 32,500KDa by SDS-PAGE the enzyme was purified to 2.47-fold with a specific activity of 1,93U/mg using ammonium sulphate precipitation and the purification increased to 5.61fold with a specific activity of 4.83 U/mg after chitin affinity binding technique. The optimum pH and Temperature of the purified enzyme activity were found to be 5.4 and 45°C respectively. The thermostability test of the enzyme showed its stability till 50°C. The purified enzyme was enhanced by Na^{+}, k^{+}, Ca^{2+}, and Mg^{2} salts and inhibited by Hg^{2+} and Pb^{2+} salts at different concentrations. The enzyme exhibited a strong inhibitory action against *Fusarium oxysporum, Fusarium solani, Fusarium moniliform, Alternaria alterna, and Candida albicans.* The enzyme may represent an important defense protein in bean seeds. The finding suggests the possible usage of the purified enzyme as an antifungal agent against some phytopathogenic fungi in the field and its gene can be cloned and expressed in other plants for the production of fungal resistance once. Besides, the anticandidal activity suggests its possible pharmaceutical applications.

INTRODUCTION

Plants are protected from fungal invasion by antifungal proteins and peptides found in a variety of affected plant tissues. Defense mechanisms to fight plant pathogens involve the synthesis of pathogenesis-related proteins that are not found in plants in normal conditions (Van Loon, *et al.,* 2006). These proteins can have a wide range of structural variations, and they are generally classified based on their biological activities and chemical structure. This categorization includes chitinases, chitin-binding proteins, ribosome-inactivating proteins, thaumatin-like proteins, peroxidases, defensins, defending-like peptides, lectins, protease inhibitors, and lipid-transfer proteins (Wong, *et al.,* 2012).

Chitinase (E.C.3.2.1.14) are glycosyl hydrolases that hydrolyze chitin into its oligomeric, dimeric, and monomeric components by cleaving the β-1,4linkage between GlcNAC. Endochitinases and exochitinases are the two classes of chitinase. Endochitinases randomly cleave chitin at internal polymer sites, resulting in the production of soluble, low

molecular weight N-acetylglucosamine multimers like chitotrose, chitotetraose, and the dimer di-acetylchitobios. There are two subcategories of exochitinases β-1,4-N-acetylglucoseaminidase, which cleaves the oligomer products of endochitinases and chitobiosidases to produce monomer N-acetylglucosamine, and chitobioses, which catalyse the release of di-acetyl chitobiose's from the nonoxidizing end of the chitin microfibril (Cohen-Kupiec and Chet 1998). It is believed that chitin, a long linear homopolymer of β 1,4-linked N-acetylglucosamine, is a crucial structural component of the fungal cell wall. Chitin makes up only 1-2% of the dry weight of the yeast cell wall (Klis.,1994 and Klis *et al*., 2002), although it is believed to make up 10-20% of the cell walls of filamentous fungi like *Neurospora* and *Aspergillus*. Chitin is broken by chitinase into oligomers, that are perfect for agricultural, industrial, and medical functions containing antitumor activity as well as elicitor action (Yuli *et al*., 2004 and Farag *et al*., 2016).

The common bean (*Phaseolus vulgaris* L.) is one of the most significant grain legumes in many parts of the world, providing more than 500 million people with a diet high in protein, dietary fiber, necessary micronutrients, and phytochemicals (Singh, *et al*., 2020). A large and diversified soil microflora, including different fungal species linked to diseases like root rot, such as an *Alternaria alternata*, can thrive and flourish in nations with subtropical climates (Were, *et al.*, 2021), and it's is easily affected by pest diseases, which can affect crop production. Van Schoonhoven and Voysest 1989, reported that more than 400 kinds of pests and 200 types of pathogens can affect it. This research aims to describe a new short method for isolation of chitinase enzyme from *Phaseolus vulgaris* plants, characterization of the isolated enzyme and test the antifungal activity of the enzyme against some pathogenic fungi.

MATERIALS AND METHODS

Plant Material:

Dry seeds of common bean (*Phaseolus vulgaris* var. *paulista*) were purchased from the (Agricultural Research Center, Giza).

Seed Protein Extraction:

Fifty grams of common bean seeds were pre-soaked for 48 hours in water at 4°C**.-** Soaked Seeds were homogenized in 500 ml of 50 mM Sodium phosphate buffer (pH 6.4). The extract was centrifuged at 14000 rpm for Fifteen minutes at 4°C. The supernatant was used for further experimentation.

Purification of Chitinase Enzyme:

a. Ammonium sulphate precipitation:

Using 80% ammonium sulphate saturation, 150 ml of protein extract was precipita ted, and the mixture was kept overnight at a temperature of 4°C. The residue was separated by centrifugation for 10 minutes at 14000 rpm at 4°C. The precipitate pellet was dialyzed and dissolved in 5 ml of a 50 mM sodium phosphate buffer with a pH of 6.4. dissolved in 30 ml of 50 mM sodium phosphate buffer (PH 6.4).

b. Chitin Affinity Binding:

Colloidal chitin was prepared using the Lee *et al*. (2009) method:

1 g of chitin powder was gradually added to 20 ml Hcl (concentrated),stirred and preserved at 4°C overnight. Following vigorous stirring, the mixture was added to 200 ml of ice-cold 95% ethanol and kept at 4°C overnight. Precipitate was obtained by filtration, and dialyzed against distilled water until the nutrient PH was reached by using dialysis tubing, and then the end volume was increased to 100 ml using ddH_2O. For chitin affinity 20 ml of protein dialysate was mixed with 10 ml of colloidal chitin in 50 mM sodium phosphate buffer with a pH of 6.4. The mixture was kept at 4°C overnight with constantly stirred. The mixture was then centrifuged for 10 minutes at 4 °C together with colloidal chitin at a speed of

