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SEPARATION AND PARTIALLY PURIFICATION OF AN ANTIFUNGAL PROTEIN ON AGAROSE GEL ELECTROPHORESIS FROM PEARL MILLET SEEDS

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ABSTRACT
One of main challenges' faced by growing crops successfully, comes from the natural enemies of plants including bacterial, viral and fungal pathogens .Antifungal proteins (AFPs) are ubiquitous components in different plant parts, belong to a group of plant defense protein that have a broad spectrum of biological activity and play a key role in plant defense against pathogenic fungi by preventing or limiting their spread. Occurrence of AFPs in seeds might be involved in protecting the seeds during resting or storage germination. Various antifungal protein have been studied from different plant seeds, that help to protect the seeds from the invading soil-borne plant pathogens. The main objective of proposed study was to isolate and partial purification of antifungal protein from Pearl millet seeds and its analysis on agarose gel electrophoresis .Pearl millet is known by the name of scientific name of "*Pennisetum glaucum.* The protein was isolated by 60% ammonium sulphatesalt fractionation method and partially purified by dialyzing the sample against sodium phosphate buffer (pH=7).The protein was analysed on 1% agarose gel electrophoresis. Single band was obtained corresponding to the molecular mass of 25KDa.Antifungal activity against Aspergillusniger was tested which had shown the inhibition of A.niger after the plates were incubated for 48 hours.

KEYWORDS: Pearl millet, antifungal protein, *Aspergillus niger*, Agarose gel electrophoresis.

INTRODUCTION

One of main challenges of growing crops successfully comes from the natural enemies of plants including viral, bacterial and fungal pathogens. The dependence in agriculture on synthetic agrichemicals including antimicrobials is undoubtedly important but not sustainable for the sake of the environment in the long run. Some plant proteins associated with defence, particularly the pathogenesis-related (or PR) proteins, may appear as the host responses not only to pathogen attack, but also to mechanical and chemical agents or different environmental stressors. (Leung WMD & Alizadesh H, 2011).

Pearl millet is known by the scientific name of *Pennisetum glaucum*. It is the most commonly grown millet in the Indian subcontinent and in West Africa. It is also known by the name of "Bajra" in India. Numerous health benefits of Pearl Millet are because of its richness in essential compounds like phytic acid and niacin which is not found commonly in other cereal grains. It is an excellent source of protein and fibre. It has very high starch content and is rich in minerals like phosphorus and zinc. It is also known for its high iron content. It is easily available in areas with erratic rains as compared to other grains due to the plants low water consumption. Due to its rich composition of minerals and proteins, Pearl Millet has many health benefits**,** beneficial in treating stomach ulcers, for heart health, bone growth development and repair, reduces cancer risk, helps in weight loss. Pearl Millet has the highest protein content for any grain.

Many antifungal proteins have been detected in plnat seeds. These proteins include chitinases (Anuratha CS, *et.al.*, 1992), β-1, 3-glucanases (Leah R. *et.al.*, 1991), thionins (Carmona MJ. *et.al*.1993), permatins (Roberts WK and Selitrennikoff CP 1986, Leach R *et.al*,1991) defensins (Terras FRG *et.al*.1992b) and nonspecific lipids transfer proteins (Terras FRG.*et.al.*,1992,a,Commue *et.al*,1995). It is suggested that these antifungal proteins may proetct the seeds from the invading soil-born plant pathogens (Commue BPA.*et.al.*, 1995). In the course of a search for antifungal proteins from plants seeds, we observed inhibition of mycelial growth of *Aspergillus niger* with extracts of Pearl millet. In this paper we had described the isolation and partial purification of an antifungal protein from Pearl millet (*Pennisetum glaucum(L.)* R.Br.) seed and its analysis on agarose gel electrophoresis.

MATERIALS AND METHODS

Seeds ofpearl millet (*Pennisetum glaucum(*L.) were obtained from Local market of Akola, Maharashtra and were ground and the powder was suspended in 200ml,5mM Sodium phosphate buffer, 10mM NaCl, 1mM EDTA, pH 7.0. and then homogenized in a blender at 4^0 C for 5min.The homogenate was filtered through cheese cloth before centrifugation at 8,433 rpm.for 15 min at 4^0 C.The pellet was discarded and the supernatant was fractionated by the addition of $(NH_4)_2SO_4$ and the fraction that percipitated at 60% saturation was collected by centrifugation at 8,500 rpm.for 10 min at 4^0 C. The percipitate was resuspended in 5mM Sodium phosphate buffer, pH 7.0 and dialyzed against the same buffer overnight.Amount of protein in all samples were determined by the Folin lowry assay using egg albumin as standard (Velazhahan R .*et.al.*, 2001). The dialysed sample was analyzed on 1% Agarose gel using MolBio Himedia prestained protein ladders (range 10-245 kDa) on horizontal gel electrophoresis unit. Sample was run at 100 volt for 2 hour.and gel was stained for protein with Coomassie brilliant blue partial.

Fungal growth inhibition assay

All experiments were carried out under sterile conditions.The protein fractions were sterilized using 0.2µm filters and used for assayof antifungal activity against Aspergillusniger, a spore suspension of Aspergillusniger was prepared and mixed with 20ml of molten Potato dextrose agar (PDA) medium and poured onto the Petri dishes (90 mm in diameter). Sterile filter paper discs (6 mm in diameter) were laid on the agar surface one cm away from periphery of the petri dish and 0.02 ml supernatant fractions (10µg of protein) were applied to the each disc.After that plates were incubated at room temp. for 48 hr.