10,000 rpm. To remove unbound substances, the precipitate was washed four times with 25 ml of 50 mM phosphate solution. The protein (chitinase) was then eluted from the precipitate using 25 ml of a PH 6.4 solution made up of 50 mM phosphate buffer and 1 M sodium chloride. In order to extract the enzyme from colloidal chitin, the mixture was stirred continuously at 4 °C overnight. After centrifugation at 10,000 rpm, The supernatant was precipitated with a 100% ammonium sulphate saturation. pellet was dialyzed and dissolved in 5 ml of a 50 mM sodium phosphate buffer with a pH of 6.4.

Chitinase Activity Measurement:

The reaction mixture contained 750 ul of colloidal chitin at 1% (w/v), and 750 ul of enzyme solutions suspended in a 0.1 mM acetate buffer solution. The reaction mixture was incubated at 40 °C for 60 minutes, centrifuged at 14000 rpm for 5 minutes, and the supernatant was used for reduced sugar analysis using the dinitrosalicylic acid (DNS) method. According to (Nawani and Kapadnis, 2005), the amount of chitinase enzyme required to release 1 u mol of GlcNAc per minute under the above-mentioned conditions is known as one unit (U) of chitinase activity.

Determination of Molecular Weight of Chitinase:

According to Laemmli's (1970) method, the molecular mass of the purified chitinase enzyme was determined by SDS-PAGE. Samples were mixed 1:4 with sample buffer before boiling for 10 minutes. Geneaid, a common protein size marker, was applied. Gels were stained with 0.1% (w/v) Coomassie brilliant blue R-250 (Sigma), 30% (v/v) methanol, and 10% (v/v) glacial acetic acid, and then destaining was done with a solution of 30% (v/v), methanol, and 10% (v/v) glacial acetic acid in distilled water. The molecular weight of chitinase was calculated by comparing it to those of the marker proteins.

Estimation of the Total Protein:

The Bradford method was used to determine The protein concentration of samples, and bovine serum albumin (Sigma) served as the standard (Bradford, 1976).

The Effects of pH and Temperature on the Enzymatic Activity of The Purified Chitinase:

The enzymatic activity of the purified enzyme was tested in the presence of pH range from pH 4.4 to pH 8.0 using 0.02 M sodium acetate buffer (pH 4.4, 5.0 and 5.4), sodium phosphate buffer 0.02 M (pH 6.0, 6.8, 7.4 and 8) and a temperature range from 30 to 80 °C. The relative activity was calculated with respect to the control where no additives were used during the reaction.

Thermostability of Chitinase:

In the method described by (Wang *et al*. 2008 a&b), the thermal stability of purified chitinase was evaluated by determining the residual activity of the enzyme solution after 30 min of incubation at different temperatures from 50 to 85 °C.

Effect of Metal Ions on Chitinase Activity:

Chitinase activity was determined by the standard assay method in the presence of Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Zn^{2+}, Hg^{2+}, Mn^{2+}, and Pb^{2+} at 1 and 2 mM. The relative activity was calculated with respect to the control where no additives were used during the reaction.

Antifungal Activity:

Fungal isolates (*Fusarium oxysporum, Fusarium solani, Fusarium moniliform, Alternaria alterna, and Candida albicans)* were obtained from the collection of fungi found in the Botany and Microbiology Department, at Minia University. For the antifungal activity test, filamentous fungi were subcultured on potato dextrose agar (PDA) medium for 7 days**,** and for the test, a 5 mm fungal plug of size was inoculated into the center of Petri dishes containing PDA medium and incubated for 48 h at 30°C, then 5 mm ware made in the gel wells, and 20 µl of purified enzyme or buffer (for control) were added, the plates then were incubated for 24 hours at 30°C. A crescentic zone of fungal inhibition is observed around the well when the enzyme has antifungal activity.

In the case of *Candida albicans*, it was subcultured in (PDA) for 24h and the fungal suspension's optical density was adjusted to reading OD 600=0.2 and 20 µl of the purified enzyme or buffer (as a control) was then added to the discs of the sterilized filter paper after spreading1ml of this suspension on PDA plates. Plates were incubated for 24h at 30°C, and the creation of clear zones surrounding the discs was evaluated.

RESULTS

Purification of Chitinase Enzyme*:*

The purification method results showed that the enzyme was purified 2.47-fold with a specific activity of 1.93 U/mg protein after ammonium sulphate precipitation. After the chitin affinity binding purification step, the enzyme purified to 5.61-fold with a specific activity of 4.38 U/mg protein as shown in Table (1).

Table 1. Purification of chitinase from *Phaseolus vulgaris.*

Step	Volume	Protein (mg)	Activity (U)	Specific activity (Umg-1)	Yield (%)	Fold of purification
Crude extract	150ml	250,58	196,28	0,78	100	1
Ammonium sulphate precipitation (80%)	50ml	97,44	188,70	1,93	96,1	2,47
Chitin affinity binding	5ml	0,284	1,245	4,38	0,6	5,61

Molecular Weight of Chitinase:

The Purified enzyme exhibited a single band on SDS –PAGE indicating that the purified chitinase is a monomeric protein as shown in Figure (1). The molecular weight of the chitinase obtained was estimated by SDS-PAGE to be 32.500 KDa. This range of molecular weight was reported for plant chitinase in many literatures.

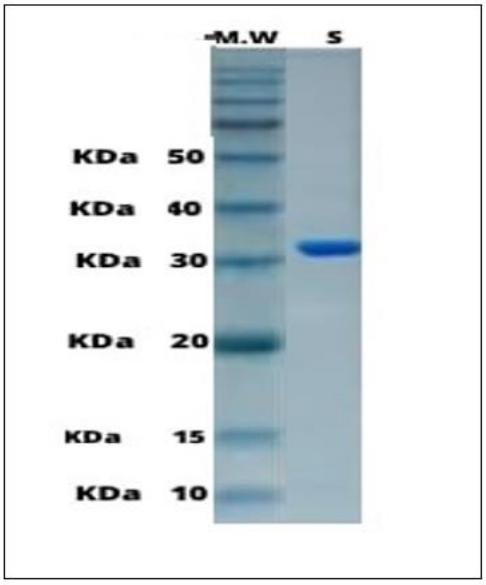

Fig 1. Purification of chitinase from *Phaseolus vulgaris* by SDS-PAGE**.**

Effect of PH on Chitinase Activity:

Relative enzyme activity as a function of pH was shown in (Fig.2). The optimum PH value was 5.4. A slight increase or decrease of pH around 5.4 would lead to a sharp reduction in the chitinase activity. The chitinase was almost inactivated at PH above 6 or below 5.

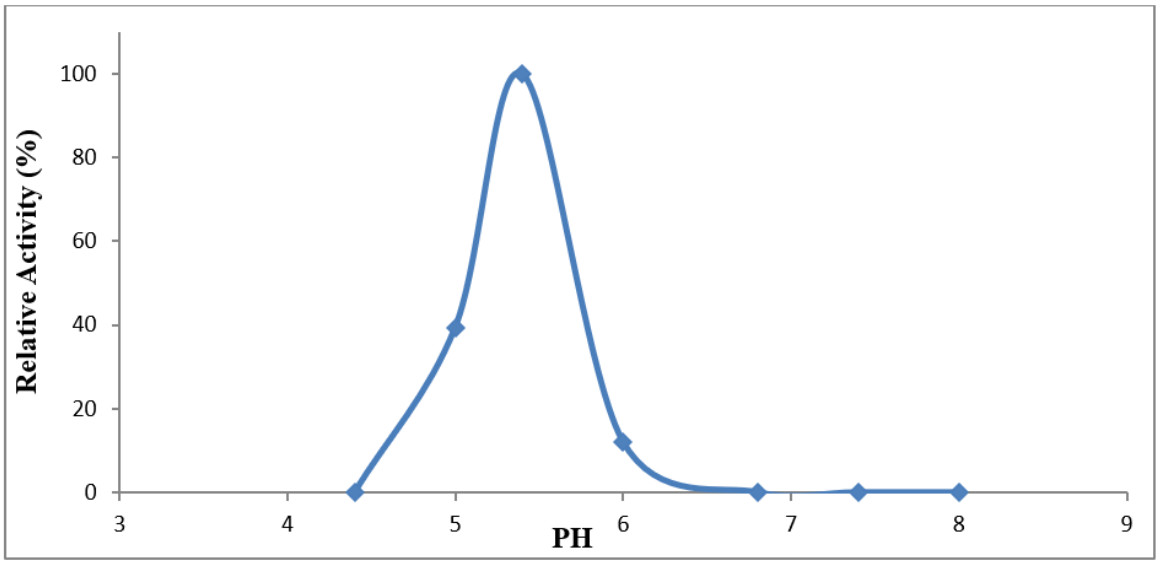

Fig. 2. Effect of pH on activity of the purified chitinase. Activity at pH 5.4 was used as standard.

Effect of Temperature on Chitinase Activity:

According to the results in (Fig.3), the chitinase relative activity indicated the optimum activity of chitinase at a temperature of 45°C, below and above this temperature there was a reduction in the enzyme activity.