RESULTS AND DISCUSSION

An antifungal protein was isolated from Pearl millet seeds by ammonium sulphate fractionation.The partial purification of protein was carried out by dialysis technique using sodium phosphate buffer(pH 7).The protein was analysed on 1% agarose gel electrophoresis. A single band of 25kDa protein was observed (Fig 1). An antifungal activity of above protein against *Aspergillus niger* was observed after the plates were incubated at room temperature for 48 hours. Antifungal proteins (AFPs) are ubiquitous components in different plant parts, belong to a group of plant defence proteins that have a broad spectrum of biological activity and play a key role in plant defence against pathogenic fungi by preventing or limiting their spread (De Wit, 1992). They can protect plants from devastating damage caused by fungal pathogens and consequently prevent economic losses (Wang HX,Ng TB.,2006). Occurrence of AFPs in seeds might be involved in protecting the seeds during resting or storage and germination (Chilosi G *et.al*.,2000 and Nobrega FM. *et.al*.,2005). These natural defence mechanisms are necessary as seeds are likely to encounter plenty of soil-borne pathogenic organisms in the soil. At the imbibitional stage, the seed coat represents a physical and chemical barrier against aggressors until favorable conditions for seed germination (Ramos AL. *et.al*.,1998). In germinating seeds, the growing radicle becomes exposed to pathogens (Witmer X *et.al*.,2003). Therefore, a post-germination defence strategy can protect germinating seeds when physical barriers are unable to protect the embryo from pathogenic organisms (Flach J. *et.al*.,1992, Rose TL .*et.al*, 2006and Yang X.*et.al*.2006).

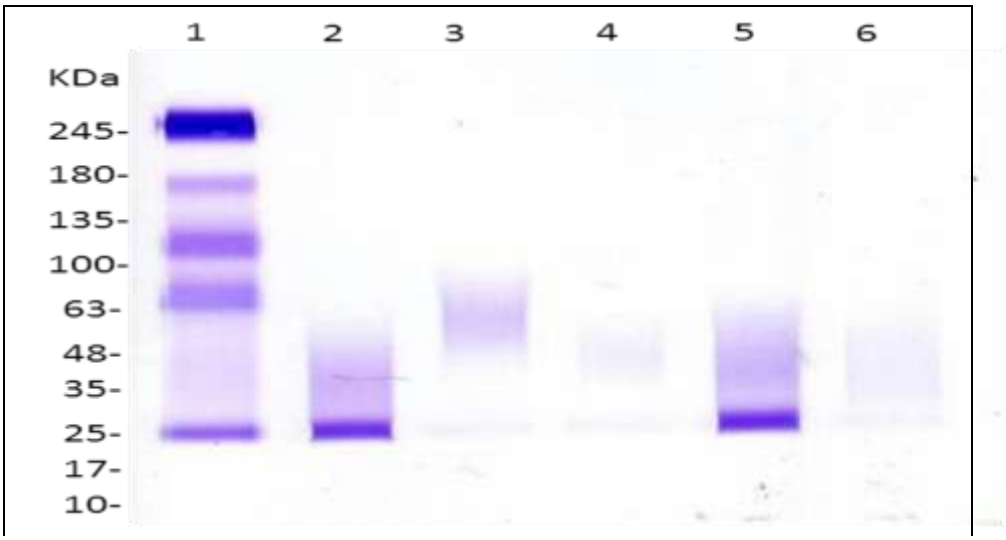

Fig.1 Coomassie brilliant blue stained 1% agarose gel electrophoresis showing Lane1-molecular mass marker, Lane 2 to 6-dialysed Antifungal protein from pearl millet seeds.

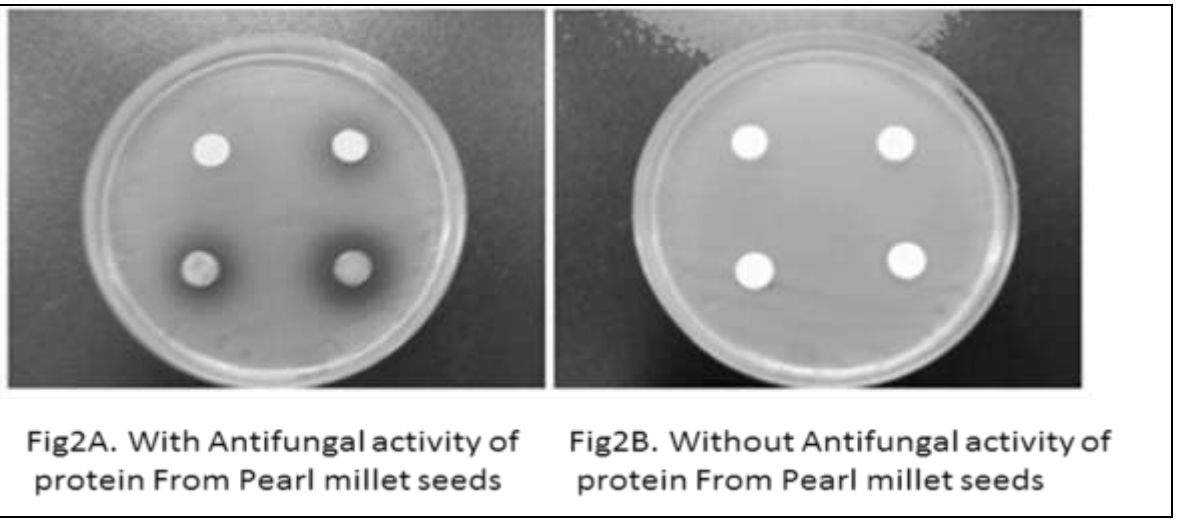

Fig2A. With Antifungal activity of protein From Pearl millet seeds

Fig2B. Without Antifungal activity of protein From Pearl millet seeds

CONCLUSION

The present findings showed that Pearl millet seeds possess the antifungal activity that may be due to the presense of such proteins. Study also proves that protein can be analysed on agarose gel electrophoresis.

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