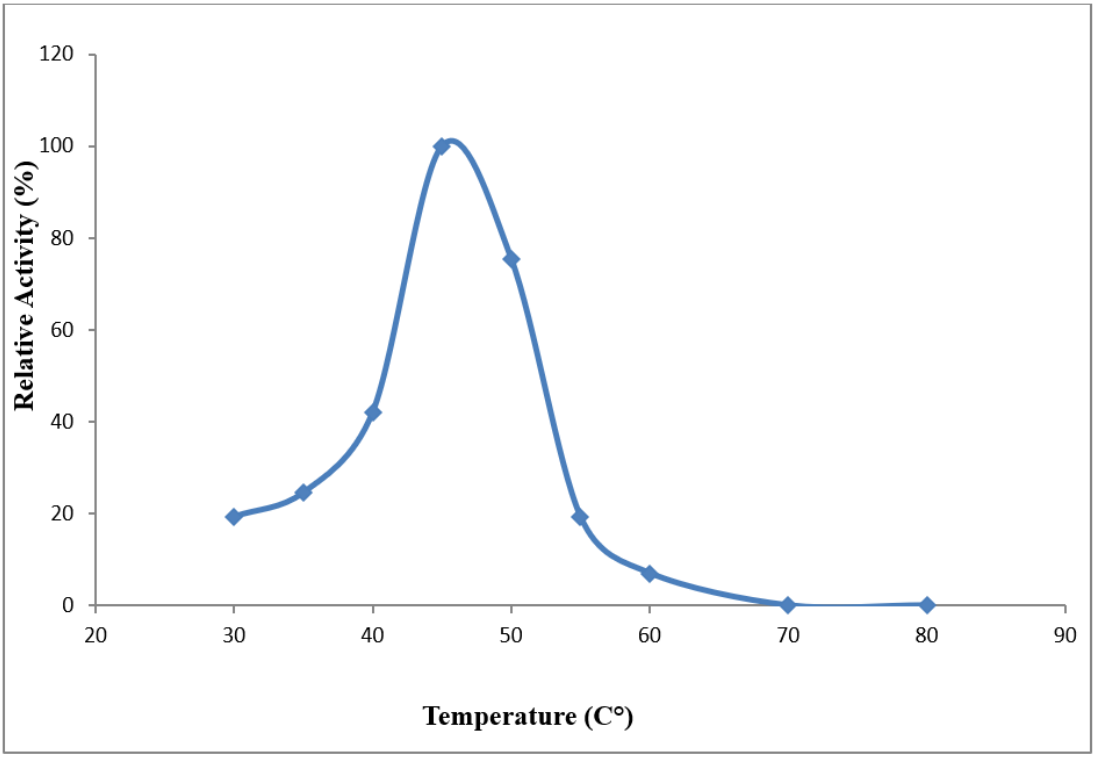

Fig .3. Effect of temperature on activity of the purified chitinase. Activity at 45 was used as standard.

Thermostability of Chitinase Enzyme:

Purified chitinase was stable from 45°C to 50°C, Incubation at 55°C for 30 min resulted in approximately half loss of the enzyme activity and it was rapidly inactivated when it incubated at a temperature above 55°C (Fig. 4).

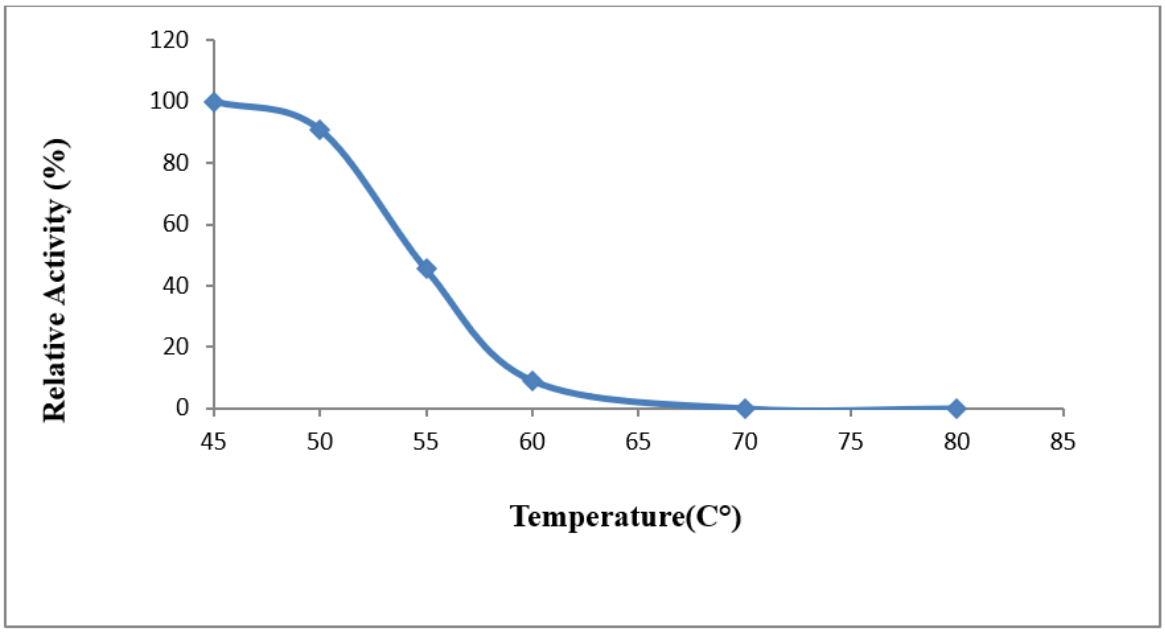

Fig. 4. Thermal stability of the purified chitinase.

Effect of Metal Ions on Chitinase Activity:

In the presence of Na^{+}, k^{+}, Zn^{2+}, Ca^{2+}, Mg^{2+}, $^{+}$and Mn^{2+} the chitinase relative activity was either retained well or slightly increased. However, the activity was inhibited by Pb^{2+} and Hg^{2+}. $Hg^{2+}$is a common inhibitor of most chitinases (Fig. 5).

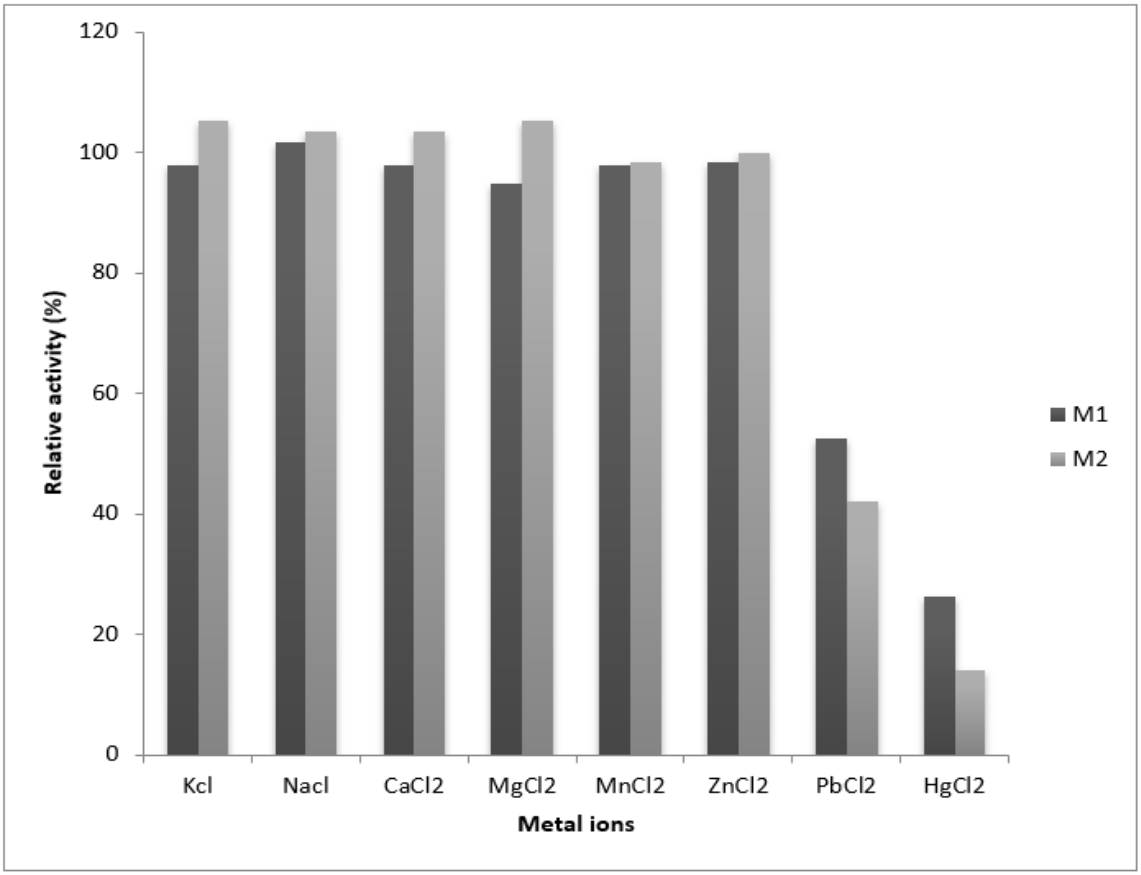

Fig.5. The effect of metal ions on *Phaseolus vulgaris* chitinase activity.

Antifungal Activity:

Antifungal activity of the purified chitinase enzyme was tested against a collection of fungi through crescents of inhibition around wells and clear zones around disks, it exhibited a very strong effect against *Alternaria alternate* (Fig.6 A), strong effect against *Fusarium moniliform, Fusarium oxysporum* (Figs.6 B& C), followed by *Fusairum solani and Candida albicans* (Figs. 6. D, and E).

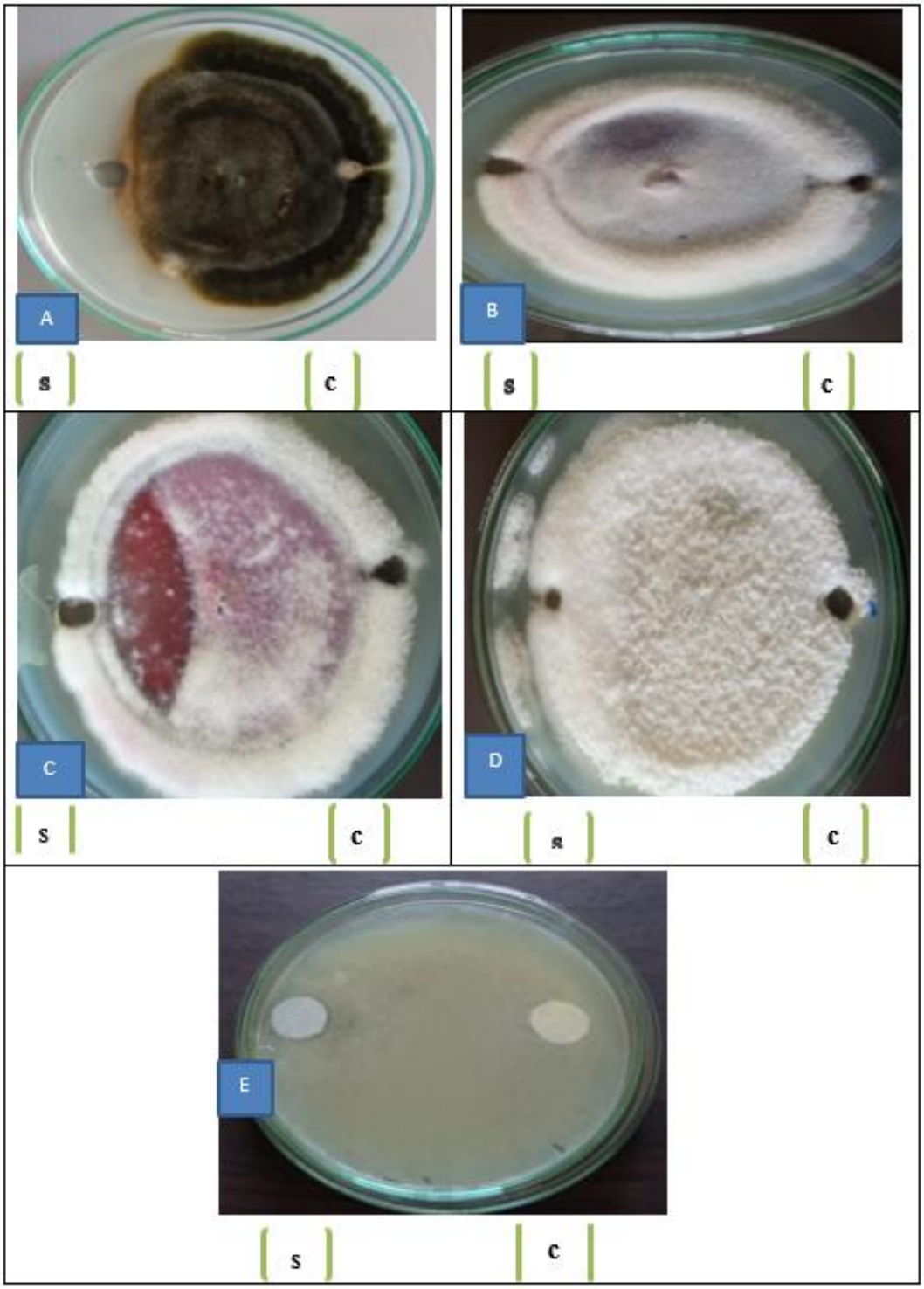

Fig .6. Antifungal activity against different fungi (A) *A. alternata*, (B) *F. moniliforme*, (C) *F. oxysporum* , (D) *F. solani*, (E) *C. Albicans*. (s) refers to sample, and (c) refers to control.

DISCUSSION

Enzymes are found in living organisms where they regulate and catalyze essential chemical reactions required for the life of organisms (Nisha and Divakaran, 2014). They are large and fragile protein molecules. They are different from inorganic catalysts, sensitive and unstable to process conditions (Biro *et al.,2008;* Buchholz *et al.*,2012). As mentioned in some reports for the purification of chitinase enzymes from different sources, the chitin affinity binding technique was used for the purification of a new chitinase from *Phaseolus vulgaris* var. *paulista*. The results indicate that chitinase was purified to 5.61fold revealing the effectiveness of this purification method. Other researchers have reported a multistep procedure for the purification of chitinase (Ye and Ng 2005), since they purified a chitinase throw three steps of chromatography (CMSephadex C-50, POROS HS and Sephadex G-75 column chromatography) from *Mung* beans to 3.9-fold. Also, (Taira *et al*., 2005) purified chitinase from *Pineapple* leaf throw four chromatography steps such as chitin affinity, (Butyl-Toyopearl 650 column, Resource Q column and HPLC on phenyl superpose column), and also (Waleed, 2022) who purified a new chitinase from cowpea seeds by using the same chitin affinity techniques.

The current results indicate the molecular weight of the isolated chitinase enzyme from *Phaseolus vulgaris* var. *paulista* of nearly 32.500 KDa. Generally, the molecular weight of chitinase ranged from 20 to 80 kDa. Sometimes, several chitinases are secreted from one strain, whereas, there are visible variations in their molecular weight and enzyme activity (Sakai *et al.,* 1998). the molecular weight for most leguminous plant chitinases was reported within the range between 25-35 kDa (Ye and Ng 2002). Some chitinases have a molecular weight near 30 kDa, while others are also over 30 kDa even up to 45 kDa in their molecular weight (Sahai and Manocha 1993; Radhajeyalakshmi *et al*. 2000).

Optimum conditions of the isolated chitinase enzyme from *Phaseolus vulgaris* var. *paulista* (pH, temperature, and thermostability) were determined using the 3,5-dinitro salicylic acid (DNS) assay. Maximum enzyme activity was found at pH 5.4, but the enzyme retained >80% of its activity between pH 5.0 and pH 6. It has been noted that other chitinases from various organisms have this acidic character. such as *Manduca sexta* (Zhu *et al*., 2001), *Beauveria bassiana* (Fan *et al*., 2015), *Ipomoea batatas* L. (Liu *et al*., 2020), *Helicoverpa armigera* (Ahmed *et al.,* 2003), *Locusta migratoria* (Li *etal.*, 2016), *Lithocolletis ringoniella* (Fan *e tal*., 2016) and *Eisenia fetida* (Ueda *et al.*, 2017). The optimum temperature for the enzyme at pH 5.4 was 40–50°C. Chitinase showed lower activity when it was tested at lower 40°C or more than 50°C, which is similar to the counterparts from *Mung bean (Phaseolus mungo*) seeds, bean (*Phaseolus vulgaris*) leaves, bean (*P. vulgaris*) seeds obtained by Wang *et al*. (2005), Boller *et al*. (1983), and Yang and Luo (1998) respectively. However, the optimum pH of the purified chitinase was somewhat different from the above-mentioned chitinases reported by either Boller *et al*. (1983) or Yang and Luo (1998). The former was at pH 6.5 and the latter was at pH 6.0, while this newly isolated chitinase was almost inactivated by 40% at pH 6.0. The chitinases often differ in their physical, chemical and biological properties, which represents the diversity of chitinases. The thermostability of the purified enzyme is similar to that of chitinase reported by Boller *et al*. (1983) but higher than those for chitinases reported by Yang and Luo (1998) and by Wang *et al.* (2005).

According to the results, the chitinase enzyme was activated by Ca, Mg, Na and K, not affected by Mn and Zn while it was inhibited by Hg^{2+} and Pb^{2+}. The activity of a chitinase from *Microbispora* sp. V2 was just inhibited by Hg^{2+}, in the presence of which only 10% activity was retained (Nawani, *et al.,* 2002). Among the two chitinases that were purified from the intestinal tract of the South American sea lion, one was inhibited with Cu^{2+}, Fe^{2+}, Hg^{2+} and Zn^{2+}, while the other one was inhibited with Fe^{2+} (Konagaya *et al*.,

2006). The lima bean chitinase was inhibited by Pb^{2+} and Hg^{2+} rather than Cu^{2+} and Fe^{2+} (Wang *et al*., 2008).

Chitinases from different sources have been reported to have antifungal activity, such as those from bacteria) Zarei *et al.*, 2011; Delfini *et al*.,2021) and plants (*Taira et al*., 2001). Our results showed antifungal activity against a series of pathogenic fungi which agree with the results obtained for plant chitinases (Boller *et al*. 1983; Yang and Luo 1998; Wang *et al.* 2008). When it comes to the antifungal mechanism of chitinase, we could go back to its nature as one of the chitinolytic enzymes. Chitin, a β-(1,4)-linked polymer of N-acetyl D-glucosamine (GlcNAc), is a majorly structural polysaccharide in fungal cell walls. Chitinases hydrolyze the chitin located in fungal cell walls and demonstrate antifungal activity, normally the hyphal morphological distortion along with stunted growth is observed for fungi with enzyme treatment (Shaoyun *et al.*, 2009) Also, the enzymatic activity of chitinase is measured with chitin as a substrate.

Conclusion:

Chitinase enzyme with M.W (32.500KDa) was purified using ammonium sulphate precipitation and chitin affinity binding technique from bean seeds. It showed also optimum pH and temperature at 5.4 and 45°C respectively. The enzyme showed activity till 50°C, enhanced by Na^{+}, k^{+}, Ca^{2+}, Mg^{2} ions and inhibited by Hg^{2+}, Pb^{2+}ions while is not affected by Zn and Mn. The enzyme exhibited strong antifungal activity against several pathogenic fungi, with possible biotechnological applications.

REFERENCES

Ahmad, T., Rajagopal, R., and Bhatnagar, R.K., (2003). Molecular characterization of chitinase from polyphagous pest *Helicoverpa armigera*. *Biochemical and Biophysical Research Communications*, 310, 188–195.

Biro, E., Nemeth, A. s., Sisak, C., Feczko, T. and GYENIS, j. (2008): Preparation of chitosan particles suitable for enzyme immobilization. *Journal of Biochemical and Biophysical Methods*,70:1240:1246.

Boller T, Gehri A, Mauch F, ad Vogeli U (1983). Chitinase in bean leaves: induction by ethylene, purification, properties, and possible function. *Planta*, 157, 22–31.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein dye binding. *Analitical Biochemistery*, 72, 248-254.

Cohen-Kupiec R, and Chet I. (1998) The molecular biology of chitin digestion. *Current Opinion in Biotechnology*, 9, 270-277.

Delfini, C. Villegas, L. Martínez, M. and Baigorí, M. (2021). Extracellular antifungal activity of Chitinase- producing Bacteria isolated from guano of insectivorous bats, *Current Microbiology*, 78, 2787–2798.

Fan,X.J., Mi, Y.X., Ren, H., Zhang,C,. Li, Y., and Xian, X.X, (2015). Cloning and functional expression of a chitinase cDNA from the apple leaf miner *moth Lithocolletis ringoniella*. *Biochemistry, Moscow.* 80, 242–250.

Farag AM, Abd-Elnabey HM, Ibrahim HAH, and El-Shenawy M. (2016). Purification, characterization and antimicrobial activity of chitinase from marine-derived *Aspergillus terreus*. *Egyptian Journal of Aquatic Research*, 42(2):185–192.

Klis FM, Mol P, Hellingwerf K, and Brul S. (2002). Dynamics of cell wall structure in *Saccharomyces cerevisiae*. *Federation of European Microbiological Societies. Review*,26: 239–256.

Klis FM. (1994). Review: cell wall assembly in yeast. *Yeast*, 10:851–869.

Konagaya, Y., Tsuchiya, C., and Sugita, H. (2006). Purification and characterization of chitinases from *Clostridium sp*. E-16 isolated from the intestinal tract of the South

American sea lion (*Otaria flavescens*). *Letters in Applied Microbiology,* 43, 187–193.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature,* 227: 680-85.

Lee, Y. G.; Chung, K. C.; Wi, S. G.; Lee, J. C. and Bae, H. J. (2009). 'Purification and properties of a chitinase from *Penicillium* sp. LYG 0704'. *Protein Expression Purification Journal,* 65, 244-50.

Li, Y.L., Song, H.F., Zhang, X.Y., Li, D.Q., Zhang, T.T., Ma, E.B., and Zhang, J.Z., (2016). Heterologous expression and characterization of two chitinase 5 enzymes from the migratory locust *Locusta migratoria*, *Insect Science*, 23, 406–416.

Liu, M, Gong, Y., Sun, H., Zhang, J., Zhang, L., Sun, J., Han, Y., Huang, J., Wu, Q., and Zhang, C., Li, Z. (2020). Characterization of a novel Chitinase from sweet potato and its fungicidal effect against *Ceratocystis fimbriata*, *Journal of Agricultural and Food Chemistry*, 68, 7591–7600.

Nawani, N. N., Kapadnis, B. P., Das, A. D., Rao, A. S., and Mahajan, S. K. (2002). Purification and characterization of a thermophilic and acidophilic chitinase from Microbispora *sp*. V2. *Journal of Applied Microbiology*, 93, 965–975.

Nawani, N.N. and Kapadnis, B.P. (2005). Optimization of chitinase production using statistics based experimental designs. *Process Biochemistry,* 40, 651-60.

Nisha, N. S. and Divakaran, J. (2014). Optimization parameters for alkaline protease production using bacterial isolates from different coastal regions of Tamil Nadu, *India International Journal of Current Microbiology and Applied Sciences*,3:500-505.

Radhajeyalakshmi R, Meena B, Thangavelu R, Deborah SD, Vidhyasekaran P, and Velazhahan R (2000). 45-kDa chitinase purified from pearl millet (*Pennisetum glaucum (L.*) shows antifungal activity. *Journal of Plant Diseases and Protection,* 107:605–616.

Sahai AS, and Manocha MS (1993). Chitinases of fungi and plants: their involvement in morphogenesis and host–parasite interaction. *Federation of European Microbiological Societies,* 11: 317–338.

Shaoyun Wang & Biao Shao & Hong Fu and Pingfan Rao (2009). Isolation of a thermostable legume chitinase and study on the antifungal activity. *Applied Microbiology and Biotechnology,* 85: 313–321.

Singh, G.; Dukariya, G.; and Kumar, (2020). Distribution, Importance and Diseases of Soybean and Common Bean: A Review. *Biotechnology Journal International,* 24, 86–98.

Taira, T., Yamagami, T., Aso ,Y., Ishiguro ,M., and Ishihara, M., (2001). Localization, accumulation, and antifungal activity of Chitinases in Rye (*Secale cereale*) seed. *Bioscience, Biotechnology, and Biochemistry,* 65, 2710–2718.

Taira, T.; Toma, N. and Ishihara, M. (2005). 'Purification, Charactrization and Antifungal activity of chitinase from Pineapple (*Anana camosus*) leaf. *Bioscience, Biotechnology, and Biochemistry,* 69: 189-96.

Ueda, M., Shioyama, T., Nakadoi, K., Nakazawa, M., Sakamoto, T., Iwamoto, T., and Sakaguchi, M. (2017). Cloning and expression of a chitinase gene from *Eisenia fetida*, *International Journal of Biological Macromolecules,* 104 1648–1655.

Van Loon, L.C., Rep, M. and Pieterse, C.M. (2006). Significance of inducible defense-related proteins in infected plants. *Annual Review of Phytopathology*, 44, 135–162.

Van Schoonhoven, A. and Voysest, O. (1989). Common beans in Latin America and their constraints in Bean production problems in the tropics (second edition) (Eds. H. F. Schwartz & M. A. Pastor-Corrales): 33-57, (CIAT, Cali,).

Waleed W. Nazeer. (2022). Anovel isolation and purification of antifungal chitinase from cow pea (*Vinga unguiculata*) and its possible biotechnological applications. *African Journal of Biological Sciences,*18(2):89-96.

Wang SY, Shao B, Ye XY, and Rao PF (2008). Purification and characterization of a chitinase from peanut (*Arachis hypogaea L*.). *Journal of Food Biochemistry,* 32:32–45.

Wang, S. Y., Zhou, J. J., Shao, B., Lu, Y. J., and Rao, P. F. (2008). A thermostable chitinase with chitin-binding activity from *Phaseolus limensis. Journal of Food Science,* 73(6), 452–457.

Wang, S.Y., Wu, J.H., Rao, P.F., NG, T.B. and YE, X.Y. (2005). A chitinase with antifungal activity from the *mung bean. Protein Expression and Purification,* 40, 230–236.

Were, S.A.; Narla, R.; Mutitu, E.W.; Muthomi, J.W.; Munyua, L.M.; Roobroeck, D.; Valauwe, B. Biochar and Vermicompost (2021). Soil Amendments Reduce Root Rot Disease of Common Bean (*Phaseolous vulgaris L.*). *African Journal of Biological Sciences,* 3, 176–196.

Wong, J.H., Ip, D.C.W., Ng, T.B., Chan, Y.S., Fang, F. and Pan, W.L. (2012). A defensing like peptide from *Phaseolus vulgaris cv. 'King Pole Bean'*. *Food Chemistry,* 135, 408-414.

Yang, H.Q. and Luo, Z.M. (1998). A study of the purification and properties of chitinase from bean seeds *(Phaseolus vulgaris*). *Journal of Hunan Agricultural University. (Chinese),* 24(3), 194–198.

Ye XY, and Ng TB (2002). Delandin, a chitinase-like protein with antifungal, HIV-1 reverse transcriptase inhibitory and mitogenic activities from the ricebean (*Delandia umbellata*). *Protein Expression and Purification,* 24:524–529.

Ye, X. and Ng, T.B. (2005). A chitinase with antifungal activity from the mung bean. *Protein Expression and Purification,* 40:230-36.

Yuli PE, Suhartono MT, Rukayadi Y, Hwang JK, and Pyunb YR. (2004). Characteristics of thermostable chitinase enzymes from the *Indonesian Bacillus sp*.13.26. *Enzyme and Microbial Technology;35*(1-2):147- 53.

Zarei, M., Aminzadeh, S., Zolgharnein,H., Safahieh, A., Daliri, M., Noghabi, K.A., Ghoroghi, A., and Motallebi, A., (2011). Characterization of a Chitinase with antifungal activity from a native *Serratia Marcescens* B4A, Braz. *Journal of Microbiology,* 42 1017–1029.

Zhu, X., Zhang, H., Fukamizo, T., Muthukrishnan, S., and Kramer, K.J., (2001). Properties of *Manduca sexta* chitinase and its C-terminal deletions. *Insect Biochemistry and Molecular Biology*, 31, 1221–1230.